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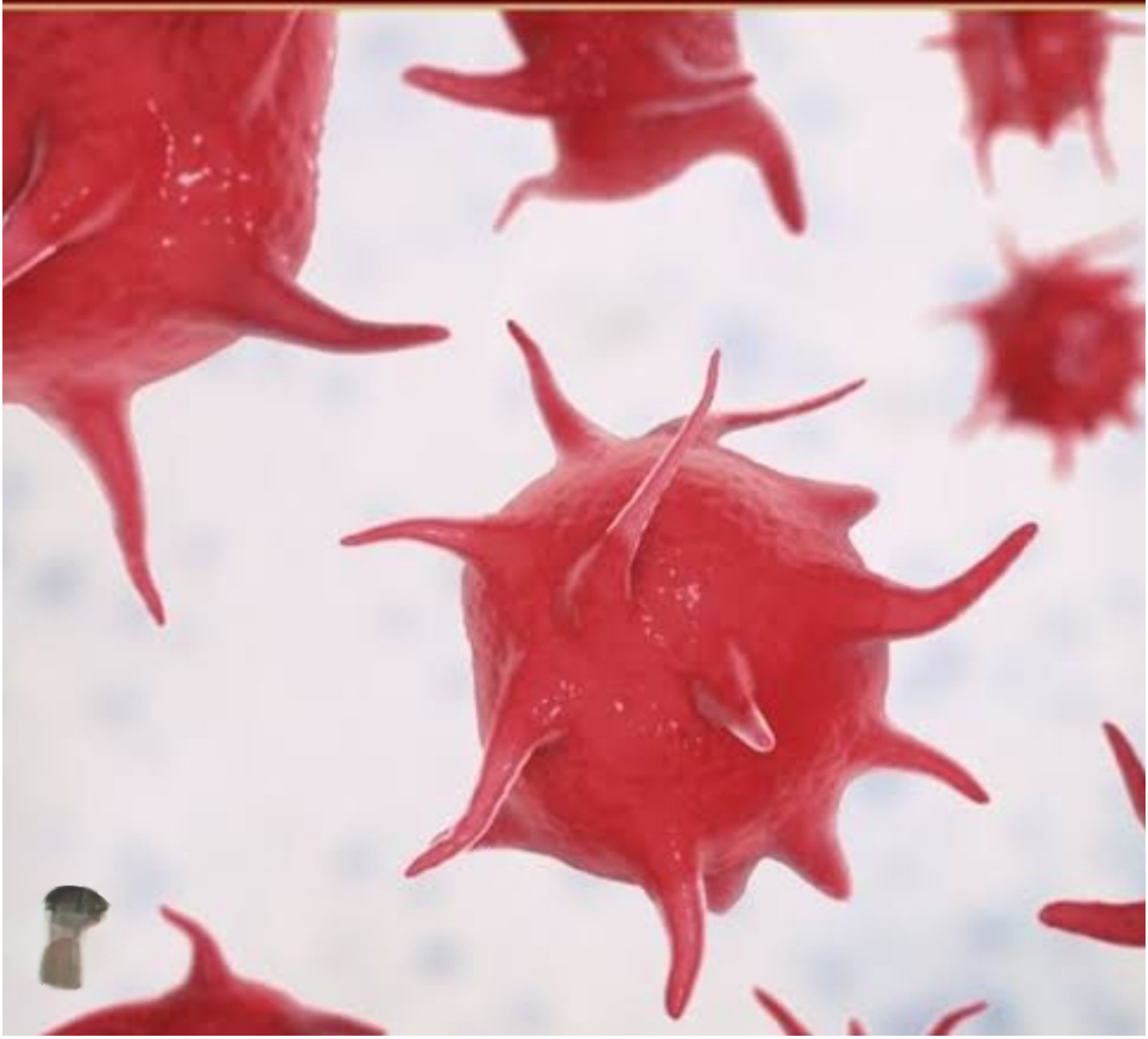
Hematology 2023

AMERICAN SOCIETY of HEMATOLOGY
Education Program™
65th ASH® Annual Meeting and Exposition
December 9-12, 2023



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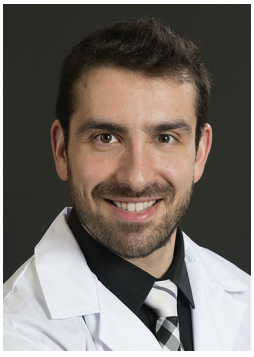
Editors' Message

Welcome to the 65th annual meeting of the American Society of Hematology. In addition to meeting friends and colleagues old and new, forging collaborations, and learning of the promising new advances in hematology, we hope you will discover that this year's meeting will not just meet but exceed the high standards for education for which the ASH Education Program is known. With a complete set of rigorously peer-reviewed, case-based, concise articles for this year's education program replete with useful figures and tables, our standards for *Hematology, the ASH Education Program* have never been higher, and we hope you will find that the quality of the content has never been better. We are also happy to say to all the bookworms out there that your pleas have been heard: the print version of *Hematology* is now once again available, this time for sale on an opt-in basis. This way, everyone wins: those who prefer to read the content online can continue to do so, and those who prefer a physical book can buy one if they wish. Best of all, no one needs to find room in their luggage for the tome, as preordered printed copies will be shipped soon after the meeting. In this volume, you will find articles for every session included in the education program, as well as a series of thought-provoking evidence-based minireviews, all written by international expert speaker-authors.

We must thank this year's education co-chairs, Dr. Jean Connors (classical hematology) and Dr. Amy DeZern (malignant hematology), for crafting a state-of-the-art education program on which this volume is based. Finally, *Hematology* is possible only thanks to the efforts of hundreds of invaluable peer reviewers and the diligent efforts of the ASH publications staff, including Ebony Stewart, Alison Beale, Jeremiah Murphy, Dax Rodulfa-Blemberg, Brian Cannon, Kenneth April, Keith Gigliello, Glenn Landis, and Nina Hoffman, among others. We are thankful for all of their work.

Enjoy, from your *Hematology* Editors!



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The *Hematology ASH Education Program* is an annual publication that provides practicing hematologists with invaluable information on the most important areas of clinical progress.

Hematology 2023 is a peer-reviewed collection of articles written by the 2023 ASH Education Program speakers and the Ham-Wasserman Lecturer. The papers showcase groundbreaking advances and new concepts in 31 different fields. Every year, the periodical provides an updated and comprehensive review of each of the topics covered in the annual meeting education sessions.

Educational objectives

1. Employ the knowledge gained regarding the diagnosis and treatment of malignant and classical hematologic disorders to improve patient care.
2. Discuss the state-of-the-art therapeutics in hematology.
3. Analyze the potential contribution of novel, not-yet-approved modalities of therapy to current evidence-based management of malignant and classical hematologic disorders.

Date of release

December 2023

Date of expiration

On December 31, 2024, the ability to earn Continuing Medical Education (CME) credit and American Board of Internal Medicine (ABIM) Maintenance of Certification (MOC) points for this product expires. This is the last date for users to claim credit for this product. For questions about credit, please contact the ASH Education Department at cme@hematology.org or call 866-828-1231 (toll-free) within the United States only or 1-202-776-0544 internationally.

Accreditation and credit designation

The American Society of Hematology (ASH) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians.

ASH designates this enduring material for a maximum of 40 *AMA PRA Category 1 Credits*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Physicians who participate in this CME activity but are not licensed in the United States are also eligible for *AMA PRA Category 1 Credit*™. To earn these credits, readers must pass two online tests (malignant and classical) based on articles from *Hematology 2023*.

ABIM MOC

Successful completion of this CME activity, which includes participation in the evaluation component, enables the participant to earn up to 40 MOC points in the ABIM's MOC program. It is the CME activity provider's responsibility to submit participant completion information to ACCME for the purpose of granting ABIM MOC credit.

Claiming CME credits and ABIM points

The estimated time to complete this educational activity is 40 hours. To claim CME credit, users must complete an evaluation of the product and a test of medical knowledge, both of which are accessed through ASH Academy on Demand (academy.hematology.org). There is a one-time processing fee for claiming CME/MOC credit. Users with scores of 80% or better on these self-assessment modules are eligible to claim credit for the activity.

To facilitate claiming of credit, the test for this product is divided into two subtests, one of which focuses on malignant hematology content with the other focusing on classical content. Successful completion of each test earns the user 20 *AMA Category 1 PRA Credits*™. Users claim CME and/or ABIM MOC credit for each test individually. You can take one or both tests, depending on your CME and MOC needs.

The malignant and classical hematology tests consist of 20 questions each. The questions in each test can be answered in one sitting, or a user can save their progress and return to complete the test at a later time.

The malignant hematology test covers information presented in the following sections:

- Are We Personalizing MDS Therapy in 2023?
- CAR T Cells in ALL: Bridge or Definitive Therapy?
- Graft-Versus-Host Disease: Is an Ounce of Prevention Worth a Pound of Cure?
- Have We Optimized Therapy Yet for Patients with AML?
- How Can We Manage High-Risk Hematologic Malignancies in the Community?
- How Do We Apply T-Cell Redirection Therapy for Multiple Myeloma? CAR T Cells and Bispecific Antibodies
- How Do We Calibrate Cellular Therapy for Lymphoma In 2023?
- How Do We Enhance Results in Rare Hematologic Malignancies?
- How Do We Extend Survival for Patients with CLL In 2023?
- How Do We Improve Outcomes in Relapsed and Refractory Multiple Myeloma in 2023?

- How Do We Tackle Remaining Clinical Challenges in CML?
- How Is the Management Paradigm Evolving for Hodgkin Lymphoma in 2023?
- Not Kids Anymore and Not Adults Yet: How Do We Treat Adolescent and Young Adult (AYA) Patients with ALL?
- What Are the Advances in Myeloproliferative Neoplasms Affecting Management?
- What Are Treatment Options for Patients with Relapsed, Refractory, or Persistent AML?
- What Makes a Good Transplant Recipient? Putting the Puzzle Pieces Together

The classical hematology test covers information presented in the following sections:

- Acquired Hemophilia A Diagnosis and Management
- Alphabet Soup: Challenging Consults on the Pediatric Units
- Energizing the Red Cell: Pyruvate Kinase Activators for Treatment of Hereditary Hemolytic Anemias
- Goldilocks and Transplant Timing in Inherited Marrow Failure Syndromes: Too Early, Too Late, Just Right?
- Ham-Wasserman Lecture
- Hematologic Toxicity of Immunotherapies
- Hematologists and the Care of Pregnant Women
- Hematologists as Lifesavers: Inpatient Hematology Emergencies
- Hemostasis in Patients with Severe Liver Disease
- Hot Topics in Blood Donation: Donor Risks and Social Justice
- Improving Outcomes for Individuals with Sickle Cell Disease: Are We Moving the Needle?
- Inherited Bone Marrow Failure Syndromes: from Pediatrics to Adult
- Ongoing Challenges in the Management of VTE
- The Iron Revolution!
- Transfusion Support in Sickle Cell Disease
- Why Am I Getting Paged at 2 AM? Microangiopathic Emergencies

Thrombotic anti-PF4 immune disorders: HIT, VITT, and beyond

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Antibodies against the chemokine platelet factor 4 (PF4) occur often, but only those that activate platelets induce severe prothrombotic disorders with associated thrombocytopenia. Heparin-induced thrombocytopenia (HIT) is the prototypic anti-PF4 disorder, mediated by strong activation of platelets through their FcγIIa (immunoglobulin G [IgG]) receptors (FcγRIIa). Concomitant pancellular activation (monocytes, neutrophils, endothelium) triggers thromboinflammation with a high risk for venous and arterial thrombosis. The classic concept of HIT is that anti-PF4/heparin IgG, recognizing antigen sites on (cationic) PF4 that form in the presence of (anionic) heparin, constitute the heparin-dependent antibodies that cause HIT. Accordingly, HIT is managed by anticoagulation with a nonheparin anticoagulant. In 2021, adenovirus vector COVID-19 vaccines triggered the rare adverse effect "vaccine-induced immune thrombotic thrombocytopenia" (VITT), also caused by anti-PF4 IgG. VITT is a predominantly heparin-*independent* platelet-activating disorder that requires both therapeutic-dose anticoagulation and inhibition of FcγRIIa-mediated platelet activation by high-dose intravenous immunoglobulin (IVIG). HIT and VITT antibodies bind to different epitopes on PF4; new immunoassays can differentiate between these distinct HIT-like and VITT-like antibodies. These studies indicate that (1) severe, atypical presentations of HIT ("autoimmune HIT") are associated with both HIT-like (heparin-dependent) and VITT-like (heparin-independent) anti-PF4 antibodies; (2) in some patients with severe acute (and sometimes chronic, recurrent) thrombosis, VITT-like antibodies can be identified independent of proximate heparin exposure or vaccination. We propose to classify anti-PF4 antibodies as type 1 (nonpathogenic, non–platelet activating), type 2 (heparin dependent, platelet activating), and type 3 (heparin independent, platelet activating). A key concept is that type 3 antibodies (autoimmune HIT, VITT) require anticoagulation plus an adjunct treatment, namely high-dose IVIG, to deescalate the severe anti-PF4 IgG-mediated hypercoagulability state.

LEARNING OBJECTIVES

- Classify the different prothrombotic disorders induced by platelet-activating anti–platelet factor 4 antibodies
- Define 3 types of anti-PF4 antibodies
- Review treatment for patients with anti-PF4 disorders and the requirement for therapeutic-dose anticoagulation and high-dose intravenous immunoglobulin (IVIG)

Introduction

Anti–platelet factor 4 (PF4) antibodies are the underlying cause of the prothrombotic disorders heparin-induced thrombocytopenia (HIT) and vaccine-induced immune thrombotic thrombocytopenia (VITT). It is increasingly recognized that anti-PF4 antibodies can also cause severe prothrombotic disease independent of ongoing heparin treatment or adenovirus vector vaccination.[1] However, only a subset of anti-PF4 antibodies are pathogenic.

Within the emerging concepts of thromboinflammation and immunothrombosis,[2] we describe the clinical presentations, serological characteristics, and pathogenesis of HIT and VITT and address the emerging evidence of prothrombotic anti-PF4 antibody disorders beyond HIT and VITT. We also propose a new nomenclature for anti-PF4 antibodies. Type 1 antibodies are non–platelet activating and usually of no pathological relevance. In contrast, type 2 and type 3 antibodies are pathogenic and activate platelets via FcγIIa receptors (FcγRIIa). Type 2 antibodies cause classic HIT and require the concomitant presence of PF4 and pharmacological concentrations of heparin (or another polyanion) to effect pathogenicity. Type 3 antibodies cause thromboinflammation by binding to PF4 alone and have been identified to underlie VITT. An

important new development is that anti-PF4 type 2 and type 3 antibodies are increasingly identified as causes of severe, atypical HIT ("autoimmune HIT") as well as thrombotic disorders beyond HIT and VITT.

Favorable outcomes in thrombotic anti-PF4 antibody disorders require early recognition and aggressive treatment. It is increasingly being recognized that the often extreme hypercoagulability state of patients with predominant anti-PF4 type 3 antibodies will not abate with therapeutic-dose nonheparin anticoagulation. An adjunctive strategy to deescalate the prothrombotic state is paramount. This is currently achieved by high-dose intravenous immunoglobulin (IVIG).

CLINICAL CASE

In 2015 a 35-year-old woman presented with severe headache and thrombocytopenia (49×10^9/L); D-dimer levels were greatly elevated (>35 000 µg/L fibrinogen equivalent units [FEU]). Cerebral vein sinus thrombosis (CVST) was confirmed by nuclear magnetic resonance. Her past medical history included a recent upper respiratory tract infection about 2 weeks prior; otherwise, she was healthy and did not take any medications other than hormonal contraception. Heparin was started, but the CVST progressed. The combination of unexplained thrombosis and thrombocytopenia prompted testing for anti-PF4 antibodies; a PF4/heparin immunoglobulin G (IgG) microtiter plate assay was strongly positive, but the confirmatory heparin-dependent platelet activation test was negative. Although platelet counts increased, the patient developed fatal secondary intracerebral bleeding.

HIT and VITT

HIT

Fifty years ago (1973), HIT was recognized as a prothrombotic disorder associated with heparin-dependent, platelet-activating antibodies. Later, the antigen target was identified as a multimolecular complex of the cationic chemokine PF4 (also named CXCL4) and negatively charged heparin. Until the early 1990s, the predominant clinical presentation of HIT was believed to be arterial thrombosis.[3] But during the era of widespread heparin thromboprophylaxis, it became evident that HIT was more often associated with venous thrombosis and pulmonary embolism, particularly after major surgery. HIT antibodies activate platelets and leukocytes via FcγRIIa and trigger massive thrombin generation. The classic view of HIT is that of a predominantly heparin-dependent, platelet-activating disorder, managed by heparin cessation and substitution with a nonheparin anticoagulant. This includes direct thrombin inhibitors (argatroban, bivalirudin) and agents with exclusive (fondaparinux, rixaroxaban, apixaban) or predominant (danaparoid) anti–factor Xa activity.[4] In addition, IVIG is emerging as an adjunct treatment to inhibit FcγRIIa-mediated platelet activation.[5] In contrast, warfarin treatment during acute HIT increases the risk for limb ischemic necrosis and amputation due to the progressive microvascular thrombosis associated with severe protein C depletion.[6] A clinical suspicion of HIT is based on clinical features, as reflected by the 4Ts scoring system (Table 1).

VITT

In 2021, recognition of a rare (1-2/100 000 vaccinations) adverse effect of adenovirus vector–based COVID-19 vaccines, VITT, greatly increased interest in anti-PF4 IgG-mediated disorders.[7-9] The characteristics of VITT are summarized in Table 2. In comparison to HIT, the risk for CVST or splanchnic vein thrombosis seems to be especially increased in VITT. Anti-PF4 antibodies in VITT differ substantially from HIT antibodies: they bind to an epitope on PF4 distinct from the HIT heparin-dependent antigen sites.[10] Indeed, heparin usually *inhibits* VITT antibody-mediated platelet activation. The current view of VITT is that of a predominantly heparin-*independent* platelet-activating disorder that requires high-dose IVIG to decrease FcγRIIa-mediated platelet activation and associated hypercoagulability together with therapeutic-dose anticoagulation.[11]

The iceberg model

A striking feature of the anti-PF4/heparin immune response is that a high proportion of heparin-exposed patients form nonpathogenic (anti-PF4 type 1) antibodies. Also, in 5% to 8% of the normal population, nonpathogenic anti-PF4 type 1 antibodies are found after COVID-19 vaccination.[12] This has enormous diagnostic relevance, given that anti-PF4 antibodies detectable by immunoassays do not necessarily indicate clinical disease. The "iceberg" model (graphical abstract) represents this concept[13]: the "tip" of the iceberg represents the clinically evident manifestations (thrombocytopenia, thrombosis) of the anti-PF4 response, in association with anti-PF4 type 2 and/or type 3 antibodies.

"Functional" (platelet activation) tests detect anti-PF4 platelet-activating antibodies (Table 3).[14] They differentiate between anti-PF4 type 1, type 2, and type 3 antibodies. For detection of anti-PF4 type 2 antibodies in HIT, heparin is added. First-generation platelet-rich plasma assays were later supplanted by washed platelet assays, with various readout modifications, mainly: the serotonin-release assay (SRA), the heparin-induced platelet activation assay (HIPA), and flow cytometry-based assays. For the detection of anti-PF4 type 3 antibodies in VITT, PF4 (instead of heparin) is added. All functional assays are restricted to reference laboratories.

The seminal discovery by Jean Amiral that HIT antibodies target PF4 paved the way for developing widely applicable enzyme immunoassays (EIAs) for detecting IgG that recognize PF4/heparin (polyanion) complexes. With widespread EIA availability, HIT entered the diagnostic mainstream. Microtiter plate-based HIT assays are also sensitive for anti-PF4 type 3 (VITT) antibodies, while commercially available rapid immunoassays generally only recognize anti-PF4 type 2 HIT antibodies (Table 3).[15]

Pathogenesis of HIT and VITT

The clinical characteristics of HIT have long puzzled clinicians and scientists. The notion that the 2 dominant anticoagulants of the 1970s and 1980s—heparin and vitamin K antagonists—induce or aggravate thrombotic complications, in the setting of thrombocytopenia, was highly counterintuitive. Today, HIT and VITT can be seen as prototypic examples of immunothrombosis and thromboinflammation.[2] These 2 evolving concepts combine immunity and hemostasis, especially the network between coagulation and the innate immune system, involving platelets,

Table 1. The 4Ts score for heparin-induced thrombocytopenia

	Score=2	Score=1	Score=0
Thrombocytopenia Compare the highest platelet count within the sequence of declining platelet counts with the lowest count to determine the % of platelet fall	• >50% platelet fall AND a nadir of ≥20×10⁹/L AND no surgery within preceding 3 days	• >50% platelet fall but surgery within preceding 3 days OR • Any combination of platelet fall and nadir that does not fit criteria for score 2 or score 0 (eg, 30% to 50% platelet fall or nadir • 10 to 19×10⁹/L	• <30% platelet fall • Any platelet fall with nadir <10×10⁹/L
Timing (of platelet count fall or thrombosis[a]) Day 0=first day of most recent heparin exposure	• Platelet fall days 5 to 10 after start of heparin • Platelet fall within 1 day of start of heparin AND exposure to heparin within past 5 to 30 days	• Consistent with platelet fall days 5 to 10 but not clear (eg, missing counts) • Platelet fall within 1 day of start of heparin AND exposure to heparin in past 31 to 100 days • Platelet fall after day 10	• Platelet fall ≤4 days without exposure to heparin past 100 days
Thrombosis (or other clinical sequelae)	• Confirmed new thrombosis (venous or arterial) • Skin necrosis at injection site • Anaphylactoid reaction to IV heparin bolus • Adrenal hemorrhage	• Recurrent venous thrombosis in a patient receiving therapeutic anticoagulants • Suspected thrombosis (awaiting confirmation with imaging) • Erythematous skin lesions at heparin injection sites	• Thrombosis not suspected
oTher cause for thrombocytopenia	• No alternative explanation for platelet fall is evident	• Possible other cause is evident: • Sepsis without proven microbial source • Thrombocytopenia associated with initiation of ventilator • Other:	• Probable other cause is present: • Within 72h of surgery • Confirmed bacteremia/fungemia • Chemotherapy or radiation within past 20 days • DIC due to non-HIT cause • Posttransfusion purpura • Thrombotic thrombocytopenic purpura • Platelet count <20×10⁹/L and given a drug implicated in causing drug-induced immune thrombocytopenia • Nonnecrotizing skin lesions at LMWH injection sites

[a]Key features of HIT are a platelet count decrease of more than 50% but, uncommonly, less than 20×10⁹/L; a typical onset in the second week of heparin treatment (between days 5 and 10; first day of immunizing heparin exposure=day 0); the occurrence of new thrombotic complications (arterial and/or venous); and the absence of another compelling explanation for the clinical features observed. Risk for HIT: score less than or equal to 3=low; 4–5=intermediate; 6–8=high.

LMWH, low-molecular-weight heparin.

Data modified from Warkentin and Cuker.[37]

monocytes, neutrophils, and—in the case of anti-PF4 antibody disorders—anti-PF4 IgG antibodies as key players. Immunothrombosis was originally designed through evolution to defend against microbial infection. It locally confines an infection by facilitating the recognition, containment, and destruction of pathogens. When these defense mechanisms get out of control, thromboinflammation develops. If misdirected, for example, by anti-PF4 type 2 and especially type 3 antibodies, thromboinflammation results in activation of the endothelium, complement, and innate immune cells, particularly granulocytes and monocytes, causing immunothrombosis.[16]

Figure 1 summarizes the self-enhancing activation cascade involving platelets, the coagulation cascade, and the innate immune system by platelet-activating anti-PF4 antibody types 2 and 3.

While the downstream effects of thromboinflammation and immunothrombosis are rather similar in HIT and VITT, the mechanisms triggering initial immunization differ. In HIT, PF4 binds to the polyanion, heparin; PF4 thereby undergoes conformational changes, expressing neoepitopes, which trigger the activation of B cells and the production of anti-PF4/polyanion antibodies. For VITT, the region on PF4 to which anti-PF4 type 3 antibodies bind is well characterized.[10] It overlaps with the binding site of heparin to PF4 and is distinct from the binding site of HIT antibodies. Which vaccine constituent(s) trigger(s) the anti-PF4 immune response and why tolerance is broken after vaccination, resulting in anti-PF4 type 3 antibodies, remain(s) unresolved. Patches of negative charge on the adenovirus vector have been suggested as PF4 binding sites,[17] but it remains unclear whether these or other vaccine constituents interact with PF4 to trigger the aberrant immune response. The ChAdOx1-nCoV-19 vaccine contains more than 2000 different proteins, many derived from the cell line in which the vaccine vector is propagated.[18] The usual straightforward approach to identify the binding partner of PF4 that causes the conformational changes inducing the immune reaction would be to incubate PF4 in the presence and absence of different potential binding partners and measure the binding of anti-PF4 antibodies to putative complexes. This

Table 2. Characteristics of VITT

Thrombocytopenia (<150×10⁹/L) or documented platelet count decrease by more than 50%
AND
D-dimer >8-fold the upper normal limit, in most assays corresponding to >4000 µg/mL (FEU)
AND
Presence of thrombosis OR typical headache that may precede CVST as described below: • severe persistent headache starting 4 or more days after vaccination (in about 10% of VITT patients, severe headache precedes CVST). Of note, headache during the first 2 days after vaccination is a common, harmless adverse event.
AND
Positive anti-PF4 antibody EIA assay and positive functional assay for PF4 dependent antibodies: • if no functional assay is available, a strong OD in the PF4 ELISA of >2.0 can be used as a surrogate marker, as this is associated with a >95% probability for the presence of platelet-activating PF4-dependent antibodies.[39] • Of note rapid assays for HIT are insensitive for VITT antibodies and a negative rapid test does not exclude VITT
AND
Onset of symptoms 4 to 30 days after vaccination (day of vaccination=day 0) with the exception of isolated DVT/PE, which may occur up to 42 days after vaccination
VITT should not be diagnosed
If an alternative diagnosis is more likely (eg, tumor, sepsis)

DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; OD, optical density; PE, pulmonary embolism.

Data modified from Pavord et al.[38]

approach, however, cannot be used in VITT because the antibodies became autoantibodies that bind to and cluster PF4 by themselves with very high affinity.[19] This phenomenon is also seen in patients with atypical clinical presentations of HIT, such as when thrombocytopenia begins, worsens, or persists in the absence of heparin.

The anti-PF4 type 2 antibody immune response in HIT (and most likely also the anti-PF4 type 3 antibody response in VITT) is a *secondary* immune response. The onset of thrombocytopenia in HIT occurs typically between days 5 and 10 (median, day 7), even in patients who have never been treated with heparin and without anti-PF4 IgM precedence. A primary immune response would require considerably more time for B-cell activation, and an immunoglobulin class switch from IgM to IgG, before the production of high-titer antibodies. One explanation for primary immunization against PF4/polyanion complexes is PF4 binding to bacteria. Gram-positive and gram-negative bacterial surfaces are strongly negatively charged, at least twice as much as eukaryotic cells. Indeed, this charge difference represents a fundamental difference between prokaryotic and eukaryotic cells. Strong negative charges are highly efficient activators of innate immunity, including the complement system, the contact phase of coagulation, and the granulocytes. However, the adaptive immune system has no receptors for negative charges. We have proposed that one of the biological roles of PF4 is to "translate" negative charge into structure.[20] After binding to strong negatively charged molecules on bacterial surfaces, PF4 undergoes conformational changes that induce anti-PF4 antibodies. The interesting evolutionary concept is that these anti-PF4/polyanion IgG antibodies can opsonize any bacterium that binds PF4, even if the immune system has never encountered that particular organism before. During treatment with heparin, this strongly charged polyanion binds to cell surfaces, and subsequently, PF4 binds and undergoes its conformational changes, exposing the "danger" epitopes to which pathogenic anti-PF4 type 2 antibodies bind.

Another unusual feature of the anti-PF4 immune response is antibody transience (rapid seroreversion). In both HIT and VITT, platelet-activating antibodies disappear in the majority of patients within 3 to 6 months,[21,22] and in some patients even faster. This feature, however, does not fit a typical secondary immune response. In addition, even PF4 knockout mice can produce anti-PF4 antibodies upon microbial challenge despite their immune system never having encountered PF4 before. Furthermore, B cells that produce anti-PF4 antibodies after nonspecific in vitro stimulation are found in the blood of nearly all humans (including newborn cord blood). Thus, at least the IgM immune response against PF4 is part of the innate immunoglobulin repertoire. Both the transience of the IgG response and the antigen-independent production of antibodies indicate that the involved B cells are most likely either B1 or marginal zone B cells,[23] as they typically produce natural antibodies that react with endogenous proteins. Many healthy individuals have natural, polyreactive IgM antibodies, which bind to PF4/heparin complexes.[24] This allows complement factor C3 binding to the natural IgM bound to PF4 complexes. B cells express the receptor for C3, allowing binding of PF4/natural IgM-C3 complexes to nearly all B cells. This also brings PF4 into close proximity to the cognate receptor on anti-PF4 antibody-producing B cells.[25]

Despite the many similarities, there is a striking difference between HIT and VITT anti-PF4 antibodies. Anti-PF4 IgG antibodies in HIT are polyclonal[26]; in VITT, the resulting antibodies appear to be mono- or oligoclonal, with a unique restriction to one haplotype of the hypervariable IgG light chain region.[27] This may hint toward a genetic predisposition for VITT, while in HIT no genetic predisposition has been identified.

Beyond HIT and VITT

The classic picture of HIT as a primarily heparin-dependent disorder was challenged over 20 years ago when patients were

Table 3. Platelet antigen and activation assays for detecting HIT and VITT antibodies

Assay	Comment
Enzyme-immunoassays (EIAs) *(None of the EIAs detect all HIT and/or all VITT antibodies)*	
PF4/heparin (in-house and commercial)	Sensitive (≥99%) for HIT and VITT
PF4/polyvinylsulfonate	Sensitive (≥99%) for HIT and VITT
Platelet lysate/heparin	Sensitive for HIT (>99%), slightly reduced sensitivity for VITT in comparison to other EIAs
Aeskulisa HIT II	Sensitive for HIT; slightly reduced sensitivity for VITT in comparison to other EIAs
Rapid immunoassays	
Particle gel immunoassay	Sensitivity for HIT >95%, but ~45% sensitivity for VITT
Lateral flow assay	Sensitivity for HIT ~90%, but ~10% sensitivity for VITT
Latex enhanced immunoturbidimetric assay	Sensitivity for HIT ~95%, but <5% sensitivity for VITT
Chemiluminescence immunoassay for anti-PF4/heparin antibodies	Sensitivity for HIT ~95%, but <5% sensitivity for VITT
Chemiluminescence immunoassay for anti-PF4 antibodies[15] (only available as research assay; status October 2023)	Sensitivity for VITT ~95%, but ~30% sensitivity for HIT (possible marker for autoimmune HIT [aHIT])
Washed platelet activation assays	
SRA	Sensitivity for HIT antibodies ~95%; ~50% sensitivity for VITT antibodies Read out: measurement of ^{14}C-radiolabeled serotonin (or other methods of serotonin measurement) released from platelets
PF4-SRA	PF4-SRA more sensitive than SRA for detecting HIT and VITT antibodies Read out: measurement of ^{14}C-radiolabeled serotonin (or other methods of serotonin measurement) released from platelets[14]
PF4/H-SRA	PF4/H-SRA is more sensitive than SRA for detecting HIT antibodies and less sensitive for VITT antibodies than PF4-SRA Read out: measurement of ^{14}C-radiolabeled serotonin (or other methods of serotonin measurement) released from platelets
HIPA	Sensitive for HIT antibodies (>95%); ~50% sensitivity for VITT antibodies Read out: platelet aggregation, assessed visually on microtiter plates
PIPA	PIPA more sensitive than HIPA for detecting VITT antibodies; sensitivity of PIPA increases when sera are tested undiluted and 1:4 diluted Read out: platelet aggregation, assessed visually on microtiter plates
PEA	PEA is more sensitive than SRA for detecting VITT antibodies Read out: flow cytometry (detection of P-selectin as platelet activation marker)
Whole-blood platelet activation assays	
PIFPA	PIFPA has high sensitivity and specificity for VITT Read out: flow cytometry (detection of P-selectin as platelet activation marker)
Multiplate	Minimal experience reported to date for diagnosis of VITT Read out: impedance aggregometry performing using multiplate instrument
Platelet procoagulant assay	Exploits synergistic platelet activation by PAR-1 agonist and HIT/VITT antibodies Read out: flow cytometry (detection of P-selectin as platelet activation marker and annexin V binding)
Platelet-rich plasma (citrated) platelet activation assay	
HitAlert	Minimal experience reported to date for diagnosis of VITT Read out: flow cytometry (detection of P-selectin as platelet activation marker)

PAR-1, protease activated receptor 1; PEA, PF4-enhanced P-selectin expression assay; PF4/H-SRA, PF4/heparin-SRA; PF4-SRA, PF4-enhanced SRA; PIFPA, PF4-induced flow cytometry–based platelet activation assay.

Data modified from Warkentin and Greinacher.[14]

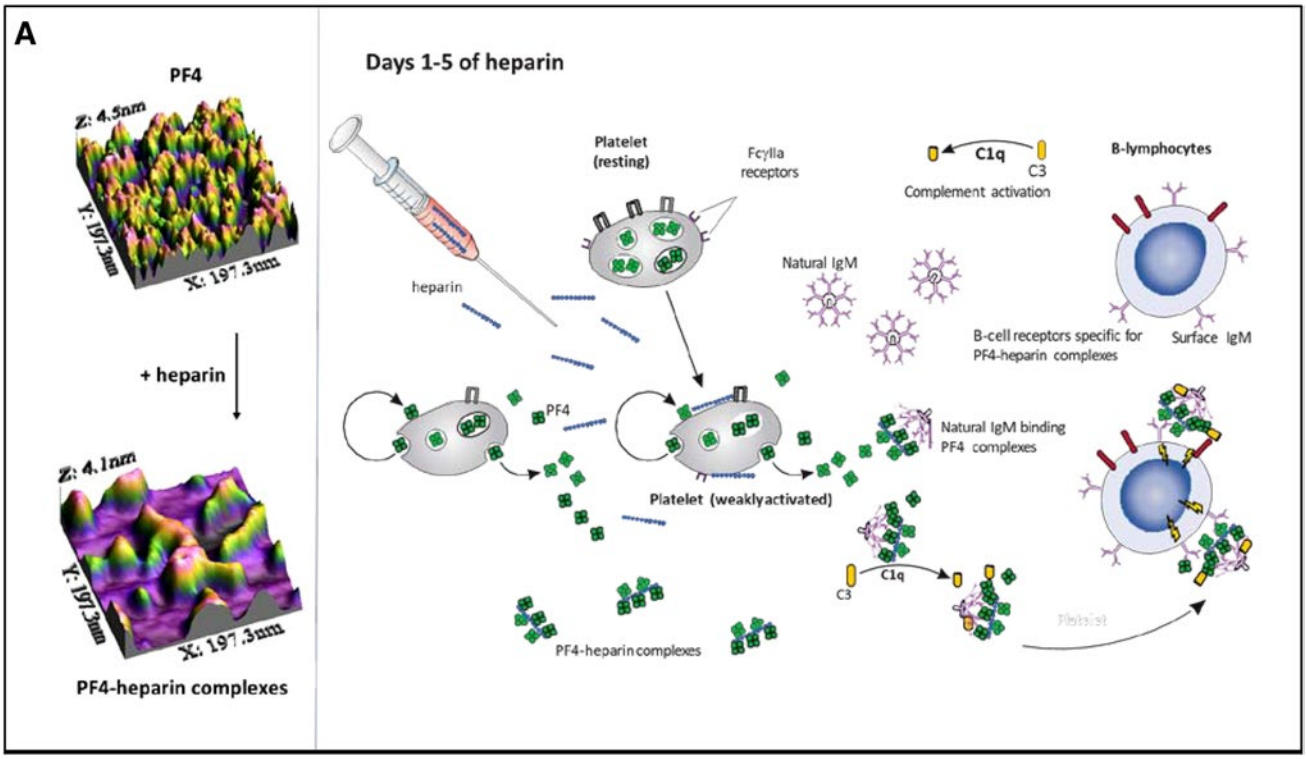

Figure 1. Current concepts of the pathogenesis of HIT and VITT. The schematic presentation shown in panel B is speculative and in large part inferred from experiments in HIT. The schematic presentation of the downstream prothrombotic process shown in panel C is largely substantiated by experimental data, some performed with VITT antibodies, others with HIT antibodies. (A) After heparin exposure, positively charged PF4 binds to negatively charged heparin; PF4/heparin complexes are formed. Natural IgM binds to the complexes, and complement factor C3 binds to the natural IgM. Complexes of PF4/heparin, natural IgM, and C3 bind to B cells via the complement receptor CR2 (CD21). B cells expressing the receptor for the HIT antigen on PF4 also bind to the PF4/heparin complexes, thus activating B cells to produce anti-PF4/heparin antibodies. The far left of the panel shows atomic force microscopy images of PF4 alone (*upper*) and formation of PF4/heparin clusters when PF4 is incubated with heparin (*lower*). (B) After vaccination, PF4 comes in contact with vaccine constituents and activates B cells. *Left:* It has been proposed that a direct inadvertent breach in the microvasculature at the vaccination site by IV injection or by disruption of VE-cadherin tight junctions by EDTA in ChAdOx1 nCoV allows vaccine constituents to enter the circulation.[19] Within the circulation, adenovirus particles can bind to platelets and can also bind PF4 released by activated platelets or from the microvascular endothelium.[17] Platelets may become activated (i) by vessel injury caused by injection of vaccine, (ii) after binding of the virions to the cell surface, or (iii) by immune complexes formed between contaminating host cell line proteins in the vaccine and natural IgG antibodies against these proteins. Whether the virions themselves or another yet unknown constituent in the vaccine causes the conformational change in PF4 is unknown. *Middle:* Once complexes with PF4 have formed, natural IgM antibodies activate complement (as it has been shown for PF4/heparin complexes[25]), which enhances their proximity to B-cell receptors. In a mouse model, upon IV injection of ChAdOx1, platelet-bound adenoviral particles are transported to the marginal zone of the spleen, where B cells are activated upon direct contact.[33] However, electron microscopy and superresolution microscopy revealed complexes between PF4 and anti-PF4 VITT antibodies with amorphous constituents of the vaccine rather than virus particles.[19] Beside the virions, other potential partners for PF4 include unassembled hexons and host cell-line proteins. However, there is little overlap in the proteins contaminating ChAdOx1 and Ad26.COV2 vaccines,[18] which both induce anti-PF4 VITT antibodies. *Right:* Eventually, complexes of PF4 and vaccine come in contact with B cells, expressing a cognate Ig receptor for PF4, either as fluid-phase complexes, as virion-PF4 complexes, or as complexes presented by platelets. (C) *From right to left:* After clonal expansion and isotype switching of one or a few B-cell clones in VITT, or polyclonal B cells in HIT, high-titer IgG anti-PF4 antibodies are released into the circulation. Immune complexes containing PF4 (or PF4/heparin complexes) and anti-PF4 IgG cluster and signal through FcRγIIA, which generates procoagulant platelets, induces platelet/neutrophil aggregates,[34] and stimulates NETosis by neutrophils.[35] DNA released by NETosis amplifies immune injury and activates complement, which deposits on the endothelium. Endothelial cells become activated, expressing tissue factor and releasing von Willebrand factor (VWF). VWF binds PF4 and subsequently anti-PF4 antibodies,[36] which in turn further activates neutrophils and further propagates thrombin generation. To reduce complexity, multimolecular PF4 complexes in VITT are shown and not the multimolecular PF4/heparin complexes in HIT as shown in panel A. HS, heparan sulfate; MPO, myeloperoxidase. Data modified from Cines and Greinacher.[32]

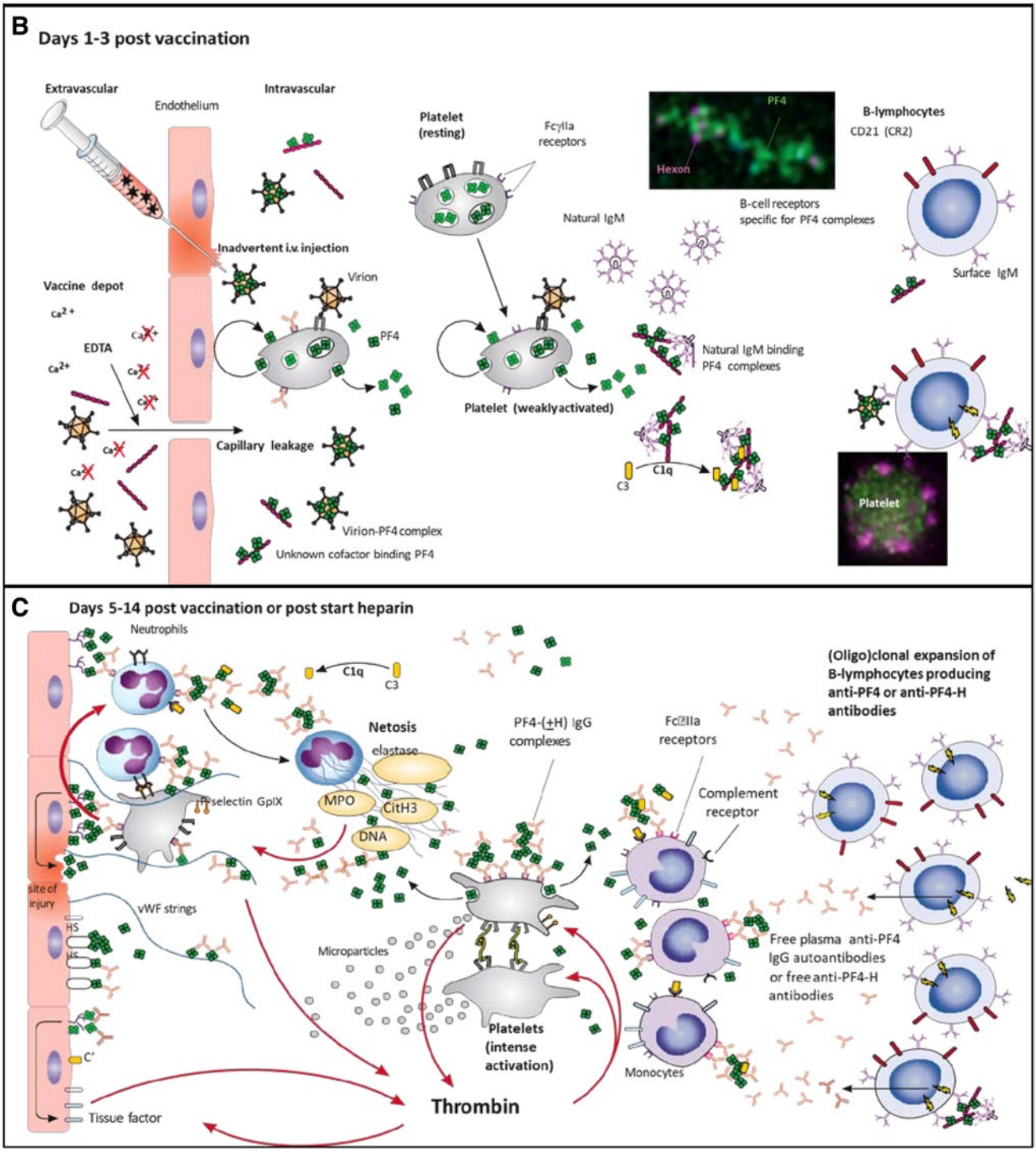

Figure 1. Continued

recognized with atypical clinical presentations. These included patients in whom the platelet count fall began or worsened after stopping heparin (delayed-onset HIT) or persisted for more than a week after stopping heparin (persisting, refractory HIT) and HIT associated with trivial exposures to heparin (heparin "flush" HIT).[28,29] We introduced the term "autoimmune HIT" (aHIT) in 2017 to indicate this group of patients, emphasizing their more severe clinical course (including overt disseminated intravascular coagulation [DIC], a higher risk of thrombosis including microthrombosis) and need for adjunct high-dose IVIG treatment.[29]

A unifying feature is that aHIT sera cause strong platelet activation in functional assays in the absence of heparin. Recent studies show that in these patients anti-PF4 type 3

antibodies are present in addition to anti-PF4 type 2 antibodies.[30] This might also explain how the additional application of PF4 enhances functional assays for HIT.[14] Sporadic reports since 2008 indicate that some patients develop thrombocytopenia and thrombosis in association with platelet-activating anti-PF4 antibodies even in the absence of proximate heparin exposure (spontaneous HIT; for review[1]). Applying assays developed for VITT, preliminary evidence indicates that in most of these patients both anti-PF4 type 2 and type 3 antibodies are found,[15,30] while in other patients, only anti-PF4 type 3 antibodies are present. The majority of spontaneous HIT patients are recognized after knee (not hip) replacement surgery, suggesting that polyanions released during this procedure may initiate the anti-PF4 response (for example, DNA and RNA released from degraded cells during application of the tourniquet). Most of the remaining patients appear to have developed this anti-PF4 antibody disorder following apparent viral infection. In other patients with monoclonal gammopathy and associated thrombocytopenia/recurrent thromboses, the paraprotein has features of anti-PF4 type 3 antibodies.[31] However, in other patients with anti-PF4 antibody-induced thrombosis, no clear proximate precipitating event is apparent. It is likely that these patients are underrecognized given that testing for anti-PF4 antibodies is usually not initiated in the absence of proximate heparin exposure or COVID-19 vaccination.

CLINICAL CASE (continued)

In 2023 we reevaluated the patient's case using repository samples: the positive anti-PF4/heparin IgG EIA and negative heparin-dependent functional assay for HIT were reconfirmed. However, the PF4-dependent functional assay (PIPA) was strongly positive. In hindsight, this patient had anti-PF4 type 3 antibodies and a VITT-mimicking severe prothrombotic disorder. The trigger was most likely the preceding upper respiratory tract infection (this case preceded the COVID-19 pandemic/vaccination campaign by several years). Today, a similar case would be managed by adjunctive high-dose IVIG in addition to anticoagulation.

Summary and future considerations

The role of pathogenic anti-PF4 antibodies is well established for HIT and VITT, among the most prothrombotic acquired disorders in medicine. Pathogenic anti-PF4 antibodies have in common the property of activating platelets strongly via the low-affinity FcγRIIa. This is fundamentally different from the nonpathogenic, non–platelet-activating antibodies found in a considerable percentage of heparin-exposed patients as well as in the normal population. We propose to name these nonpathogenic, non–platelet-activating antibodies "anti-PF4 type 1 antibodies." Pathogenic anti-PF4 antibodies most often recognize PF4/polyanion complexes and induce classic HIT (anti-PF4 type 2 antibodies). In contrast, antibodies causing VITT recognize the same site on PF4 to which polyanions (eg, heparin) bind and can cluster PF4 in the absence of polyanions (anti-PF4 type 3 antibodies). Increasingly, prothrombotic disorders caused by anti-PF4 antibodies independent of the presence of heparin are being recognized. Here, anti-PF4 type 2 and type 3 antibodies, in variable proportions, are usually identified (Table 4). Currently, these antibodies can be differentiated by functional assays.

Fifty years of HIT research facilitated the rapid identification of anti-PF4 antibodies as the underlying cause of VITT and provided guidance for diagnosis and effective treatment. The lessons learned from VITT indicate that in patients who present with thrombocytopenia and severe venous and/or arterial thrombosis, the presence of anti-PF4 type 2 and especially type 3 antibodies should be considered as indicating a

Table 4. Anti-PF4 antibodies and associated disorders

	Antibody target (PF4/heparin and PF4) and type of antibodies		
Nonpathogenic antibodies	unknown sites on PF4		**Type 1**
Platelet-activating anti-PF4 disorders	PF4/H	PF4	
HEPARIN TRIGGER			
Classic HIT (induced by heparin, low-molecular-weight heparin, and other polyanions)	√		**Type 2**
aHIT Delayed-onset HIT (onset or worsening of thrombocytopenia after stopping heparin) Persisting (refractory) HIT (delay in platelet count recovery after stopping heparin, eg, 7 or more days) Heparin "flush" HIT (HIT triggered by exposure to small concentrations of heparin) Unusually severe HIT (severe thrombocytopenia with overt DIC)	√	√	**Type 2+3**
NONHEPARIN (OR UNKNOWN) TRIGGER			
VITT		√	**Type 3**
Spontaneous thrombosis and thrombocytopenia	√	√	**Type 2+3**
Paraprotein-associated chronic thrombocytopenia/thrombosis		√	**Type 3**
Adenovirus-associated thrombosis and thrombocytopenia		√	**Type 3**

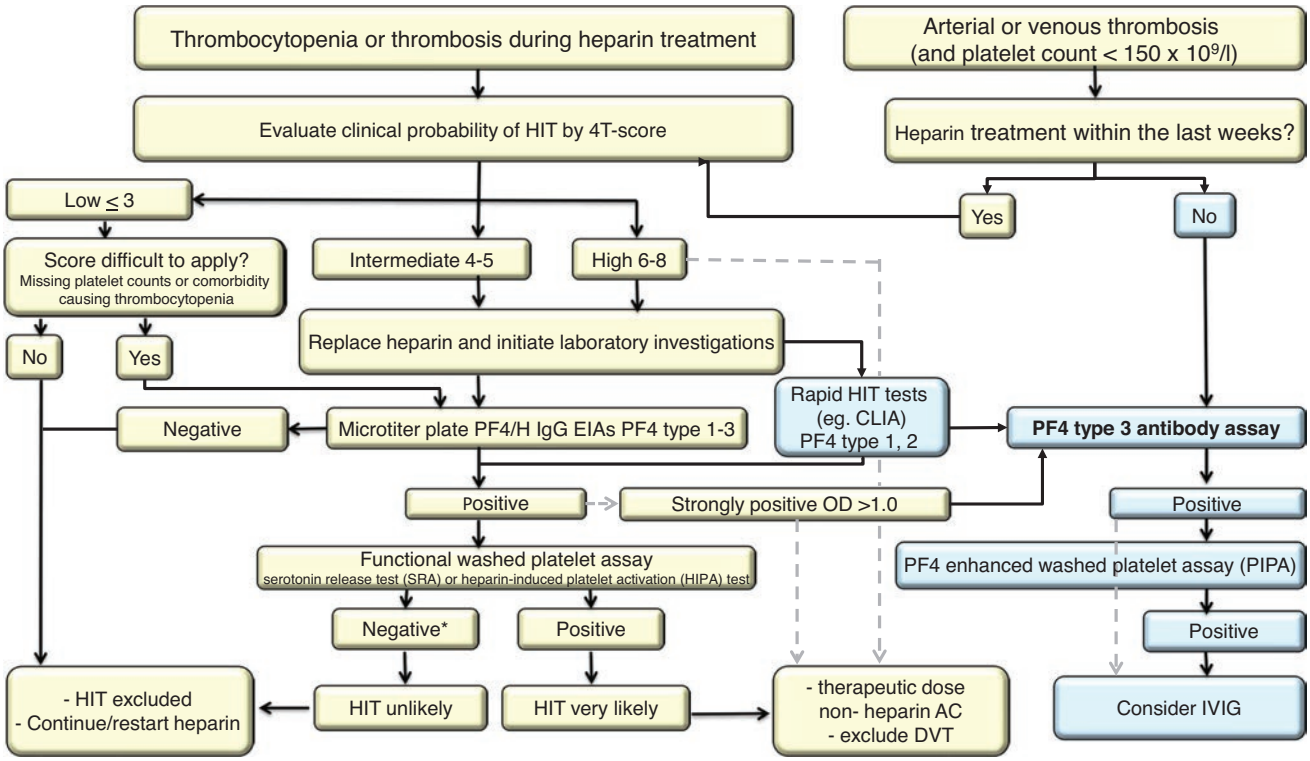

Figure 2. Flow chart for the diagnosis of anti–PF4-antibody-induced prothrombotic disorders. Anti–PF4 antibody-mediated disorders should be clinically diagnosed and laboratory testing only performed upon reasonable clinically suspicion. The yellow part of the figure presents the current diagnostic flow for heparin-induced thrombocytopenia. Anti-PF4/heparin antibody antigen tests are differentiated between microtiter plate-based EIAs and rapid HIT tests (Table 2). The light-blue part of the figure shows the workflow when clinically applicable immunoassays for anti-PF4 type 3 antibodies are available (currently under development). As the blue section of the workflow is untested, the decision to test for anti-PF4 antibodies should include consideration of the pretest probability: if there are other obvious reasons to explain thrombosis and thrombocytopenia, such as cancer or intensive care unit treatment, testing is probably not indicated. The dotted lines show the workflow based on clinical considerations, bypassing some diagnostics steps; for example, a typical presentation of HIT with a high 4Ts score of 6, 7, or 8 points should immediately prompt therapeutic-dose alternative anticoagulation, and laboratory test results are used to confirm the diagnosis. *Functional assays can be more sensitive if PF4 is added. Clinically nonrelevant anti-PF4 type 1 antibodies (detected in up to 20% of heparin-treated patients and up to 8% of COVID-19 vaccinated individuals) do not require treatment change. AC, anticoagulation; CLIA, chemiluminescence-based immunoassay; DVT, deep vein thrombosis; OD, optical density.

potential IgG-mediated prothrombotic disorder even in the absence of proximate heparin exposure or vaccination (Figure 2).

The challenge is now to identify how frequently anti-PF4 type 3 antibodies contribute to severe complications in HIT, including atypical forms of HIT, and in patients who present with thrombosis and/or thrombocytopenia independent of heparin treatment or vaccination. New immunoassays can differentiate between HIT-like and VITT-like antibodies in the research laboratory. Widely applicable assays that can differentiate among these various anti-PF4 disorders are needed to evaluate systematically the prevalence of these antibodies in different patient cohorts. From VITT we have learned that the outcome of patients with anti-PF4 type 3 antibodies can be improved (in addition to anticoagulation) by adjunctive treatment with IVIG to deescalate the FcγRIIa-dependent cell activation that triggers massive hypercoagulability. Potentially, the inhibition of FcγRIIa signal transduction might be an attractive new therapeutic approach in patients with thrombotic anti-PF4 type 3 antibody immune disorders beyond HIT and VITT.[40]

Note added in proof

Recent studies[40–42] have further implicated VITT-like anti-PF4 antibodies in chronic autoimmune anti-PF4 disorder and acute post-adenovirus infection anti-PF4 disorder.

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Conflict-of-interest disclosure

Andreas Greinacher: grants and nonfinancial support: Aspen, Boehringer Ingelheim, MSD, Bristol Myers Squibb, Paringenix, Bayer Healthcare, Gore Inc., Rovi, Sagent, Biomarin/Prosensa, Portola, Ergomed, GTH e.V.; personal fees: Aspen, Boehringer Ingelheim, MSD, Macopharma, Bristol Myers Squibb, Chromatec, Werfen (Instrumentation Laboratory).

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Paradigm Pharmaceuticals, Octapharma, Veralox Therapeutics; research funding: Werfen (Instrumentation Laboratory).

Off-label drug use

Apixaban, bivalirudin, danaparoid, fondaparinux, rivaroxaban, and high-dose IVIG are "off-label" for treatment of HIT.

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Diagnosis and laboratory monitoring of hemophilia A

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Acquired hemophilia A (AHA) is a rare disorder in which autoantibodies against factor VIII (FVIII) lead to a bleeding phenotype that varies from life-threatening to no bleeding at all. Prolonged activated partial thromboplastin times (APTT) in patients with a bleeding phenotype should be investigated to rule out AHA and should never be ignored in a preprocedure patient. Most inhibitors in AHA are heat and time dependent, so mixing studies performed only on an immediate mix are not useful: both lupus anticoagulants and treatment with direct oral anticoagulants can coexist with AHA and confound the diagnosis. Assays for intrinsic coagulation factors and von Willebrand factor should always be performed, regardless of the results of mixing studies. A Bethesda or modified Bethesda assay should be performed to quantify any inhibitor, and if susoctocog alfa (rpFVIII) is available, then an assay for cross-reacting antibodies should also be performed. At diagnosis and until complete remission, if the FVIII in the patient sample is >5 IU/dL, heat inactivation should be performed before the inhibitor assays are performed. While there are no conventional tests available to measure the effects of FVIII bypassing therapies, newer therapies may require monitoring, or their effects may need to be considered when choosing appropriate assays. Measurement of rpFVIII requires a 1-stage clotting assay, and measurement of patient FVIII while on emicizumab requires a chromogenic assay that does not contain human FX. Close communication is required between the treating clinicians and the laboratory to ensure that the correct tests are performed while patients are receiving treatments.

LEARNING OBJECTIVES

- Understand the laboratory assays used for the differential diagnosis of acquired hemophilia A
- Understand the assay considerations for laboratory monitoring of treatment and recovery in AHA with current and future treatment options

Laboratory diagnosis of acquired hemophilia A

Acquired hemophilia A (AHA), in which there are autoantibodies (inhibitors) against clotting factor VIII (FVIII), is a rare disorder that can occur in men or women.[1] The bleeding phenotype varies from life-threatening to mild bleeding, and, in approximately 10% of presentations, no bleeding at all.[2]

In a patient with a bleeding phenotype, a prolonged activated partial thromboplastin time (APTT) should be investigated to rule out AHA, and in a preprocedure patient, APTT should never be ignored.[2] Suspicion of AHA is increased in patients over the age of 60,[3] during pregnancy, or for 1 year after giving birth.[4]

CLINICAL CASE (Presentation)

A 53-year-old female presented to the emergency department (ED) with a recent history of bruising but no symptoms of bleeding. Her APTT was 82 seconds (local reference range 21–31 seconds) with a normal prothrombin time and fibrinogen of 4.3 g/L. Liver function tests were normal. She had previously delivered 4 children by Caesarean section, and there was no known malignancy.

Mixing studies

Plasma mixing studies (MSs) are performed to aid in identifying the cause of a prolonged APTT, which can be due to the presence of a factor deficiency (eg, FVIII deficiency) or an inhibitor (eg, a lupus anticoagulant [LA]).[5]

The APTT performed on a 50:50 mix of patient's plasma with normal plasma and tested immediately was 33 seconds. There are up to 6 ways to define the presence of a correction in MS, with no consensus.[5] The best method for an interpreting MS will depend on the normal plasma used and the factor-, LA- and anticoagulant-

sensitivity of the reagent. Locally, a result of 33 seconds or below is corrected, and, therefore, the immediate MS in our patient corrected.

However, most inhibitors detected in AHA are heat and time dependent, and MSs require incubation for 2 hours.[6] In AHA, MSs tested immediately are likely to be similar to those seen in patients with a single factor deficiency and no inhibitor.[5] In our patient, the APTT in the incubated MS was 75 seconds, confirming the presence of a time-dependent inhibitor.

Of the last 50 AHA patients presenting at our center, 9 (18%) had immediate-acting inhibitors, which have the potential to bind to the FVIII in the deficient plasmas used in 1-stage clotting factor assays (OSCAs), prolonging clotting times and showing an apparent reduction in factors IX (FIX), XI (FXI), and/or XII (FXII).[7] These interferences can be identified by using multiple samples dilutions[8] and by repeating the OSCA using higher dilutions an accurate result may be determined.

Patients presenting with AHA may also be taking direct oral anticoagulants. In particular, the presence of oral direct FXa inhibitors (DFXaIs) may be difficult to ascertain by screening tests alone, and MSs in the presence of DFXaIs are likely to show noncorrection in both immediate and incubated mixing studies. A suitably calibrated assay to detect the presence of DFXaIs may be useful.

MSs are poorly standardized and cannot be used in isolation to establish or exclude AHA.[9] Further investigation is always required, and specific factor activity assays should be performed in parallel to facilitate early diagnosis.[2]

Factor assays

Assays for FVIII, FIX, FXI, and von Willebrand factor (VWF) (activity and antigen)[10] must be performed at presentation, regardless of the results of mixing studies,[9] as it cannot be assumed that AHA is the only possible diagnosis. Occasionally, patients with mild hemophilia A or B, mild von Willebrand disease (VWD), or deficiencies of FXI may reach old age without being diagnosed or having an APTT measured. Acquired von Willebrand syndrome and acquired FXI deficiency may also be encountered. An FVIII to VWF antigen ratio may be useful in differentiating type 1 VWD from AHA, mild hemophilia A, and type 2N VWD. Although not associated with a bleeding phenotype, deficiency of FXII is a frequent cause of a prolonged APTT, so an FXII assay may be included in the initial investigations.

In our patient, FVIII was <1 IU/dL by both an OSCA and a chromogenic substrate assay (CSA); the VWF:GpIbM activity assay was 160 IU/dL, and VWF:Ag was 220 IU/dL. Factors IX, XI and XII were normal.

Of the last 50 AHA patients presenting at our center, the median FVIII level using a CSA was 2 IU/dL (range <1 to 40 IU/dL): 13 (26%) had FVIII <1 IU/dL, and 12 (24%) had FVIII between 10 and 25 IU/dL, similar to the observations of the European Acquired Haemophilia Registry.[3] Only 2 of these 50 patients had discrepancies between their OSCA and CSA (13 and 2 IU/dL; 8 and 22 IU/dL); the choice of assay did not make any difference to the AHA diagnosis. Laboratories using LA-sensitive reagents in OSCA may see falsely reduced FVIII levels;[2] these laboratories should confirm all reduced FVIII in OSCAs by also using a CSA, as these assays are usually insensitive to LA.[11,12]

The presence of DFXaIs may cause FVIII to appear reduced on a CSA, and, depending on the reagent and type and concentration of DFXaI, the OSCA may also be affected. Along with a prolonged APTT and noncorrection in the MS, there is potential for the misdiagnosis of AHA in these patients. The use of an activated carbon product to remove DFXaIs from the sample *in vitro* could be considered; although the effect of these products on OSCAs and CSAs for FVIII has been described,[13] there are limited data on the use of these assays in patients with AHA. In our patient, there was no history of DFXaI use.

Assays for LA using APTT are difficult to interpret in AHA, but dilute Russell viper venom time results are not affected. The coexistence of an antiphospholipid antibody is not unusual in AHA, as both are autoimmune disorders that may coexist in the same patient. In our patient, the dilute Russell's viper venom time was normal.

All OSCA factor assays should be performed at multiple dilutions.[8] This will allow the accurate quantitation of factor activities in FIX, FXI, and FXII assays in the presence of very strong or immediate-acting inhibitors to FVIII, in the presence of high doses of DFXaIs, or in the presence of a strong LA.

Inhibitor assays

If a reduced FVIII (<50 IU/dL) is detected,[3,14] a standard Bethesda[15] or Nijmegen-modified Bethesda[16] should be performed. Patient plasma is incubated with normal plasma[15] or buffered normal plasma[16] as a source of FVIII, and the amount of FVIII measurable in the mixture after 2 hours is compared to a negative control (inhibitor-free FVIII-deficient plasma incubated with normal plasma). This residual FVIII is expressed as a percentage ([patient ÷ control] ×100). Samples should be titrated in FVIII-deficient plasma until the inhibitor is not detectable in the dilution tested.

If the amount of FVIII in the patient sample is above 5 IU/dL, incubating the sample for 30 minutes at 56°C will denature all the endogenous FVIII present in the sample and leave the inhibitor suitable for use in the Bethesda assay,[17] improving assay sensitivity.[18]

In patients with congenital hemophilia A, alloantibodies to FVIII show linear type 1 kinetics in the Bethesda assay, whereas the autoantibodies in AHA often display complex, nonlinear type 2 kinetics.[19] The results for our patient with type 2 kinetics are shown in Table 1 and Figure 1.

Some low-titer inhibitors with type 2 kinetics may not be detectable in neat plasma but may be detectable in the next titration. Therefore, it may be advisable to test neat plasma and plasma diluted by at least ½ in all cases.

Measurement of residual FVIII after the incubation step in the Bethesda assay can be by OSCA or CSA. The CSA is less likely to be susceptible to interference from an LA than the OSCA if the local APTT reagent in use is LA-sensitive;[20] both assays are susceptible to interference from direct oral anticoagulants. There is a significant risk that if the presence of a DFXaI is not detected, a patient could have a prolonged APTT, noncorrection in the MSs, a reduced FVIII, and a positive Bethesda assay, resulting in an erroneous diagnosis of AHA.

Table 1. Bethesda assay results in patient with AHA

Sample dilution	Residual FVIII (%)	Inhibitor titer in this dilution	Inhibitor titer (BU/mL)
Neat	27.6	>2.00	>2.0
2	38.5	1.38	[1.38×2=] **2.8**
4	45.4	1.14	[1.14×4=] **4.6**
8	*50.3*	*0.99*	[0.99×8=] ***7.9***
16	57.7	0.79	[0.79×16=] **12.6**
32	71.6	<0.50	<16.0

The type 2 kinetics of the autoantibody gives the appearance that the inhibitor gets stronger the more it is diluted. For consistency, the dilution that gives closest to 50% residual FVIII (in italics) is used to determine the inhibitor titer that is reported to clinicians—in this case, 7.9 BU/mL.

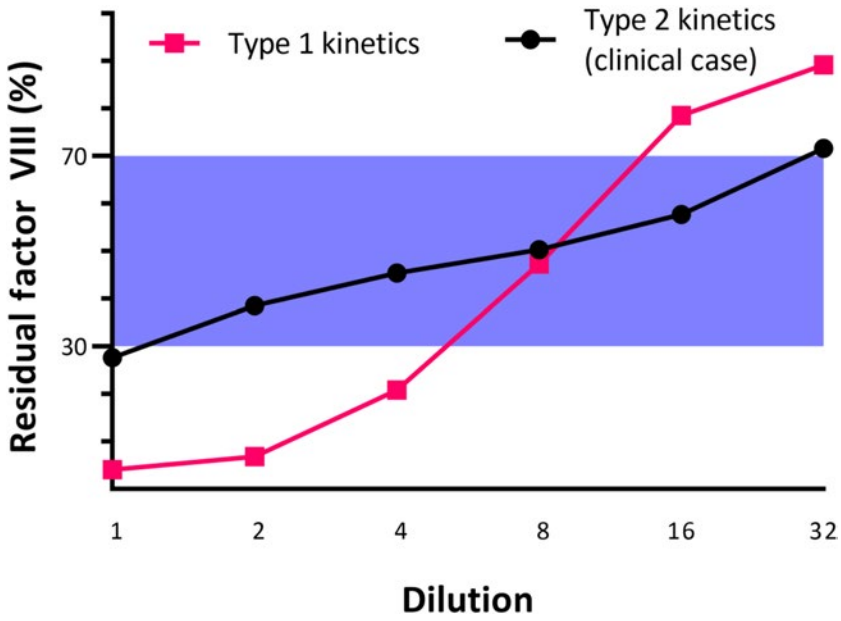

Figure 1. Residual FVIII against a sample dilution for a type 1 inhibitor often seen in congenital hemophilia (pink line) and against the type 2 inhibitor seen in a clinical case (black line). The blue shaded area denotes the area between 30% and 70% residual FVIII, where the Bethesda assay is considered to be accurate. For type 1 inhibitors, only 1 or 2 dilutions will give interpretable results (and the same calculated titer), whereas for type 2 inhibitors, multiple dilutions will give interpretable results, and the calculated titer will be different for each one.

Our patient had an inhibitor that was quantitated at 7.9 BU/mL at presentation with type 2 kinetics.

Of the last 50 patients diagnosed with AHA at our center, the median inhibitor titer on presentation was 9.5 BU/mL (range 1.4 to 2224 BU/mL): 10 had type 1 kinetics and 40 had type 2 kinetics. Recent international guidelines[2] suggest that those with FVIII <1 IU/dL or an inhibitor titer >20 BU/mL are less likely to achieve partial remission in the first 21 days if treated with corticosteroids alone and should therefore receive combined immunotherapy.

Enzyme-linked immunosorbent assay

Commercially-available anti-FVIII enzyme-linked immunosorbent assay (ELISA) has been shown to be sensitive and specific for diagnosing AHA and may be used as an adjunct or alternative to a Bethesda assay,[2,21] particularly if there is suspicion of LA or DFXaI interference in the Bethesda assay. Native or heat-inactivated plasma or serum can be used.

It should be noted that positive anti-FVIII results can be found with ELISA in a significant number of individuals who do not have congenital or acquired hemophilila A.[22]

Porcine inhibitor assays

Patients with AHA may be given recombinant porcine FVIII, susoctocog alfa (rpFVIII), to treat bleeds or to cover minor or major surgery. If rpFVIII is an available treatment option, newly diagnosed AHA patients should have another inhibitor assay performed on the same sample, using rpFVIII as the source of FVIII in the Bethesda assay.

In studies, cross-reacting anti-rpFVIII antibodies were detectable in 44%[23] and 49%[24] of newly presenting patients with AHA. A patient may be suitable to receive rpFVIII to treat bleeds or to

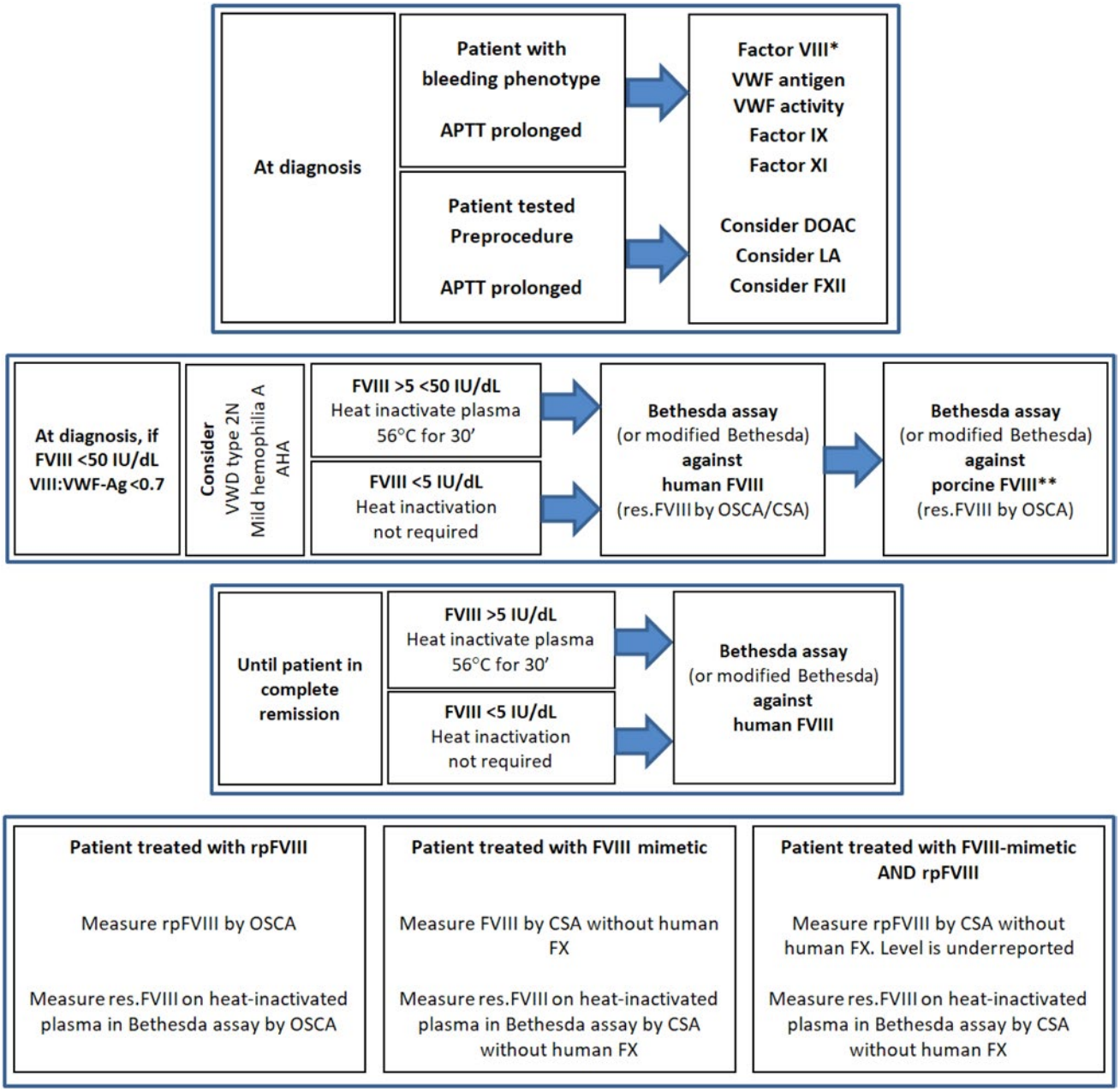

Figure 2. Algorithm for Acquired Haemophilia A diagnosis and monitoring. *One-stage clotting assay and/or chromogenic substrate assay. **Only required if treatment with susoctocog alfa is available locally. AHA, acquired haemophilia A; APTT, activated partial thromboplastin time; CSA, chromogenic substrate assay; DOAC, direct oral anticoagulant; FVIII, factor VIII; FX, factor X; LA, lupus anticoagulant; OSCA, one-stage clotting assay; res.FVIII, residual FVIII; rpFVIII, susoctocog alfa; VII:VWF-Ag, factor VIII to von Willebrand factor antigen ratio; VWF, von Willebrand factor.

cover minor or major surgery if there is no anti-rpFVIII inhibitor detectable. Patients with anti-rpFVIII inhibitors below 20 BU/mL can receive rpFVIII but are likely to require more to maintain trough levels above 50 IU/dL.[25]

Anti-rpFVIII inhibitors may exhibit type 1 or type 2 kinetics. An OSCA method is preferred for the measurement of residual rpFVIII in the Bethesda assay.[26]

When our patient presented, rpFVIII was not licensed; when the drug became available, her anti–human FVIII inhibitor level was 1.3 BU/mL, but no anti-rpFVIII inhibitor was detectable.

A suggested testing algorithm for patients suspected of AHA is shown in Figure 2.

CLINICAL CASE (Monitoring)

Monitoring of patients on bypassing agents

No specific tests exist for monitoring treatment with activated prothrombin complex concentrates or recombinant activated

factor VII. Thrombin generation assays can be used, but despite attempts to standardize testing, results in patients with hemophilia may not be comparable between those with the same FVIII activity;[27] results in an individual may show improvement with therapy, but there are no therapeutic targets for thrombin generation assay parameters.

Remission/relapse

Partial remission has been defined as FVIII >50 IU/dL and no bleeding after stopping any hemostatic treatment for at least 24 hours.[14] Complete remission is defined as normal FVIII with no detectable inhibitor and ~~either~~ (1) immunosuppression either stopped or reduced to levels used before AHA diagnosis or (2) prednisolone <15 mg/d and all other immunosuppression stopped.[14] Neither of these definitions specifies whether the inhibitor should be measured by a Bethesda, Nijmegen-modified, or ELISA method, nor whether heat-inactivated samples should be used. Inhibitors often persist in patients with AHA even when FVIII levels have reached normal levels,[18,28] so local practice is to use heat inactivation in a Nijmegen-modified assay.

Laboratories should continue to monitor FVIII levels and test for the presence of an inhibitor regardless of FVIII level until immunosuppression is stopped.

Repeated measure of inhibitors during recovery

In our patient, FVIII levels exceeded 50 IU/dL in less than 4 weeks (month 1), and no inhibitor was detected (Figure 3). At that time, heat inactivation of samples was not performed, making inhibitors difficult to detect in a Bethesda assay when FVIII levels exceed ~20 IU/dL.

A prednisolone wean was commenced, but FVIII levels immediately dropped. Immunosuppression continued, and brief recoveries in FVIII and reductions in inhibitor titer (without heat-inactivation of plasma) were seen 7 to 14 months after the patient first presented (Figure 3).

Heat inactivation of samples became routine practice in this laboratory around 13 months after our patient first presented (Figure 3): this coincided with a drop in FVIII and a low-titer inhibitor being detectable from that point onwards.

Over the course of the next 5½ years (18 months onwards), our patient continued to receive immunosuppression, with FVIII occasionally peeking into the reference range, but her inhibitor remained detectable throughout (Figure 3) and complete remission was never achieved.

CLINICAL CASE (Undergoing surgery)

Nine years after diagnosis, our patient presented to the ED with abdominal pain secondary to a benign ovarian cyst. During an ED admission 4 weeks later, clinicians found that the cyst had doubled in size and, despite a normal CA125 level, it appeared to be malignant on computed tomography scanning. Peritoneal metastases were also identified, and she required surgery. Immunosuppression with prednisolone was reinitiated 2 weeks before a laparotomy, and FVIII levels reached 221 IU/dL by the day of surgery. No additional products were required for the surgery nor for when she required repair of a colonic anastomosis 7 days later. Stage 3C ovarian carcinoma was diagnosed with metastases in the bowel and bladder, which were removed. She had an episode of septic shock 2 weeks after surgery when her FVIII levels reached 436 IU/dL (Figure 4).

Treatment of patients with susocotocog alfa

Patients with AHA who are given rpFVIII to treat bleeds or to cover minor or major surgery are given a standard dose of 200 U/kg followed by repeat doses to maintain levels in the target range,[2] although there is evidence that a dose of 100 U/kg may be sufficient.[29,30] Frequent monitoring of rpFVIII levels at trough and peak is required.[31,32]

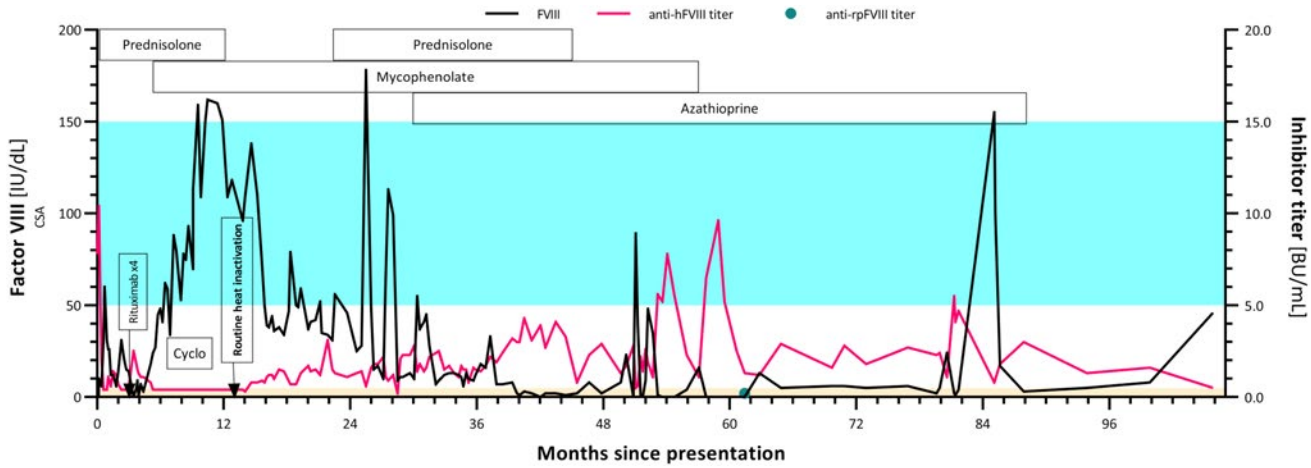

Figure 3. FVIII activity (left axis) and inhibitor titer (right axis) from diagnosis to first partial remission. Shaded blue area represents the reference range for factor VIII activity; shaded orange area represents the reference range for the Bethesda assay. anti-hFVIII, antihuman factor VIII; anti-rpFVIII, antirecombinant porcine factor VIII; CSA, chromogenic substrate assay; Cyclo, cyclophosphamide; FVIII, factor VIII.

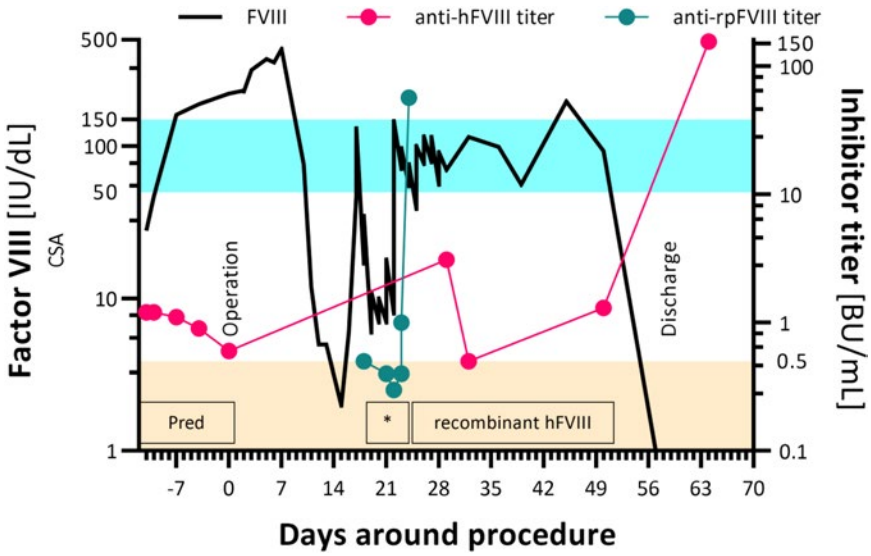

Figure 4. Factor VIII activity (left axis) and inhibitor titer (right axis) during hospital stay, 10 years after diagnosis: note logarithmic scale on *y*-axes. Shaded blue area represents the reference range for factor VIII activity; shaded orange area represents the reference range for the Bethesda assay. *Denotes the time when the patient received susoctocog alfa. anti-hFVIII, anti-human factor VIII; anti-rpFVIII, anti–recombinant porcine factor VIII; CSA, chromogenic substrate assay; FVIII, factor VIII; Pred, prednisolone.

The OSCA should be used to measure rpFVIII,[26] and VWF level in the FVIII-deficient plasma used may also be required.[33] In a field study,[34] the mean recovery for rpFVIII by CSA was 53% (range 44%-61%), and the mean ratio of OSCA:CSA was 1.85, with interlaboratory variation being higher. Recovery of rpFVIII should be measured 2 to 3 times per week to evaluate for the development of inhibitors against rpFVIII[26]; if recovery of rpFVIII is less than expected, retesting for antihuman and anti-rpFVIII may be considered. When rpFVIII was assessed in a prospective, single-arm clinical study, 5 out of 18 (28%) patients who did not have detectable anti-rpFVIII inhibitors at baseline developed anti-rpFVIII antibodies after 8-85 days.[25]

In our patient, FVIII levels dropped 3 weeks after surgery, and rpFVIII prophylaxis was started. Immediately before prophylaxis, her anti-rpFVIII inhibitor result was negative, but a week later, the anti-rpFVIII titer jumped to 56.8 BU/mL; prophylaxis was switched to recombinant human FVIII. A week later, her FVIII became undetectable, and her antihuman FVIII inhibitor peaked at 155.2 BU/mL (Figure 4). However, she was successfully discharged home, and chemotherapy was planned.

Three weeks following discharge she was found to have liver and lymph node metastases, after which she was palliated. She died 5 weeks later.

Other treatments

Treatment of patients with factor VIII mimetics

Emicizumab is a recombinant bispecific monoclonal antibody with FVIII-mimetic activity that is licensed for use in patients with congenital hemophilia A with or without inhibitors. In some case reports and case series, emicizumab has been used off-label in patients with AHA.[35] A prospective multicenter phase 3 study of emicizumab prophylaxis in AHA has recently commenced, and it is likely that this drug will be used to prevent bleeds in AHA.[36]

Emicizumab does not require monitoring, but FVIII levels may need to be measured in AHA to assess if patients are in partial or complete remission. Emicizumab interferes with APTT-based OSCAs and also with CSAs that use human FX.[37,38] Therefore, CSAs that use bovine FX must be used to calculate both the patient's own FVIII and the residual FVIII in any Bethesda inhibitor assay.[39]

Heat inactivation up to 58°C does not denature emicizumab.[40]

Patients on emicizumab who bleed and are given rpFVIII

Emicizumab-calibrated modified OSCAs and CSAs that include human FX in the reagents are known to lead to overestimation of FVIII of up to 89% when compared to recombinant human FVIII,[41] and it is highly likely that this will also apply to assays of rpFVIII in the presence of emicizumab. Therefore, although the preferred assay for rpFVIII is an OSCA, the only assays that available to measure rpFVIII levels in these patients will be CSAs that do not contain human FX reagents.[26] When monitoring such patients, it will be important to remember that CSA underestimates rpFVIII by 44%-61%.[34]

Other FVIII-mimetics may become available in the future: consideration of their mode of action will be needed when selecting appropriate assays.

Conclusion

Acquired hemophilia A is a rare disease that sometimes relies on a laboratory diagnosis to be correctly made, especially in patients with a mild or absent bleeding phenotype at presentation. This includes a full investigation of a prolonged APTT regardless of the results of mixing studies, including assessing for FVIII, FIX, FXI, FXII, VWF activity, and VWF antigen, with consideration being given to the coexisting presence of oral anticoagulants and/or LA.

An inhibitor assay using a Bethesda or modified Bethesda assay should be performed using human plasma FVIII as the source of FVIII: residual FVIII can be measured using an OSCA or a CSA, although a CSA may be more specific. If rpFVIII is a potential treatment option, it should be used as the source of FVIII in an additional inhibitor assay, and an OSCA should be used to measure residual FVIII. An ELISA for anti-FVIII antibodies can be performed if it is not possible to exclude the presence of oral anticoagulants or LA.

Careful choice of laboratory assays is required for newer therapies for AHA, as measurement of rpFVIII requires an OSCA assay, and measurement of patient FVIII (or residual FVIII in a Bethesda or modified Bethesda assay) while on factor VIII mimetics requires a CSA that does not contain human FX. Compromises need to be made if patients receive rpFVIII to treat bleeds while on factor VIII mimetics, and clinicians and laboratories need to work closely together to ensure that the correct assays are performed and interpreted correctly.

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Off-label drug use

Sean Platton: the use of emicizumab for patients with AHA is off-label.

Suthesh Sivapalaratnam: the use of emicizumab for patients with AHA is off-label.

Priyanka Raheja: the use of emicizumab for patients with AHA is off-label.

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Immunotherapy of acquired hemophilia A

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Acquired hemophilia A (AHA) is an autoimmune disorder characterized by the formation of autoantibodies that neutralize the function of coagulation factor VIII. Immunosuppressive therapy (IST) with glucocorticoids, cyclophosphamide, rituximab, or combinations thereof is the standard of care to suppress autoantibody formation and induce remission of AHA. About 80% of patients achieve remission over the course of a few weeks to several months. However, patients with AHA are often elderly and frail and have adverse events from IST. Therefore, guidelines suggest an individualized approach using caution in elderly and frail patients. Prophylaxis with emicizumab may reduce the need for early and aggressive IST in the future.

LEARNING OBJECTIVES
- Review guidelines on the use of immunosuppression in acquired hemophilia A
- Discuss new concepts for reduced intensity or delayed immunosuppression

CLINICAL CASE

A 75-year-old woman is admitted for anemia and an extended forearm muscle bleed after venipuncture. During workup, her activated partial thromboplastin time is prolonged to 90 seconds. Factor VIII (FVIII) activity is reduced to <1% of normal, and acquired hemophilia A (AHA) is confirmed by the Nijmegen-modified Bethesda assay demonstrating an FVIII inhibitor of 28 BU/mL. Her previous medical history includes rheumatoid arthritis, arterial hypertension, and chronic obstructive pulmonary disorder; her concomitant medications include prednisolone 7.5 mg per day, methotrexate 10 mg per week, ramipril, and hydrochlorothiazide. Her general condition is compromised, with a World Health Organization (WHO) performance status of 3. She receives recombinant factor VIIa for 5 days to control the muscle bleed. A decision is made to postpone immunosuppressive therapy (IST) until her general condition has improved, and she receives prophylaxis with emicizumab. She receives outpatient care in the Hemophilia Care Center on a weekly basis and starts IST 4 weeks later, consisting of dexamethasone 40 mg per os (PO) (days 1–4 and 15–18) and rituximab 375 mg/m^2 intravenous (IV) (days 1, 8, 15, and 22). FVIII activity slowly improves, and remission is achieved after 6 weeks.

Introduction

AHA is a severe bleeding disorder caused by autoantibodies that neutralize the activity of coagulation FVIII. Anti-FVIII autoantibodies, also known as "inhibitors," can develop in previously healthy individuals, regardless of age or sex.[1,2] Two peaks in AHA incidence are typically observed: one associated with pregnancy and another one with advanced age, typically >65 years. Of patients with AHA, 30% to 50% have underlying disorders, most commonly autoimmune disorders or malignancy.

Patients with AHA often have comorbidities and medications, such as antithrombotic agents or immunosuppressants, and require an individualized therapeutic approach. In contrast to congenital hemophilia, comparative clinical studies are not available in AHA, largely as a result of the rarity of the disorder. Care decisions are often based on the clinical experience of treating physicians, and expert center referral is recommended to provide the best possible care.

Traditionally, the therapeutic approach to AHA includes 2 distinct goals, one being the immediate control of acute bleeding through hemostatic medication, while the other is directed toward long-term eradication of autoantibody inhibitors through IST.[3]

Rationale for immunosuppressive therapy

The autoimmune disorder in AHA is polyclonal, as evidenced by the presence of anti-FVIII antibodies of different

affinities, immunoglobulin classes and subtypes, and FVIII epitopes.[4,5] Most patients also have autoantibodies against targets other than FVIII, suggesting a general disturbance of immune regulation.[6] Monoclonal gammopathy, which is a typical condition associated with the acquired von Willebrand syndrome and is often unresponsive to standard immunosuppressive regimens, is rarely seen as an underlying disorder of AHA.

The indication for autoantibody eradication with IST is based on historic observational studies that have documented inhibitor-related mortality rates of 22% to 64%.[7-9] More recent registry studies reported low mortality rates from bleeding (3%-9%) in patients receiving IST.[10-14] However, the same studies reported that complications of IST, in particular infections, have become a leading cause of death among patients today. Therefore, recent guidelines suggested caution when using immunosuppressive therapy in elderly and frail patients.[2]

Goals of IST and definition of remission

The goal of IST is to reduce the risk of future bleeding by inducing remission of AHA. Spontaneous remission has been observed in patients not treated with IST, but this outcome is rare and unpredictable.[15] Definitions of remission vary across studies and registries. The UK surveillance study defined complete remission (CR) as FVIII normal, inhibitor undetectable, and immunosuppression stopped or reduced to doses used before AHA developed without relapse.[10] The GTH-AH 01/2010 study also included a definition for partial remission (PR): FVIII restored to >50% and no bleeding after stopping any hemostatic treatment for at least 24 hours.[12]

Guidelines on the use of IST in AHA

Two comprehensive guidelines have been published in the past 5 years.[1,2] Both appeared in the preemicizumab era, when IST was the only way to protect patients from future bleeding episodes.

First-line options suggested were glucocorticoids alone or in combination with either cyclophosphamide or rituximab. Upfront combination therapy was suggested for patients with FVIII <1% or inhibitor titers >20 BU/mL, who were observed to need longer times to PR (median, 5-6 weeks) compared to patients with higher FVIII or lower inhibitor titers (3-4 weeks) in the observational GTH-AH 01/2010 study.[12] The CREHA study compared glucocorticoids with cyclophosphamide against glucocorticoids with rituximab in first-line therapy of AHA, but results have not been published at the time of this review.[16]

Second-line IST is suggested if no decline in the inhibitor titer or rise in the baseline FVIII level is seen after 3 to 5 weeks of first-line therapy. Options include adding cyclophosphamide or rituximab, whichever was not used during first-line therapy, or alternative agents such as mycophenolate mofetil.[17]

Dosing recommendations for glucocorticoids, cyclophosphamide, mycophenolate mofetil, and rituximab are provided in Table 1.

High-dose IV immunoglobulin and immune tolerance regimens with high-dose FVIII, which were developed for inhibitors in congenital hemophilia A, are generally not recommended in AHA.

Efficacy of IST and monitoring

Complete remission is achieved by 60% to 90% of patients according to observational studies (Table 2). Overall, the rate of achieving CR appeared lower with glucocorticoids alone compared to combination therapies. However, results must be interpreted with caution owing to the retrospective design of most studies. Time to achieve remission varied between a few weeks and many months. Throughout this period, the risk of bleeding

Table 1. Immunosuppressive drugs used to treat AHA

Class or drug	2019 guideline recommended dosing[2]	Alternative dosing (CyDRi)[20]
Glucocorticoids	Prednisolone or prednisone 1 mg/kg/d PO for a maximum of 4–6 weeks (followed by tapered withdrawal)	Dexamethasone 40 mg PO on days 1, 8, 15, and 22
Cyclophosphamide	1.5–2 mg/kg/d PO for a maximum of 6 weeks	1000 mg IV on days 1 and 22
Rituximab	375 mg/m^2 IV weekly for a maximum of 4 cycles	100 mg IV on days 1, 8, 15, and 22
Mycophenolate mofetil	1 g/d for 1 week, followed by 2 g/d	—

Table 2. Efficacy of IST according to selected observational studies

Study	N	Median age, y	Partial remission	Complete remission
EACH2 registry[26]	294	75	NR	GC: 58% GC + Cy: 80% GC + Ri: 64%
GTH-AH 01/2010[12]	102	74	83%	61%
Spanish registry[14]	151	74	NR	84%
Dutch cohort study[13]	143	73	NR	79%
Chinese national registry[27]	187	52	GC: 70% GC + Cy: 92%	GC: 49% GC + Cy: 83%
Budapest (CyDRi)[20]	32	77	NR	91%

Cy, cyclophosphamide; GC, glucocorticoid; NR, not reported; Ri, rituximab.

remains high, as it has been observed that only achieving a FVIII level above 50% is fully protective.[18]

Close monitoring of FVIII levels is recommended until patients achieve CR, for 2 reasons: first, IST should be withdrawn or tapered as soon as PR is achieved; second, relapse occurs in approximately 20% of patients, most often early after achieving PR. After achieving CR, monthly monitoring is recommended during the first 6 months, every 2 to 3 months up to 12 months, and every 6 months during the second year and beyond, if possible.[2]

Monitoring of inhibitor levels can be helpful to guide IST. In particular, failure to decrease inhibitor levels during 2 to 4 weeks of first-line IST may guide the decision to use second-line options.

Risks of adverse events due to IST

Patient with AHA are often elderly and frail at the time of diagnosis. Glucocorticoids and other immunosuppressive drugs carry a high risk of adverse events, particularly in frail patients. The GTH-AH 01/2010 study followed a strictly prospective design and documented high rates of IST-related adverse events and mortality.[12] Among the 34 deaths recorded in this study, the most common cause was infection (n=16), followed by cardiovascular disorders (n=6), underlying disorders (n=3), and bleeding (n=3). Fourteen deaths were directly attributed to IST. These findings indicate that mortality related to IST, especially infection, surpasses the current risk of fatal bleeding in AHA. Patients with a poor WHO performance status (>2) upon presentation had a 4-fold higher risk of mortality (Figure 1).

Therefore, careful consideration of the need for and contraindications to IST, as well as its intensity and timing, is crucial for frail patients with AHA. IST should be discontinued if severe side effects occur during treatment.

Data on antibiotic and antiviral prophylaxis are scarce, but the high risk of infection may justify its use in patients at risk. Cotrimoxazole (960 mg PO 3 times weekly) and low-dose acyclovir (400 mg PO once daily) might be reasonable options.

Prognostic factors for outcomes of IST

Inhibitor titer at baseline appears to be an important prognostic factor for achieving CR. This was already identified in a meta-analysis of 249 patients published in 2003[9] and also in a recently published registry from The Netherlands.[13] In the latter study, patients with baseline inhibitor <20 BU/mL and mild bleeding at presentation had a 61% chance of CR with glucocorticoid therapy alone, compared to 7% of patients with >20 BU/mL and severe bleeding. Similar results were published from the GTH-AH 01/2010 study.[12] Predictors for mortality were advanced age (>75 years), malignancy, and intensive care unit admission in the Dutch study.[13] In the German-Austrian study, poor WHO performance status and malignancy predicted mortality.[12]

Newer IST regimens

The development of less toxic IST regimens remains a priority for research in IST. Mycophenolate mofetil was initiated either as first-line therapy in combination with glucocorticoids or as subsequent additional therapy in a single-center study of 11 patients.[17] With the exception of 1 patient, who died from underlying lymphoma, all patients were alive and in PR at 1 year of follow-up. Two patients developed transient neutropenia, and infections occurred in 4 patients at 1 year.

Another single-center study of 25 patients with AHA used dexamethasone pulses of 40 mg (4 days in weeks 1, 2, 5, and 6) instead of daily prednisolone and stratified additional immunosuppressive drugs according to baseline characteristics.[19] Results

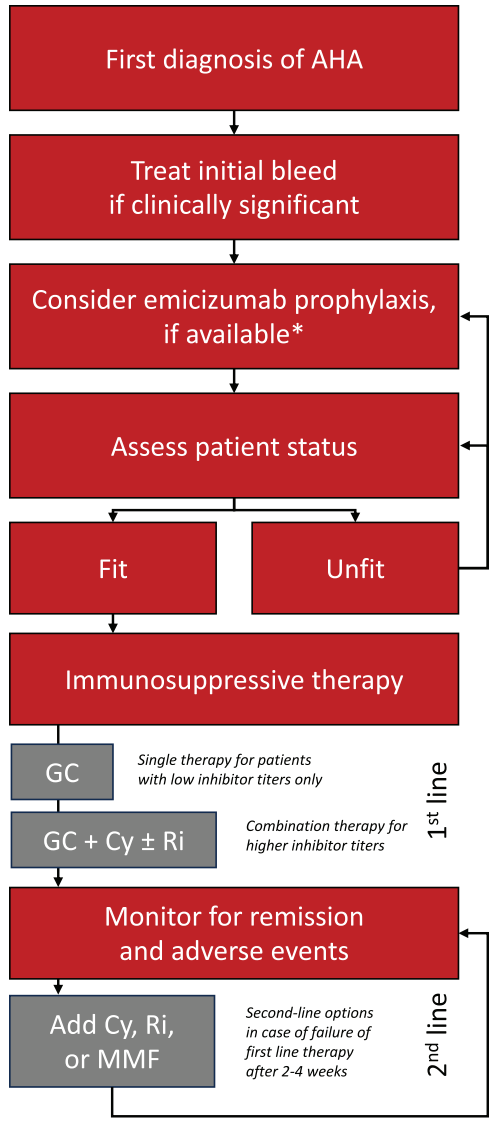

Figure 1. Suggested treatment algorithm. At the time of first diagnosis, most patients with AHA have active bleeding that requires hemostatic therapy. Once bleeding is under control, emicizumab prophylaxis should be considered to reduce the risk of bleed relapse and subsequent bleeding. Patients who are fit for IST should be offered first-line glucocorticoids (GCs), cyclophosphamide (Cy), and/or rituximab (Ri) (see text for details). Mycophenolate mofetil (MMF) is a second-line option. Patients who are not fit for IST should be reassessed in regular intervals and considered to be receive bleed prophylaxis with emicizumab. *Note: emicizumab is currently not licensed for AHA, except in Japan.

were similar to the GTH-AH 01/2010 study. Sixteen patients had grade 3 or 4 infection, and 2 patients died from infection.

A first-line combination therapy of glucocorticoids (dexamethasone 40 mg PO, days 1, 8, 15, and 22), cyclophosphamide (1000 mg IV, days 1 and 22), and rituximab (100 mg, days 1, 8, 15, and 22), designated as the CyDRi regimen, was recently reported from 2 centers in Budapest, Hungary.[20] Of the 32 patients who were retrospectively identified and had received this regimen, 31 achieved CR. Infections were reported in 5 patients and treatment-related mortality in only 1 patient.

Special situations

Based on limited data, an approach similar to other patients was suggested in patients with pregnancy-associated AHA, although careful consideration may be required with the use of cytotoxic agents. The same applies to younger patients with AHA who are still in the reproductive age.

The usual principles of management, including IST, also apply to patients with malignancy-associated AHA.[21] In patients with active solid tumors, IST will often be required to achieve remission before surgical resection or even biopsy of suspected lesions becomes feasible. About half of the malignancies associated with AHA are hematologic neoplasms.[22] In these cases, treatment of the malignancy may in part overlap with IST (including glucocorticoids, rituximab, and cyclophosphamide), and the regimen should attempt to sufficiently treat both the underlying condition and AHA.

Patients with autoimmune disorders can develop AHA while already on immunosuppressive drugs, and combination therapies will often be required to induce remission. After achieving PR in these patients, IST would be reduced to doses of glucocorticoids or other immunosuppressants used before to control the underlying autoimmune disorder.

Future directions

Initial reports on the use of emicizumab in AHA are promising.[23,24] Provided the efficacy and safety of emicizumab in patients with AHA are similar to patients with congenital hemophilia A, the drug reduces the requirement for early IST.[25] Postponing IST until patients have recovered from sequelae of the initial bleeding symptoms, surgery, and frailty could reduce the risk of infectious complications and improve mortality. Likewise, emicizumab could reduce the risk of bleeding complications until remission of AHA is achieved. Prospective clinical trials are under way to address these questions.

Of similar importance is the development of safer IST protocols. Promising results from retrospective observations should be confirmed by prospective studies. These should also address the use of antibacterial and antiviral prophylaxis to reduce the risk of infection.

Conflict-of-interest disclosure

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Off-label drug use

Andreas Tiede: emicizumab, rituximab, mycofenolate mofetil, cyclophosphamide.

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The role of emicizumab in acquired hemophilia A

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Acquired hemophilia is a rare bleeding disorder that predominantly affects older people with potential underlying comorbidities, including cardiovascular and thrombotic risk factors. The current standard therapies with hemostatic agents for acute bleeding and immunosuppression often require inpatient management, are not approved for routine bleeding prophylaxis, and contribute to the high mortality in this population. Emicizumab is a factor VIII (FVIII) mimetic approved for bleeding prophylaxis in congenital hemophilia A with and without FVIII inhibitors. Given subcutaneously, it may allow easier outpatient bleeding prophylaxis and reduce intensity of immunosuppression. This article summarizes the currently available data on the efficacy and safety of emicizumab in acquired hemophilia A.

LEARNING OBJECTIVES

- Recognize the current challenges of current treatment of acquired hemophilia A
- Summarize the current experience with emicizumab in AHA
- Describe the current safety profile of emicizumab use in AHA

CLINICAL CASE 1

One month after the birth of her second child, a 34-year-old gravida 2, para 2 woman noticed easy bruising and swelling of her left arm and leg that slowly resolved (Figure 1. A/B). Approximately 10 months postpartum, she reported to the emergency department with acute onset right arm swelling with severe pain and numbness (Figure 1. C). She underwent emergent fasciotomy for compartment syndrome, which was complicated by severe intraoperative bleeding that required 14 units of red blood cells (RBCs) over the next week. She was subsequently diagnosed with acquired hemophilia A with a factor VIII (FVIII) activity of 6 IU/dL and a FVIII inhibitor of 11 Bethesda units (BU). Treatment with activated prothrombin complex concentrate (aPCC) was initiated with significant improvement in bleeding, but she continued to saturate her bandages every 2 to 4 hours and continued to require RBC transfusions. Her bleeding improved after switching hemostatic treatment from aPCC to recombinant porcine FVIII concentrate. However, 6 days after switching, her bleeding increased again and a porcine FVIII inhibitor measured 22 BU.

Introduction to acquired hemophilia A (AHA) and the challenges of currently available therapy

AHA is a rare autoimmune condition caused by an IgG autoantibody directed toward endogenous FVIII. This condition is idiopathic in about half of all cases but can be associated with other autoimmune diseases, malignancies, pregnancy, and certain drugs (particularly alemtuzumab, clopidogrel, and omalizumab).[1] There also have been several case reports suggesting a potential association with COVID vaccination.[2,3]

Bleeding pattern and severity in AHA

Most patients present with spontaneous bleeding that is cutaneous or intramuscular or with retroperitoneal bleeding, but bleeding can also occur with trauma or procedures.[4] Many bleeds are severe at presentation[5] with significant drop in hemoglobin (Hb) necessitating blood transfusions, and they can quickly become limb-, organ-, or life-threatening.

Efficacy and limitation of currently available hemostatic therapies for AHA

Recombinant activated factor VII (rFVIIa) and aPCC are frequently used for acute hemostatic management of AHA

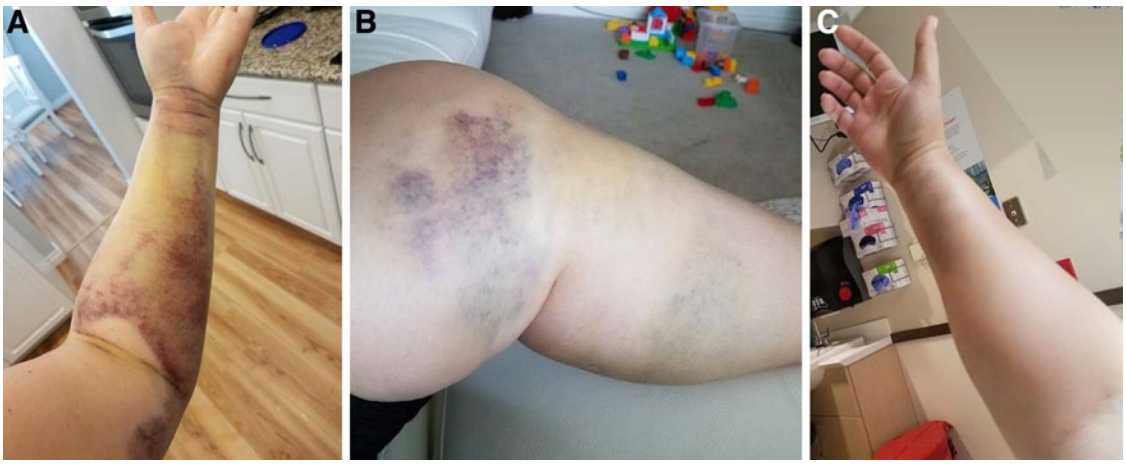

Figure 1. Bleeding in a case of postpartum-associated AHA. (A) Soft tissue bleeding in left arm; (B) soft tissue bleeding in left leg; (C) soft tissue bleeding resulting in compartment syndrome in right forearm.

Table 1. Current hemostatic agents recommended for AHA

	Recombinant factor VII activated (rFVIIa)	Activated prothrombin complex concentrate (aPCC)	Recombinant porcine factor VIII (rpFVIII)
	Eptacog alfa, NovoSeven®RT	Anti-inhibitor coagulant complex, FEIBA®	Antihemophilic factor (recombinant), porcine sequence, Obizur®
Indication	• Treatment of bleeding episodes and perioperative management in adults with acquired hemophilia[30]	• Hemophilia A and B patients with inhibitors[31]	• On-demand treatment and control of bleeding episodes in adults with acquired hemophilia A[32]
Dosing	• 70–90 mcg/kg IV every 2–3 hours until hemostasis achieved[30]	• 50–100 U/kg IV[31] every 6–12 hours	• Initial dose 200 U/kg IV then titrate based on Factor VIII (FVIII) levels[32]
Limitation of use	• Serious arterial and venous thrombotic events following administration have been reported[30]	• Thromboembolic events reported during postmarketing surveillance, particularly following the administration of high doses and/or in patients with thrombotic risk factors[31] • Contraindicated in acute thrombosis or embolism (including myocardial infarction)[31]	• Safety and efficacy has not been established in patients with a baseline anti-porcine factor VIII inhibitor titer >20 BU[32]

(Table 1). These bypassing hemostatic agents were developed for patients with congenital hemophilia A and inhibitors. Due to their short half-lives and intravenous route of administration, both rFVIIa and aPCC often require hospitalization. Neither is approved for hemostatic prophylaxis outside the perioperative setting, and hemostatic under- or overcorrection cannot be monitored by a laboratory assay. Both contain activated factor VII, contributing to a potential risk of unwanted thrombosis.

Recombinant porcine FVIII (rpFVIII, Obizur) is approved for both on-demand hemostatic treatment and prophylaxis in AHA. However, some patients present with anti-FVIII autoantibodies that cross-react with rpFVIII and reduce efficacy. Patients with AHA whose autoantibodies do not cross-react at presentation will often develop a de novo antibody against rpFVIII after ongoing exposure (typically 8 to 85 days after starting rpFVIII).[6] This unfortunately makes rpFVIII efficacious in only a proportion of patients with AHA and for a limited duration. Waning efficacy should be closely monitored with FVIII activity levels. Once rpFVIII is no longer effective, patients with AHA must be restarted on bypassing agents for ongoing or recurrent bleeding.

The current international guidelines for AHA recommend hemostatic treatment with rFVIIa, aPCC, or rpFVIII rather than human FVIII concentrate for clinically significant bleeding and/or invasive procedures or surgeries.[7] The efficacy of human FVIII is reduced by the anti-FVIII autoantibodies in patients with AHA. In a registry of 501 patients with AHA, FVIII products had a 70.1% rate of controlling frontline bleeds compared with 91.8% for the bypassing agents.[8] Desmopressin (DDAVP) can be used to release endogenous FVIII stores but has limited utility in the presence of an autoantibody against FVIII.

The rebleeding risk after initial hemostasis and need for immunosuppression therapy

Approximately 50% of patients experience recurrent bleeding as per the prospective GTH-AH 01/2010 study of 102 subjects with AHA.[9] The bleeding risk is particularly high in patients with FVIII activity of <20 IU/dL. Due to the high risk of recurrent bleeding and rarity of spontaneous remissions, immunosuppression is standard of care for patients with AHA.[7] Frontline combination immunosuppression has historically been favored to try to

irradicate the inhibitor as quickly as possible given the difficulties in treating bleeds with the available hemostatic agents, especially in the outpatient setting. Treatment recommendations for first-line immunosuppression (IST) traditionally have been glucocorticoids with cyclophosphamide or rituximab depending on the severity of the inhibitor and FVIII level.[7,10] Adverse events from immunosuppression, however, are common, with a high mortality in those with secondary infections (30% of adverse events in the GTH/AH study were probably or definitely related to IST and 54% of those with infections died).[11] Improved outpatient-based bleeding prophylaxis could reduce the intensity of immunosuppression and the risks of adverse events.

CLINICAL CASE 1 (continued)

After 3 weeks in the hospital, the patient achieved good temporary hemostasis with rpFVIII concentrate. Her fasciotomy was closed surgically and she did not require further RBC transfusion. She started on prednisone and cyclophosphamide, but her FVIII inhibitor titer remained detectable at 11 BU with an FVIII activity of 8 IU/dL. She developed a detectable anti-porcine FVIII inhibitor, and the efficacy of rpFVIII was waning. She remained at high risk of rebleeding but had a strong desire to return home to her 2 young children. To provide an outpatient hemostatic prophylaxis, the off-label use of emicizumab was started.

Efficacy of emicizumab for bleeding treatment and prophylaxis

Emicizumab is a bispecific antibody that binds activated factor IX and factor X mimicking the function of FVIII. Currently, emicizumab is approved for hemostatic prophylaxis for congenital hemophilia A with and without inhibitors.[12,13] Anti-FVIII antibodies do not impact the efficacy of emicizumab, making it an appealing treatment for AHA. Unlike other available hemostatic options for AHA, emicizumab has a half-life of 28 days and can be administered subcutaneously.

Animal model of emicizumab in AHA and the experience in congenital hemophilia A

An early model of AHA in cynomolgus monkeys showed bleeding reduction in animals receiving weekly emicizumab (ACE910) prophylaxis.[14] This study was the basis for phase 1/2 studies[15] and an extensive phase 3 clinical trial program in congenital hemophilia A (HAVEN 1-4) leading to the FDA approval for prophylaxis in congenital hemophilia A.

Based on these trials, emicizumab 3 mg/kg for 4 weekly loading doses was approved for congenital hemophilia A. This results in steady state drug levels that can be maintained with 1.5 mg/kg every week, 3 mg/kg every 2 weeks, or 6 mg/kg every 4 weeks. In vitro thrombin generation studies suggest emicizumab is equivalent to an FVIII activity of over 15 IU/dL. There seems to be a ceiling effect; emicizumab does not fully correct hemostasis to that of someone without a bleeding disorder, but rather improves the clinical bleeding phenotype from severe to mild. For patients with severe congenital hemophilia A with and without inhibitors, emicizumab has revolutionized outpatient bleeding prophylaxis, resulting in significantly reduced breakthrough bleeding.[12,16,17] The annual bleeding rate appears to continue to improve with longer term use of emicizumab. Additionally, emicizumab has reduced hospitalization days and the need for bypassing hemostatic therapies in patients with congenital hemophilia A and inhibitors.[18]

Current experience with emicizumab in people with AHA

Since the approval of emicizumab for congenital hemophilia A, there has been conceptual adaptation and use for AHA. Knoebl et al. retrospectively described 12 cases (median age 74, range 51-87 years, 50% women) of off-label use of emicizumab that showed clinical improvement of bleeding, even in patients with severe or surgical bleeding. Bleeding stopped in patients with insufficient hemostasis on bypassing agents within 4 days of emicizumab initiation, and no new or breakthrough bleeding was noted after day 2 of emicizumab therapy.[19]

A systematic review by Thomas et al. compiled 12 case reports with 33 patients with AHA treated with emicizumab.[20] Emicizumab was initiated for active/recurring bleeding in most cases (90.9%). One patient was switched from aPCC to emicizumab after developing a myocardial infarction, and another patient was started on emicizumab to enable dual antiplatelet therapy after coronary stent placement. All reported patients had a clinical response without further spontaneous bleeding after starting emicizumab.

A retrospective survey of 87 hemophilia treatment centers in the United States in 2021 revealed that of 358 patients with AHA treated at 32 centers between 2016 and 2021, 40 patients had received off-label emicizumab. An initial look at 24 patients where data were available showed that the majority (17 of 24) were started on emicizumab to provide bleeding prophylaxis and 15 of 24 started to facilitate a transition to outpatient treatment. In 9 of 24 emicizumab was started because of failure of the current hemostatic management. After patients started emicizumab, the use of other hemostatic agents reduced from 75% to 17%, RBC transfusion from 50% to 8% and hospitalization days from a median of 10 (range 0-60) to 3 (range 0-30).[21]

A prospective, multicenter, open-label study of emicizumab prophylaxis in 12 adult patients led to approval of the drug for the treatment of AHA in Japan. Eleven of these patients completed the study and experienced 77 major bleeds prior to starting emicizumab. Although there were no major bleeding events, 45% (5 of 11) experienced some bleeding after emicizumab initiation. While 8 of 11 patients had used bypassing agents prior to emicizumab, only 3 patients received rFVIIa while on emicizumab, and the number of patients needing blood transfusions decreased from 7 to 3.[22]

Additionally, there are 2 parallel prospective multicenter trials to study the efficacy and safety of emicizumab in AHA over a 12-week period; 1 in Germany and Austria recently completed enrollment (NCT04188639), and 1 in the United States is still enrolling (NCT05345197).

Emicizumab dosing in AHA

There is no standard emicizumab dosing regimen for patients with AHA. In the retrospective case series by Knoebl et al., all 12 patients received a loading dose of 3 mg/kg weekly for 2 to 3 doses followed by maintenance dosing of 1.5 mg/kg.[19] In Thomas's review of an additional 21 cases, 5 patients received

Table 2. Considerations when choosing hemostatic agents to treat breakthrough bleeding on emicizumab

	Recombinant factor VII activated (rFVIIa)	Activated prothrombin complex concentrate (aPCC)	Recombinant porcine factor VIII (rpFVIII)
Safety	• May increase risk of thrombosis (limited data in AHA); for congenital hemophilia A, the combination of emicizumab and rFVIIa was safe	• Thromboembolic events and thrombotic microangiopathies were reported with dose of >100 U/kg/24 hours and administered for >24 hours and the use of aPCC in people on emicizumab prophylaxis is relatively contraindicated	• Coadministration of rpFVIII and emicizumab has been reported in case series and theoretically does not have an additive effect as both compete for the same binding site
Limitation of use	• rFVIIa should only be reserved for acute severe breakthrough bleeding	• Avoid with emicizumab	• The chromogenic FVIII assay underestimated rpFVIII (Obizur) activity[26]

the standard loading dose, where 15 had received an altered loading dose.[20]

A loading dose of 6 mg/kg has been entertained to reach steady state more expediently. Monthly doses of 6 mg/kg were found safe and without thrombotic complications in the HAVEN 4 study.[23] A dosing regimen of 6 mg/kg on day 1 and 3 mg/kg on day 2, followed by 1.5 mg/kg weekly starting on day 8, was investigated in the prospective study in Japan and suggested steady state emicizumab concentration after 1 week.[22]

The impact of body mass index (BMI) on dosing of emicizumab has not been reported. While the activated partial thromboplastin time (aPTT) has been shown to correct within 1 to 3 days after initiating emicizumab,[19] we have observed that normalization of the aPTT can take up to 5 days in patients with morbid obesity.

Laboratory monitoring

AHA is diagnosed and response to treatment is monitored by measuring the FVIII activity and FVIII inhibitor titer. Traditionally, this has been performed with an aPTT-based FVIII activity and the aPTT-based inhibitor assay, Nijmegen-Bethesda assay (NBA). Emicizumab interferes with the aPTT and aPTT-based assays, making traditional one-stage FVIII activity levels uninterpretable (Table 2). In a study of 12 patients with AHA, emicizumab normalized the aPTT within 1 to 3 days of administration[19] even when the endogenous FVIII was still low. For patients on emicizumab, a chromogenic FVIII assay with bovine reagents must be used to monitor the patient's endogenous FVIII activity. Inhibitor titers can be measured using an ELISA-based assay, which has higher sensitivity (1.0) but lower specificity (0.83) than the NBA and is not impacted by substances that interfere with the aPTT, such as lupus anticoagulants and emicizumab.[24] Currently, an emicizumab "level" is reported by some centers using the chromogenic FVIII with human reagents; however, this is not standard of care and is not recommended by the manufacturer of emicizumab for patients with congenital hemophilia A.

CLINICAL CASE 2

A 94-year-old man with hypertension, aortic stenosis, and a remote history of bladder cancer was diagnosed with AHA after presentation with deltoid and lower extremity hematomas. He started on off-label emicizumab with good bleeding control and improvement of the hematomas. On week 8 of treatment, he became increasingly anemic (hemoglobin 6.1 g/dL) and presented with maroon blood per rectum. He was hospitalized and transfused with 3 units of RBCs.

Treatment of breakthrough bleeding on emicizumab

While there is a clear suggestion that emicizumab may be an effective prophylactic agent to reduce subsequent or recurrent bleeding in AHA, emicizumab should not be used for acute bleeding due to its delayed bioavailability via the subcutaneous route of administration. As of now, there are no pharmacokinetic data of emicizumab associated with intravenous administration. Considering limitations for the current hemostatic agents available for AHA (Table 2), rFVIIa should probably be used over rpFVIII and aPCC for breakthrough bleeding. The combination of aPCC and emicizumab carries a black box warning due to the risk of thrombotic events and thrombotic microangiopathy seen in trials of congenital hemophilia A. Combining emicizumab with rFVIIa 90 mg/kg appears safe and in vitro data[25] suggest higher doses may be feasible, but thromboembolic complications have been reported.

Laboratory monitoring of rpFVIII limits its use with emicizumab. rpFVIII is an effective hemostatic option in AHA[6] and can be monitored with the one-stage, aPTT-based FVIII clotting assay, which is uninterpretable in patients on emicizumab. The chromogenic FVIII assay was shown to underestimate rpFVIII (Obizur) activity.[26] Considering that the one-stage assay is unreliable in patients on emicizumab, monitoring of FVIII activity for rpFVIII therapy is challenging. Concurrent use of rpFVIII with emicizumab can be considered for acute bleeding, with the caveat that a target chromogenic FVIII activity has not been established, since it gives falsely low results (underestimates) with rpFVIII.

CLINICAL CASE 2 (continued)

The patient continued to have GI bleeding, and a decision was made to add tranexamic acid to his hemostatic management. However, he continued to have GI bleeding requiring transfusions, for which rFVIIa 90 µg/kg was initiated. He received 14 doses of rFVIIa over 6 days and then developed acute right-sided ataxia. rFVIIa was discontinued and his stoke symptoms

resolved without residual deficits. A capsule endoscopy demonstrated telangiectasias in mid jejunum/proximal ileum for which he started octreotide with good bleeding control.

Adverse events of special interest

Common adverse events to emicizumab include injection site reactions, headaches, and arthralgias. Additional adverse events must be considered in patients with AHA on emicizumab given the high rate of comorbidities and advanced age of this population. AHA is a condition associated with high morbidity and mortality, likely due to the advanced age of patients, their underlying comorbidities, the use of IST, and, less often, bleeding.[10,27] As would be expected in this population, thromboembolic events, especially when bypassing agents are used, are not uncommon despite the AHA state.[10]

Thromboembolism

In the case series by Knoebl et al., a 79-year-old experienced a minor stroke on day 16 of emicizumab after receiving rFVIIa 90 µg/kg prior to a procedure.[19] Of the 24 patients at the US centers, 1 developed a lower extremity deep vein thrombosis (DVT) while on weekly emicizumab therapy.[21] The prospective Japanese study of 12 patients reported 1 event of an incidental lower extremity DVT on day 16 of emicizumab treatment that resolved without treatment.[22]

From our experience so far, we would anticipate that breakthrough bleeding can occur on emicizumab and that patients need additional hemostatic prophylaxis for invasive procedures. From the early experience during the HAVEN 1 study, we know that the use of aPCC is contraindicated in patients on emicizumab and can lead to thrombosis and thrombotic microangiopathies.[12] The coadministration of emicizumab and rFVIIa, however, was safe in people with congenital hemophilia A.[28] Prospective assessment of thromboembolic events for people receiving emicizumab for AHA is ongoing in the above-mentioned parallel clinical studies.

Anti-drug antibodies

Data from the congenital hemophilia studies showed that 5.1% (34 of 668) of people exposed to emicizumab developed anti-emicizumab antibodies. Of these antidrug antibodies, 41.2% were transient, 52.9% were neutralizing in vitro, and only 0.6% resulted in decreased emicizumab plasma levels.[29] In the prospective Japanese study, 1 of 12 (8.3%) developed an antidrug antibody, but it did not have a clear impact on the pharmacokinetics.[22]

CLINICAL CASE 3

A 76-year-old man with poorly controlled hypertension and diabetes had prolonged aPTT for at least 3 years. He had no abnormal bleeding and was never further evaluated or treated for his prolonged aPTT. He then emergently presented with an incarcerated inguinal hernia that required surgery. He was diagnosed with AHA with an FVIII activity of 5 IU/dL and FVIII inhibitor of 11.9 BU. He received aPCC perioperatively and did well. Postoperatively, he was seen in clinic and started on cyclophosphamide 100 mg PO daily for immunosuppression. He was felt to be a poor candidate for high-dose corticosteroid due to his history of hypertension and diabetes. Fourteen days later, he presented to the emergency department with nausea and ankle swelling and was found to be hyponatremic with sodium levels of 115 mmol/L (normal 136–145 mmol/L) with mildly elevated liver enzymes. Cyclophosphamide was held and he received supportive care with resolution of the hyponatremia and transaminitis, but 5 days later he developed massive, spontaneous bilateral iliopsoas bleeding resulting in bilateral leg weakness and loss of sensation in both anterior thighs, profoundly impacting ambulation and self-care.

Impact of emicizumab on immunosuppression

While approximately 10% of patients with AHA do not initially present with bleeding,[7] a lack of bleeding history does not negate future bleeding risk or eliminate the need for immunosuppression. Historically, early use of immunosuppression for AHA was essential to eradicate the inhibitor and control bleeding. However, immunosuppression-related adverse events such as infections are a main driver of the high morbidity and mortality of AHA. With effective outpatient bleeding prophylaxis, emicizumab reduces the urgency to eradicate the inhibitor, allowing reduced-intensity immunosuppression. In a survey of adult hematologists at 87 US hemophilia treatment centers, 70% of providers with experience with emicizumab for AHA reported using emicizumab to delay or decrease immunosuppression.[21] In Knoebl's case series, all patients started on emicizumab were deemed to be poor candidates for immunosuppression.[19] The safety of postponing immunosuppression with emicizumab may be answered in a planned analysis between the 2 ongoing clinical trials in Europe (NCT04188639) with delayed initiation of IST and the United States (NCT05345197) with standard initiation of IST.

CLINICAL CASE 3 (continued)

The patient required several RBC transfusions and was treated with rFVIIa initially and then aPCC with stabilization. He was not able to care for himself at home and needed in-patient rehabilitation, which proved difficult because he continued on aPCC every 12 hours to prevent further bleeding. Further, the next line IST, rituximab, was difficult to administer in the rehab setting. Considering the ongoing need for a bleeding prophylaxis that could be given in rehab as well as a potential delay in further IST, he was started on off-label emicizumab with an accelerated loading dose. This allowed for transition to rehab, where he did not have recurrent bleeding and regained strength, allowing for transition to home 2 months later. He then received rituximab 100 mg weekly×4 doses and cleared his inhibitor 6 weeks later.

Duration of emicizumab use in AHA

Monitoring patients with AHA for partial and complete remission of the inhibitor is essential and will guide the duration of emicizumab therapy. Laboratory monitoring on emicizumab

Table 3. Impact of emicizumab on labs

Lab	Impact of emicizumab	Recommended alternative
aPTT	False decrease	Do not use while on emicizumab
One-stage FVIII (PTT based)*	False increase	Chromogenic FVIII with bovine reagents (measure infused and/or endogenous FVIII)
FVIII inhibitor titer with clotting-based assays (Bethesda)	Uninterpretable	Chromogenic Bethesda assay with bovine reagents
Activated clotting time (ACT)	False decrease	Anti-Xa activity
Activated protein C resistance (aPTT based)	Uninterpretable	Factor V Leiden genetic testing (if clinically appropriate)

*The one-stage FVIII activity will overestimate the impact of emicizumab and will not be able to separate the endogenous FVIII activity from the impact of emicizumab on the assay. Emicizumab impacts all single-factor assays that are performed with one-stage, aPTT-based assays (such as factor IX activity).

was discussed by Platton et al. in an earlier chapter and has special considerations (Table 3). Emicizumab may be discontinued once the endogenous FVIII level improves; however, there is no strict FVIII threshold for discontinuing emicizumab and there is a variety in clinical practice: emicizumab was discontinued when FVIII ranged from 10% to 86% in 26 patients with AHA in a systematic review by Thomas et al.[20] In our practice, we continue emicizumab if there is still a persistent inhibitor and low FVIII level, especially if the patient has a history of bleeding as it can recur without prophylaxis. Data from 2 prospective clinical trials in Europe (NCT04188639) and the United States (NCT05345197) may help guide emicizumab dosing and stopping thresholds. Due to the long half-life of emicizumab, some effect of the drug can likely be anticipated until 5 months after discontinuation.

Conclusion

Although currently off label for AHA, emicizumab is poised to revolutionize treatment through effective bleeding prophylaxis that can be given in the outpatient setting, reducing hospitalizations and the need for bypassing agents. Unlike rpFVIII, the FVIII autoantibodies do not impact the use of emicizumab, and antidrug antibodies are rare. By reducing the risk of bleeding, emicizumab may shift the treatment goals of AHA away from high-dose immunosuppression, reducing the risk of adverse events such as infections. Emicizumab cannot be used for breakthrough bleeding, and care must still be taken to monitor for thrombotic events with concurrent use of bypassing agents, especially given the high rate of comorbidities in the AHA population. Two ongoing prospective parallel studies in Europe and the United States will answer important questions about the efficacy and safety of emicizumab in AHA.

Conflict-of-interest disclosure

Jacqueline Poston is a consultant for TeraImmune.

Rebecca Kruse-Jarres is an educational speaker for Genentech, is a scientific advisor for Regeneron and Roche, and received research funding from Genentech.

Off-label drug use

Jacqueline Poston: Off-label use of emicizumab was discussed.
Rebecca Kruse-Jarres: Off-label use of emicizumab was discussed.

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Inflamed—HLH, MAS, or something else?

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Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of excessive and maladaptive inflammation. Primary HLH is most frequently encountered in young children, and, without timely recognition and therapy, can lead to multiorgan failure and death. It is most often diagnosed using the HLH-2004 criteria and by identifying pathological mutations. However, the HLH-2004 criteria are not specific for HLH, and patients can easily fulfill these diagnostic criteria in other proinflammatory states in which HLH-therapy would not be indicated, including hematologic malignancies, infections, and rheumatologic disease. Therefore, great care must be taken to ensure that the specific disease associated with features of HLH is accurately recognized, as consequences of improper treatment can be catastrophic. We propose a diagnostic pathway for patients for whom HLH is on the differential (visual abstract). Importantly, in situations in which the initial diagnostic workup is equivocal or unrevealing, reevaluation for occult malignancy, infection, or rheumatologic disease would be prudent, as occult presentations may be missed on primary evaluation. Temporizing medications can be used in critically ill patients while awaiting secondary evaluation. By using this framework, clinicians will be able to more reliably discern primary HLH from other pro-inflammatory states and thus provide timely, appropriate disease-specific therapy.

LEARNING OBJECTIVES

- Recognize the limitations of the current diagnostic paradigms for HLH, identifying the various pathologies that can manifest as HLH
- Formulate diagnostic and therapeutic approaches for patients with suspected HLH

Hemophagocytic lymphohistiocytosis (HLH) is not a single disease; rather, the term describes a state of systemic inflammation that is disproportionate, maladaptive, or sometimes merely accompanying another disease.[1,2] This state may arise from an inherited genetic lesion, from an acquired source of pathologic immune activation, or from a combination therein. Commonly used terminology has sought to distinguish between those cases attributed principally to genetic causes (which have been labeled as *primary HLH*) and those cases attributed largely to acquired causes of immune activation (which have been labeled as *secondary HLH*). This nomenclature may cause some confusion, as many cases likely arise from a combination of such inherited and acquired factors. Many of the genetic deficits which may precipitate or predispose a person to HLH affect perforin-mediated lymphocyte cytotoxicity, and this subset of primary HLH is often referred to as *familial HLH* (FHL).[3–5] In such cases, the ensuing defective lymphocytes are vulnerable to uncontrolled activation, liberating cytokines such as interferon gamma, which, in turn, leads to macrophage activation. Additional genetic variants that activate lymphocytes and macrophages via different mechanisms (eg, XIAP deficiency), along with certain specific and increasingly recognized genetic mediators of granule-mediated cytotoxic dysfunction (eg, *RAB27A, LYST*), are grouped with FHL under primary HLH (Figure 1). Activated macrophages and cytokines can cause life-threatening organ dysfunction, making rapid recognition and treatment critical.[6]

The Histiocyte Society led 2 clinical trials that improved the outcomes for children with primary HLH. The most recent trial, HLH-2004, enrolled patients aged 18 years and younger who either had genetically confirmed HLH or fulfilled 5 of 8 clinical criteria suggestive of HLH: fever, splenomegaly, cytopenia affecting at least 2 lineages, hyperferritinemia, hypofibrinogenemia or

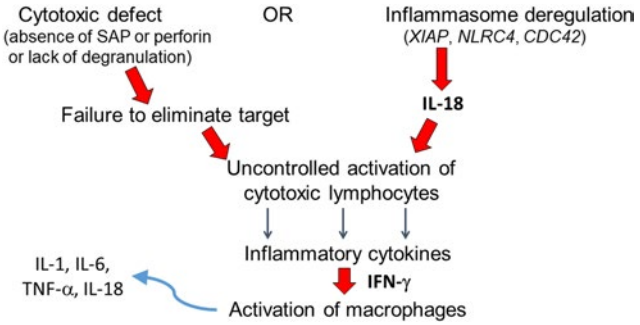

Figure 1. Pathogenesis of primary HLH and MAS. Genetic variants associated with primary HLH result in defective lymphocyte cytotoxicity (left pathway) or inflammasome degranulation, liberating IL-18 (right). Either mechanism culminates in uncontrolled activation of cytotoxic lymphocytes. The pathogenesis of MAS also involves IL-18-mediated activation of lymphocytes (all HLH patients may have elevated IL-18 levels; however, the increase may be particularly profound among those with MAS). Activated lymphocytes secrete inflammatory cytokines such as IFN-γ, activating macrophages. Activated macrophages in turn secrete additional inflammatory cytokines.

hypertriglyceridemia, hemophagocytosis in bone marrow or other tissue, reduced natural killer (NK)-cell cytotoxicity, or elevated sCD25 (sIL2Rα).[7,8] These enrollment criteria were based on the experience and opinion of clinicians rather than on rigorous data. The sensitivity and specificity of these criteria for HLH remain unknown as there remains no gold standard for the diagnosis of HLH. However, the mere presence of features listed in these enrollment criteria has been adapted by much of the medical community as being diagnostic and pathognomonic of HLH.

When patients manifest these criteria in the absence of causal genetic defects, the condition is often called secondary HLH.[1,9] Secondary HLH may be understood as an extraordinary inflammatory response to a proinflammatory trigger. Conditions which can lead to manifestations of secondary HLH are many and varied including infections, malignancies (most often lymphoma), and autoimmune disorders (including macrophage activation syndrome [MAS]). Although patients with all these conditions can also suffer life-threatening organ dysfunction, lumping these disparate pathologies under 1 umbrella term of secondary HLH is not scientifically justified.[10] Treating patients with these varying diseases with therapies meant for primary HLH can lead to disastrous consequences.[11] Making these distinctions at the bedside is a monumental challenge for clinicians. We present real-life cases to elucidate a practical approach to patients with suspected HLH.

CASE 1

A previously heathy 2-month-old female was brought to the emergency department with persistent fever and decreased oral intake. She was noted to be listless, febrile, hypoxic, tachycardic, and tachypneic and to have hepatosplenomegaly. Laboratory studies were notable for pancytopenia, hyperferritinemia, hypertriglyceridemia, and hypofibrinogenemia (Table 1). She was resuscitated with intravenous fluids and treated with broad-spectrum antibiotics. Bone marrow biopsy showed normal hematopoiesis without increase in histiocytic or hemophagocytic forms.

CASE 2

A previously healthy 2-year-old boy was brought to the emergency department with symptoms of fever, abdominal distension, and reduced oral intake. An evaluation revealed fever, tachycardia, pallor, hepatomegaly, and splenomegaly. Laboratory studies revealed pancytopenia, hyperuricemia, and hyperferritinemia (Table 1). He was treated with intravenous fluids and antibiotics. Over the next hours, fibrinogen decreased and urine output declined, requiring continuous renal replacement therapy. Bone marrow morphology showed prominent hemophagocytosis but otherwise normal hematopoiesis.

These 2 cases exemplify urgent life-threatening situations wherein simple tools such as the HLH-2004 criteria rapidly draw HLH into the differential diagnosis. Subsequent results showed elevated sCD25 levels in both children (Table 1), further raising the concern for HLH. Given the young age and absence of

Table 1. Laboratory data

	Case 1	Case 2	Case 3	Case 4
Absolute neutrophil count (×10³ per μL)	0.14	0.89	1.9	0.08
Hemoglobin (g/dL)	7.4	8.7	7.8	8.1
Platelet count (×10³per μL)	26	26	84	91
Fibrinogen (mg/dL)	50	163→75	759	167
Ferritin (ng/mL)	7000	18 000	4512	>99 000
Triglycerides (mg/dL)	NR	153	192	178
sCD25 (units/mL)	88 228	71 439	34 750	1907
Uric acid (mg/dL)	NR	9.1	4.3	NR
LDH (units/L)	NR	1783	273	494

NR, not reported.

leukemia, primary HLH was considered most likely in both cases. The first child (2-month-old female) was treated with dexamethasone and etoposide and rapidly improved. Genetic studies revealed her to be homozygous for a variant of unknown significance in the *PRF1* gene. Flow cytometry on peripheral blood lymphocytes showed absent perforin protein expression in NK and cytotoxic T lymphocytes (Figure 2), confirming the diagnosis of primary HLH caused by perforin deficiency. This illustrates the utility and power of flow cytometry assays (flow) in diagnosing primary HLH. In all cases of suspected HLH, we use flow for perforin and CD107a degranulation, the latter assessing the integrity of the lytic-granule secretory pathway.[12] Normal degranulation depends on several proteins, many of which are encoded by genes implicated in primary HLH: *UNC13D, STX11, STXBP2, LYST, RAB27A,* and *AP3B1*.[12] In males, we also add flow for SAP and XIAP. The biggest advantages of these assays are rapid turnaround time and precision. Moreover, CD107a degranulation is a functional assay, and the combination of perforin flow and CD107a has been shown to be more reliable than the NK-cell cytotoxicity assay to diagnose primary HLH.[13] These assays are also helpful in situations wherein the results of genetic tests are equivocal, as above.

The second child (2-year-old), in addition to meeting the HLH-2004 criteria, also had hyperuricemia and rapidly developed renal failure, features not typical of primary HLH. His bone marrow aspirate and biopsy did not show leukemia, and full body CT scans only showed the known hepatomegaly and splenomegaly. Strikingly, quantitative polymerase chain reaction (PCR) for Epstein–Barr virus (EBV) on his peripheral blood demonstrated 25×10^6 copies/mL. His bone marrow biopsy showed an expanded population of CD8+ T lymphocytes, most of which were also positive for EBV-encoded-RNA, thus establishing the diagnosis of systemic EBV-positive T-cell lymphoma of childhood.[14] Quantitative PCR on sorted lymphocytes showed the burden of T-lymphocyte EBV to be 700-fold and 23-fold higher than that associated with B lymphocytes or NK cells, respectively. His condition did not improve with dexamethasone treatment per the HLH-94 protocol. With the EBV results, treatment with multiagent chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone) was initiated, and he rapidly recovered. All genetic and flow studies for primary HLH were normal. Following another cycle of chemotherapy with the same agents, he underwent allogeneic bone marrow transplant and is thriving. The association of EBV infection with HLH is well known.[15] In primary HLH, EBV infection is recognized as a recurrent trigger of disease activation. There are other rare immunex deficiencies associated with severe EBV infection manifesting clinical features of HLH.[16] In all these situations, EBV is associated with B lymphocytes. On the other hand, EBV infection of NK or T lymphocytes causes a spectrum of lymphoproliferative disorders (LPDs), from chronic smoldering mononucleosis-like illness (chronic active EBV) to fulminant lymphoma as in the second patient all without any identifiable genetic defects.[14] Interestingly, EBV-driven NK- and T-cell LPDs are more common in people of East Asian and Indigenous American ethnicities. All these disorders can manifest clinical features fulfilling HLH-2004 and are frequently referred to as EBV-HLH or secondary HLH due to EBV.[15,17] This broad term includes a disparate array of B- and NK/T-cell disorders, which are distinct from primary HLH, and require differing therapies. For example, treatment with the B-lymphocyte-directed anti-CD20 monoclonal antibody rituximab is beneficial in B-lymphocyte EBV disorders but not in T- or NK-cell LPDs.[18] Rapid recognition and intervention with correct multiagent chemo-

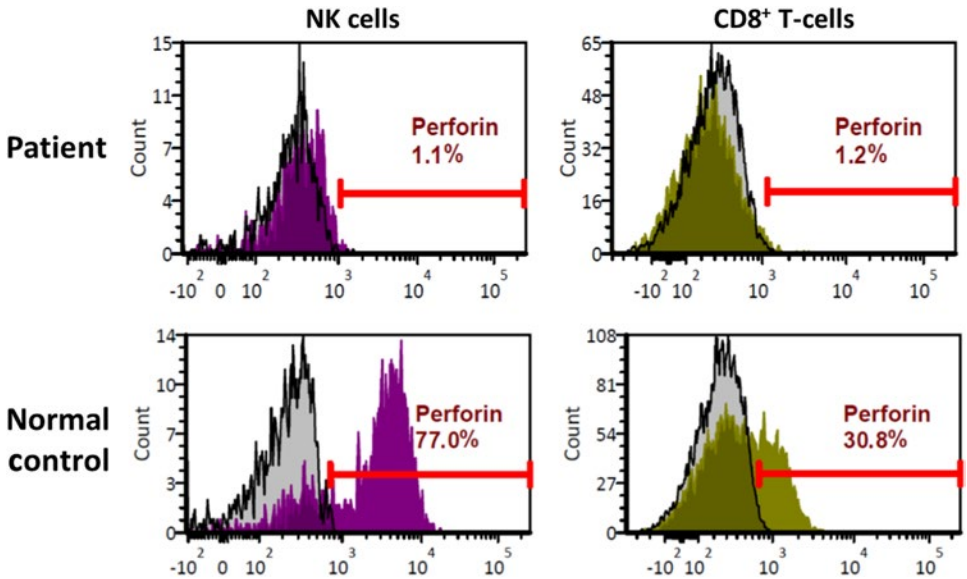

Figure 2. Perforin deficiency detected by flow cytometry. Histogram plots depicting flow cytometry analysis of peripheral blood lymphocytes from the patient described in case 1. Upper panels depict patient cells, while the lower panels depict normal controls. Results for natural killer (NK) cells are shown on the left, while those for CD8+ T lymphocytes are shown on the right. Isotype controls are represented by the gray histograms, and perforin antibody detection is represented by the histograms in magenta (NK) or green (CD8+).

therapy are crucial in fulminant NK/T-cell lymphomas. It is thus imperative to determine the specific condition in each patient.

CASE 3

A previously healthy 23-year-old developed persistent fever and fatigue. Investigations for infections were negative. Fevers persisted and laboratory studies revealed cytopenia and elevated liver enzymes. Bone marrow biopsy showed a nonspecific small population (5%) of atypical lymphocytes but was otherwise normal. Imaging studies demonstrated hepatosplenomegaly and lymphadenopathy. Lymph node needle-biopsy showed no malignancy or infection. With elevated sCD25 and ferritin (Table 1), he was diagnosed with HLH and treated with dexamethasone and intravenous immunoglobulin (IVIG). Symptoms promptly resolved but as dexamethasone was tapered, fever and fatigue recurred. He was then treated with dexamethasone and etoposide per HLH-94, with resolution of symptoms. Genetic studies for HLH revealed no variants. As treatment was weaned again, fever, fatigue, and cytopenia recurred, and he was referred for bone marrow transplant. His family requested a second opinion. In a search for illnesses that can mimic HLH, we performed a positron emission tomography scan showing innumerable fluorodeoxyglucose-avid osseous lesions and lymph nodes. An excisional lymph node biopsy revealed classic Hodgkin lymphoma. Chemotherapy for Hodgkin lymphoma was initiated with prompt resolution of all clinical signs of disease.

CASE 4

A 4-year-old boy was admitted to the hospital with fever, vomiting, bloody diarrhea, and dehydration. While hospitalized, he developed hepatosplenomegaly, pancytopenia, elevated ferritin, and triglycerides (Table 1). Bone marrow aspiration demonstrated hemophagocytosis with increased histiocytes. Having fulfilled criteria for HLH, therapy was initiated with dexamethasone and anakinra. The fever resolved, but signs of liver failure appeared with hyperbilirubinemia and coagulopathy. Additional therapy with etoposide was planned, but his family requested a second opinion. Given the bloody diarrhea and vomiting, we suspected an infection, and investigations revealed significant adenoviremia with a viral load of >200×10^6 copies/mL. Adenovirus-specific T-cell therapy was given, but his condition continued to worsen with rapid development of multiorgan failure; he died shortly thereafter. Postmortem examination determined the cause of death as disseminated adenovirus infection resulting in multiorgan failure. No genetic mutations were identified.

Cases 3 and 4 highlight the nonspecific nature of the HLH-2004 criteria, which resulted in unfortunate delays in correct diagnoses and potentially contributed to the death of the fourth patient. These occurrences are common and now account for the majority of referrals to specialized centers for patients with challenging or treatment-refractory HLH (Figure 3).[11] The HLH-2004 criteria were created as the eligibility requirement for the clinical trial HLH-2004, and their specificity for primary HLH has never been shown. All the features listed in the criteria can be seen in many systemic inflammatory conditions, such as disseminated infections or malignancies like lymphoma. The specificity of these criteria for primary HLH is likely low.[10] Contrary to popular belief and despite the eponym, hemophagocytosis is neither specific nor sensitive for primary HLH. Additionally, the NK-cytotoxicity assay also has poor specificity.[13] One of the goals of the HLH-2004 guidelines was to utilize tests readily available at most centers.[7] While these criteria and the subsequent HScore have certainly helped move HLH toward the forefront of clinicians' minds, our experience shows that in the diagnosis of primary HLH, these tools are strikingly nonspecific.[10,11] The problem is not simply one of semantics or nomenclature. The prevailing dogma is that as soon as the diagnosis of HLH is reached, based on these nonspecific tools, it signals an urgent need for therapy with potent immunosuppresive agents such as high-dose corticosteroids, closely followed by etoposide. Such algorithmic decision-making often leads to inappropriate application of primary HLH–directed therapies in conditions in which such treatments may cause harm. Furthermore, the HLH-2004 trial was restricted to children up to the age of 18.[8] There are no data on the outcome of applying such criteria to adults.[19] The term *secondary HLH* is not a specific diagnosis but rather an amorphous, catchall description that can be applied to a variety of diseases. Since malignancies like lymphoma (as well as many of the other potential triggers of secondary HLH) are more common in adults compared to children, we suspect that majority of adults evaluated for primary HLH are likely manifesting other acute inflammatory diseases. Additional confusion arises when patients known to have an infection or lymphoma are also diagnosed with HLH on the basis of fulfillment of the HLH-2004 criteria or the HScore. Clinicians then face the conundrum of which condition to treat first—HLH or the trigger disease. In the vast majority of such situations, the label of HLH is attached simply because nonspecific criteria have been fulfilled, and HLH-directed therapy with steroids and etoposide is not needed. In the case of infections, such therapy is actually contraindicated. Rather, treatment should be

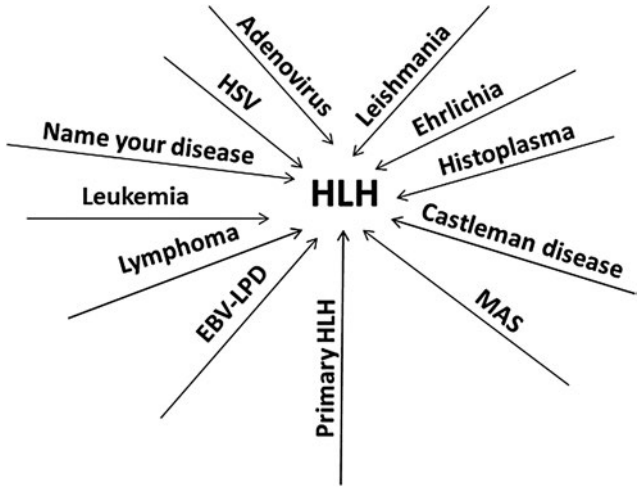

Figure 3. Many diseases can manifest clinical features of HLH. Depicted are the diseases we have seen as mistaken for and treated as primary HLH, akin to the many proverbial roads leading to Rome. HSV, herpes simplex virus.

directed at the primary pathology. In certain malignancies, liver dysfunction precludes chemotherapy. Many of these illnesses also fulfill the HLH criteria, and these patients are then often treated with steroids, sometimes with etoposide, resulting in improved liver function. While such a sequence could be interpreted as malignancy-associated HLH with response to HLH-therapy, steroids and etoposide are also efficacious in treating the vast majority of hematologic malignancies, and abatement of the cancer would then result in resolution of HLH.[11] A recent study highlighted a subset of patients with hematologic malignancies associated with higher levels of ferritin and sCD25 and higher risk of mortality.[20] It remains unclear if inflammation is directly implicated in the worse outcomes, or if these are biomarkers of treatment-refractory malignancies.

Discussion: our diagnostic approach

Many patients with a wide variety of proinflammatory illnesses may fulfill the HLH-2004 criteria or the HScore.[10] The fulfillment of these criteria is not sufficient to diagnose HLH nor to initiate HLH-directed therapies.[11] Awareness of the nonspecific nature of these tools and of the base rates of primary HLH (rare), infections, and lymphomas (common) are key components of accurate diagnosis. While testing for primary HLH should be pursued in the appropriate context, all cases of suspected HLH must also be examined for potential alternative diagnoses, including malignancies, infections, and rheumatologic disease. Broad HLH-directed testing includes assessment of ferritin, fibrinogen, sCD25, CXCL9, and IL-18, but clinicians must recognize that none of these tests is particularly specific for HLH.[21] However, normal ferritin, normal sCD25, normal CXCL9, or elevated fibrinogen make HLH less likely. Evaluation for primary HLH includes flow for perforin, CD107a, SAP, and XIAP (the last two for males) and a comprehensive gene panel for IEIs or WES.[12,22] While results are being awaited, ongoing assessment for other potential causes of hyperinflammation are prudent, such as bone marrow examination for leukemia positron emission tomography and computed tomography (and subsequent tissue biopsy if indicated) for lymphoma, and cultures and PCR/antigen assays for infections. Withholding steroids and immune suppression until biopsies have been obtained and infections ruled out should be strongly considered. Empiric treatment with antimicrobials should be pursued until infectious etiologies are sufficiently excluded. Certainly, if malignancy, infection, or other specific etiology is identified, the corresponding treatment should promptly be instituted.[11] The finding of substantially high IL-18 may suggest MAS or an inflammasome disorder, and appropriate rheumatologic diagnostics may then be indicated.[23] Abnormal CD107a, perforin, SAP, or XIAP expression makes familial HLH likely.[12,22] These findings should be confirmed via genetic testing, and treatment should be considered with HLH-directed therapy (such as HLH-94 and anticytokine therapy) as warranted based on the clinical condition. If all these tests fail to yield a specific diagnosis, patients should be thoroughly reevaluated for occult malignancy, infection, or rheumatologic process. At the same time, whole exome sequencing may be considered. If a patient is clinically deteriorating while the workup is in progress and diagnosis remains uncertain, temporizing therapies including glucocorticoids, anticytokine agents, and/or IVIG may be considered.[24-27] It is critical to recognize that most patients demonstrating HLH physiology will eventually be found to have an occult inciting condition (be it malignant, infectious, or rheumatologic) upon further evaluation. It should also be noted that many such cases may have multiple factors contributing to excessive inflammation and that even presentations of primary HLH may have an acquired trigger; therefore, even when primary HLH is identified, a search for triggering conditions should be undertaken. Although the past 2 decades have seen a tremendous increase in the awareness of HLH, they have also seen confusion regarding its accurate diagnosis and appropriate treatment. Although current screening tools, including the HLH-2004 criteria and H-Score, are diagnostically sensitive, they are also highly nonspecific, and these criteria may be fulfilled by many other severe illnesses. Evaluating suspected cases of HLH requires thoroughly investigating for other more common causes of excessive inflammation (including malignancy or infection) and using more specific testing for primary HLH (including flow cytometric and genetic testing). Such an approach may avoid missed diagnoses and inappropriate treatment of potential HLH mimics.

Disclosures

Ashish Kumar is a consultant for SOBI, Springworks therapeutics, and OPNA.
Eily Cournoyer: no competing financial interests to declare.
Leonard Naymagon: no competing financial interests to declare.

Off-label drug use

Ashish Kumar: There is nothing to disclose.
Eily Cournoyer: There is nothing to disclose.
Leonard Naymagon: There is nothing to disclose.

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Too many white cells—TAM, JMML, or something else?

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Leukocytosis is a common finding in pediatric patients, and the differential diagnosis can be broad, including benign reactive leukocytosis and malignant myeloproliferative disorders. Transient abnormal myelopoiesis is a myeloproliferative disorder that occurs in young infants with constitutional trisomy 21 and somatic *GATA1* mutations. Most patients are observed, but outcomes span the spectrum from spontaneous resolution to life-threatening complications. Juvenile myelomonocytic leukemia is a highly aggressive myeloproliferative disorder associated with altered RAS-pathway signaling that occurs in infants and young children. Treatment typically involves hematopoietic stem cell transplantation, but certain patients can be observed. Early recognition of these and other myeloproliferative disorders is important and requires a clinician to be aware of these diagnoses and have a clear understanding of their presentations. This paper discusses the presentation and evaluation of leukocytosis when myeloproliferative disorders are part of the differential and reviews different concepts regarding treatment strategies.

LEARNING OBJECTIVES

- To describe the differential diagnosis of leukocytosis in a young child
- To recognize the signs and symptoms consistent with an underlying myeloproliferative disorder
- To discuss treatment options for TAM and JMML

Introduction

Leukocytosis is a common condition for which pediatric hematology/oncology providers are consulted. Leukocytosis is defined as an elevated white blood cell (WBC) count for age (Table 1). The term *leukemoid reaction* describes a WBC count >50×10^3/μL with neutrophils and immature myeloid forms typically occurring in the setting of an (often pyogenic) infection, while hyperleukocytosis typically refers to a WBC count >100×10^3/μL.[1] Leukocytosis may be caused by an elevation in any WBC subtype, and the differential diagnosis for leukocytosis can often be narrowed based on which cell type is elevated (Figure 1).[1,2] Leukocytosis can be a primary disease process (such as in malignant or myeloproliferative disorders) or occur secondary to an identifiable underlying etiology (eg, infection).

The workup for pediatric patients with leukocytosis depends on their presentation. All patients require a thorough history and physical examination. A complete blood count (CBC) with differential and peripheral blood smear are important to evaluate the WBC differential and morphology as well as other hematologic parameters (including hemoglobin and platelet count). Additional workup may be required depending on the results of these evaluations and clinical suspicion for a primary versus a secondary disease process.

CASE 1

You are consulted by the neonatal intensive care unit (NICU) regarding a 5-day-old full-term infant with trisomy 21 (T21) found to have leukocytosis on a CBC sent during an infectious workup. The patient's clinical history is notable for a prenatal diagnosis of T21; the postnatal course has been notable for respiratory distress transiently requiring positive pressure and a low-grade fever prompting the recent infectious workup. Physical examination is notable for stigmata of T21 and hepatomegaly. Review of the patient's laboratory results reveals leukocytosis with WBC 60.8×10^3/μL

Table 1. Normal white blood cell ranges by age

Age	Total white blood cells (×10³/μL) Mean (± 2SD)
1–3 days	18.9 (9.4–34)
2 weeks	11.4 (5–20)
1 month	10.8 (4–19.5)
6 months-2 years	10.6 (6–17)
2–6 years	8.5 (5–15.5)
6–12 years	8.1 (4.5–13.5)
12–18 years	7.8 (4.5–13.5)

Adapted from The Harriet Lane Handbook, 23rd Edition.

and 40% blasts, with normal hemoglobin and platelet counts. The patient has been started on broad-spectrum antibiotics and pediatric hematology/oncology providers are consulted for evaluation of leukocytosis.

Approaching this patient

This patient is a neonate presenting with fever and leukocytosis. Infection—particularly serious bacterial infection—is an important consideration in any infant presenting with fever, and infants may have a left-shift with immature forms on peripheral smear in this setting. However, in this infant, the increased peripheral blast count is suggestive of a more serious underlying etiology. Key to this case is the patient's history of T21 (Down syndrome; DS): since these patients have an increased risk of developing myeloproliferative disorders and acute leukemia at young ages, these disorders should be high on the differential diagnosis of leukocytosis in an infant with T21.

Abnormal myelopoiesis in trisomy 21

Patients with T21 are at significantly increased risk for leukemia: while the overall risk of acute leukemia in patients with T21 is 18-fold higher than in the general population, the risk of acute myeloid leukemia in T21 patients aged <5 years is almost 400 times greater.[3,4] Abnormal myelopoiesis in patients with T21 is driven by mutations in the hematopoietic transcription factor *GATA1* and comprises 2 conditions: transient abnormal myelopoiesis (TAM) and myeloid leukemia of Down syndrome (ML-DS), both of which are classified by the World Health Organization (WHO) as "myeloid proliferations associated with Down syndrome."[5] Although these conditions share a genetic driver, the clinical features and management differ, and both must be considered in a patient with T21 and leukocytosis.

Transient abnormal myelopoiesis

Transient abnormal myelopoiesis (previously transient myeloproliferative disorder) is a clonal hematopoietic disorder unique to patients with T21 (Table 2). TAM is characterized by an increase in circulating blast cells harboring somatic mutations in *GATA1*.[6,7] Without appropriate *GATA1* signaling, normal hematopoietic development is disrupted, leading to the phenotype seen in TAM.[8] TAM has been estimated to occur in 10%-30% of infants with T21.[9] Infants with *GATA1* mutations who have blast percentages <10% and lack the clinical features of classic TAM have been designated as having *silent TAM*.[8]

Patients with TAM typically present within the first week of life, but may be diagnosed up to 3 months of age.[10] While ~25% of patients with TAM are clinically asymptomatic at presentation, patients more commonly present with a constellation of symptoms caused by organ infiltration of malignant cells, including hepatomegaly, pleural/pericardial effusions, and skin rash.[10,11] Laboratory findings characteristically include leukocytosis and an increased peripheral blast count exceeding that

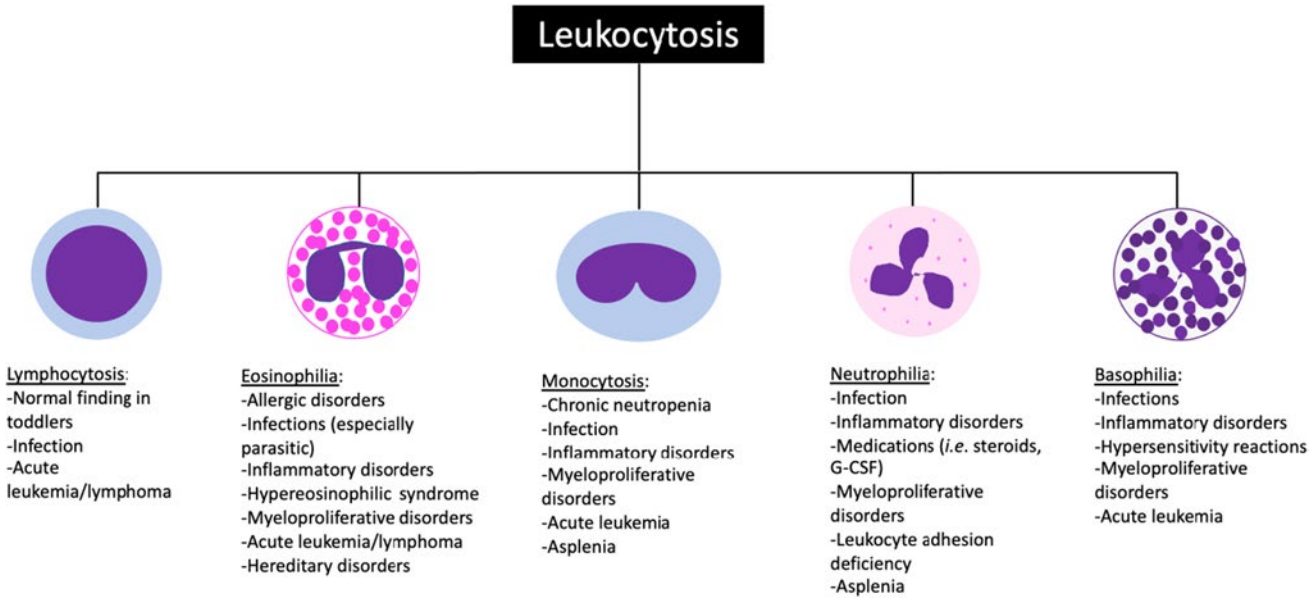

Figure 1. Common causes of leukocytosis in pediatric patients. The differential diagnosis can often be narrowed down based on which white blood cell subtype is elevated. G-CSF, granulocyte colony stimulating factor.

Table 2. Key features of TAM and JMML

	TAM	JMML
Clinical features	Diagnosis of trisomy 21	Male to female ratio 2:1
	Age ≤3 months	Most common in early childhood
	Jaundice and/or HSM are common	Splenomegaly in almost all patients
	Rash and effusions are less frequent	Lymphadenopathy and fever are common
Laboratory findings	Leukocytosis	Leukocytosis
	Circulating blasts (higher in blood than BM)	Monocytosis (≥1×10^9/L)
		Blast count <20% in blood and BM
	Anemia is rare	Thrombocytopenia is common
Genetic features	*GATA1* mutation	Germline mutations/LOH: *CBL*, *NF1*
		Or
		Somatic mutations: *PTPN11, KRAS, NRAS, RRAS*

BM, bone marrow; HSM, hepatosplenomegaly; JMML, juvenile myelomonocytic leukemia; LOH, loss of heterozygosity; TAM, transient abnormal myelopoiesis.

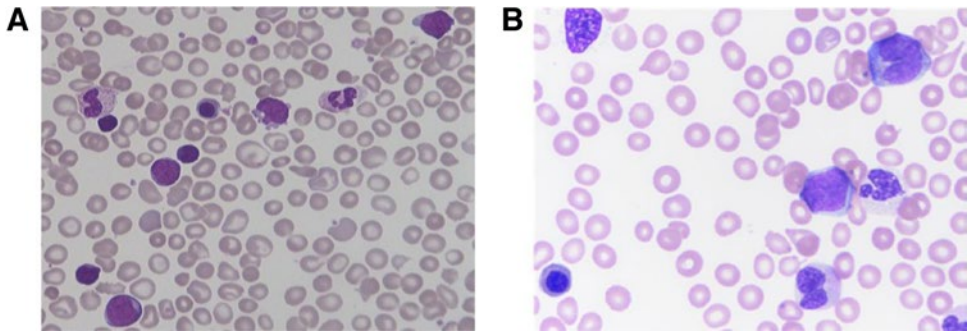

Figure 2. Peripheral blood findings in TAM and JMML. Peripheral blood smears of (A) TAM showing pleiomorphic blasts and additional immature myeloid cells, and (B) JMML showing leukoerythroblastosis with circulating myelomonocytic cells. Both smears show dysmorphic and nucleated red blood cells.

of the marrow; hemoglobin is typically normal, and platelet count is usually normal or slightly reduced.[10,11] Patients may also demonstrate abnormal liver function and/or coagulation testing due to hepatic infiltration. Peripheral smear shows pleiomorphic circulating blast cells with a unique megakaryocytic immunophenotype (Figure 2A).[12] Bone marrow evaluation is not required to diagnose TAM but is often done in the setting of peripheral blasts to rule out leukemia.

Most patients with TAM experience spontaneous resolution of symptoms without intervention. In a prospective study by the Children's Oncology Group, 79% of patients experienced resolution of peripheral blasts in a median of 36 days and of all TAM symptoms in a median of 49 days without treatment.[10] A smaller percentage of patients with TAM require intervention, typically with low-dose cytarabine. However, there is no consensus on which patients should receive treatment: some have reported improved survival by treating patients with poor prognostic features including leukocytosis or liver dysfunction, while others reserve treatment for patients with life-threatening symptoms.[10,13,14] Following symptom resolution, patients with TAM require close monitoring, as 16%-23% will develop ML-DS.[10,11,13] Treatment with cytarabine has not been shown to prevent progression from TAM to ML-DS, although data suggest that systemic steroids may be beneficial.[15,16]

Myeloid leukemia of Down syndrome

ML-DS occurs in patients with a history of TAM or silent TAM when the aberrant *GATA1* clone acquires additional oncogenic mutations.[17,18] This leads to a megakaryoblastic leukemia that is distinct from the one occurring in non-DS patients.[12,19] ML-DS onset is typically at 1-2 years of age (almost always before 4 years), and patients typically present with progressive pancytopenia.[20] Patients with ML-DS are treated with cytotoxic chemotherapies at reduced doses; this is because the leukemic blasts of ML-DS patients show increased chemotherapy sensitivity and because patients with T21 suffer increased toxic morbidity from standard-dose chemotherapies.[21,22] Treatment regimens for ML-DS typically include anthracyclines and cytarabine, though there is

disagreement about dosing intensity: a recent Children's Oncology Group study suggests that high-dose cytarabine is necessary, while Japanese studies have shown similar outcomes using lower-dose therapy.[22,23] The prognosis for patients with ML-DS is highly favorable with survival rates >85%, although patients with relapsed or refractory disease have poor outcomes.[21-24]

Infantile myeloproliferative disease

It has recently been recognized that a rare subset of neonates without Down syndrome can present with a transient myeloproliferative disorder, termed *infantile myeloproliferative disease* (IMD).[25] Though the number of reported IMD cases is small, recurrent mutations have been identified, including somatic T21 and *GATA1*. While IMD can often be managed with a "watch and wait" approach, it is important to differentiate these cases from more aggressive leukemias, as has been described in recent consensus guidelines.[25]

Differential considerations

Patients with T21 presenting with leukocytosis should always be evaluated for infection, as these patients often have immune dysregulation.[26] Patients with T21 are also at higher risk for acute lymphoblastic leukemia (DS-ALL) than the general pediatric population, although this is quite rare in young infants.[8] DS-ALL demonstrates unique biologic features compared with non-DS-ALL, and patients with DS-ALL experience worse outcomes—driven both by higher treatment-related mortality and higher rates of relapse—than non-DS-ALL patients.[27,28] Additional conditions that may cause nonspecific findings similar to those seen in TAM, such as hepatomegaly, should also be ruled out.

CASE 1 (Outcome)

The patient was given a presumptive diagnosis of TAM. Given the patient's good clinical status and lack of life-threatening symptoms, the decision was made for close monitoring without intervention. The patient was discharged from the NICU once the infectious workup was negative and was followed closely by pediatric hematologists/oncologists. Serial CBCs revealed resolution of peripheral blasts and normalization of WBC count within 4 weeks. The patient is now 2 years old and continues to be closely monitored due to the ongoing risk of progression to ML-DS, though all recent CBCs have been normal.

CASE 2

An 8-week-old boy with a history of a 1-week NICU admission for respiratory distress is admitted to the pediatric inpatient unit for evaluation and management of a left neck abscess. The patient was initially noted to have dysmorphic features during his NICU course, including low-set ears, down-slanting palpebral fissures, cryptorchidism, and an abnormal hearing test; genetic testing for Noonan syndrome is pending. A CBC reveals WBC 41.3×10^3/μL, hemoglobin 10 g/dL, and platelet count 65×10^3/μL; the differential blood count is notable for neutrophilia (absolute neutrophil count 19.4×10^3/μL) and monocytosis (absolute monocyte count 11.2×10^3/μL). A review of prior CBCs reveals persistent leukocytosis with monocytosis and thrombocytopenia since birth. Pediatric hematologist/oncologist are consulted for evaluation for leukocytosis.

Approaching this patient

When thinking about potential causes for this child's leukocytosis, it is reasonable to attribute a left-shifted, increased WBC count to an infection. However, this infant has had leukocytosis since birth, which is concerning for an underlying disorder. In addition, while thrombocytopenia could be due to infection, it could also be due to an underlying marrow disorder. A critical component of this child's history and exam is the finding of dysmorphic features concerning for Noonan syndrome and the presence of abnormal blood counts since birth. The suspicion of a myeloproliferative disorder should be high in a child with an underlying since many genetic conditions are associated with an increased risk of developing myeloid malignancies.

Juvenile myelomonocytic leukemia

Juvenile myelomonocytic leukemia (JMML) is a clonal hematopoietic disorder characterized by excessive proliferation of granulocytic and monocytic lineage cells.[29] It was previously classified by the WHO as a myeloproliferative/myelodysplastic overlap syndrome but is now recognized as a distinct myeloproliferative condition of childhood in both the recently-updated WHO diagnostic criteria and the newly described International Consensus Classification system.[5,30] Overactive RAS-pathway signaling drives JMML, and the majority of patients (~90%) are found to have alterations in a RAS-pathway gene, including *PTPN11*, *NRAS*, *KRAS*, *CBL*, and *NF1*.[30]

JMML predominantly affects infants and young children, with a median age of 1.8 years at diagnosis.[31] The incidence of JMML is estimated between 0.69 and 1.3 per million children per year, approximately 2%-3% of pediatric hematologic malignancies,[32,33] and is twice as common in males.[31] Clinical features of JMML are caused by organ infiltration by malignant monocytic cells. Presenting symptoms commonly include pallor, fever, infection, bleeding, cough, and malaise, while physical findings include hepatosplenomegaly (in most patients), lymphadenopathy, skin rash, café au lait spots, and xanthomas.[31] Peripheral smear demonstrates profound monocytosis, often with dysplasia, circulating myeloid precursors, and erythroblasts (Figure 2B).[33] Bone marrow findings often include hypercellularity with a predominance of myeloid lineage cells at all stages of maturation, dysplasia which can be seen across all hematopoietic lineages, and occasionally reticulin fibrosis.[33-35]

The diagnostic criteria for JMML are detailed by the WHO and the International Consensus Classification system and require both clinical and genetic findings.[5,30] Clinical and lab features include splenomegaly, monocytosis, and peripheral and bone marrow blast counts below 20% (Table 2). Somatic alterations consistent with JMML include mutations in *PTPN11*, *KRAS*, *NRAS*, or *RRAS*. Germline heterozygous mutations in *NF1* (or a neurofibromatosis diagnosis) or *CBL* can lead to loss of heterozygosity in the bone marrow in JMML, most commonly through a mechanism of uniparental disomy. It is critical to distinguish between germline and somatic mutations, particularly in the setting of *PTPN11*, *NRAS*, or *KRAS* mutations, which cause Noonan syndrome when present in the germline but can be consistent with

JMML when present as somatic alterations. Skin or marrow fibroblasts are ideal tissue sources for germline testing; buccal swabs are a reasonable alternative but may have tumor contamination due to monocyte infiltration.[36]

JMML-like neoplasms

There are defined JMML-like conditions that do not meet JMML diagnostic criteria. Noonan syndrome-associated myeloproliferative disorder (NAMD) is a distinct entity from JMML occurring in infants with Noonan syndrome and germline mutations in *PTPN11*, *NRAS*, *KRAS*, or *RIT1*.[30] Clinically, NAMD may be milder, or may look just like JMML, including a blast percentage below 20% and hepatosplenomegaly. A subset of patients with a clinical presentation consistent with JMML but who lack a canonical RAS-pathway mutation will be found to harbor rearrangements in *ALK*, *ROS1*, or *FLT3*, and typically have monosomy 7 in addition to their fusion.[37]

Management of JMML and related disorders

The management of patients with JMML and JMML-like neoplasms varies based on clinical presentation, prognostic factors, and genetic drivers.[35] Many children with JMML require hematopoietic stem cell transplant (HSCT), including those with *PTPN11* and *NF1* mutations or those with *KRAS* and *NRAS* mutations who have high-risk features including high DNA methylation.[38] These patients are typically treated with 5-azacytidine with or without chemotherapy prior to HSCT. Patients with non-RAS-pathway mutations may receive targeted therapy (ie, ALK or FLT3 inhibitors) with or without chemotherapy followed by HSCT.[37] Lower-risk patients who can be closely observed without intervention if they are clinically stable include patients with germline *CBL* mutations, patients with *KRAS*- and *NRAS*-mutant JMML who have low DNA methylation, and children with NAMD. For lower-risk patients who have visceral organ involvement leading to failure to thrive, respiratory distress, or diarrhea, treatment with oral 6-mercaptopurine or low-dose cytarabine as a temporizing measure is often indicated. Some JMML patients may be able to be treated with 5-azacytidine alone and avoid HSCT, but it is currently challenging to identify these patients at diagnosis.[35]

Differential considerations

Additional disorders that can mimic JMML or a JMML-like illness include certain viral or atypical infections (such as cytomegalovirus, human herpesvirus 6, mycobacteria, or toxoplasma).[39] Of note, JMML often presents concomitantly with viral infections. Other rare etiologies include leukocyte adhesion deficiency and infantile malignant osteopetrosis.[40] In the setting of peripheral blasts, it is important to make sure acute myeloid leukemia and chronic myeloid leukemia are ruled out. Some patients with *KMT2A* or *NUP98* rearrangements or t(8;16)(p11;p13) may have features resembling JMML; due to clinical and biological differences, these disorders are classified as acute myeloid leukemia even when blasts are less than 20%.[5,35,41,42]

CASE 2 (Outcome)

The patient was treated with antibiotics resulting in resolution of the abscess and leukocytosis, but monocytosis persisted. Genetic testing revealed a germline heterozygous mutation in *CBL* and copy neutral loss of heterozygosity in the blood, thereby identifying a case of *CBL*-related JMML. Caused by germline mutations in the *CBL* gene, CBL syndrome has been identified as an underlying genetic predisposition to JMML.[43] The clinical features of patients with CBL syndrome closely mirror those seen in classic Noonan syndrome, leading to the designation as a Noonan syndrome-like disorder.[44] While CBL syndrome is caused by a heterozygous *CBL* mutation, the leukemia cells in patients with CBL syndrome harbor biallelic *CBL* mutations due to acquired uniparental disomy.[45] JMML in patients with CBL syndrome usually follows a benign course, and patients may experience spontaneous disease regression; however, this must be differentiated from patients with somatic *CBL* mutations, who tend to present with more aggressive disease. Given that this patient was found to have a germline *CBL* mutation, he was monitored closely without intervention and was noted to have spontaneous resolution of all symptoms by 3 years of age.

Conclusion

While leukocytosis in young children may be due to various conditions, it is important to remember the unique myeloproliferative disorders of childhood. Clinicians must have a high index of suspicion for these disorders, as they can be aggressive and life-threatening. In infants and young children with myeloid-predominant leukocytosis, thorough evaluation to ensure timely identification of myeloproliferative disorders of childhood is essential for proper management and follow-up.

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Conflict-of-interest disclosure

Alexandra Satty: no competing financial interests to declare.
Elliot Stieglitz: no competing financial interests to declare.
Nicole Kucine: no competing financial interests to declare.

Off-label drug use

Alexandra Satty: discussion of off-label use of drugs is included.
Elliot Stieglitz: discussion of off-label use of drugs is included.
Nicole Kucine: discussion of off-label use of drugs is included.

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Where have all the platelets gone? HIT, DIC, or something else?

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Thrombocytopenia in ill children is common; accurately diagnosing the underlying etiology is challenging and essential for appropriate management. Triggers for accelerated consumption of platelets are numerous; common downstream mechanisms of clearance include platelet trapping in microvascular thrombi, phagocytosis, and platelet activation. Thrombocytopenia with microangiopathic hemolytic anemia (MAHA) is frequently due to disseminated intravascular coagulation. Thrombotic microangiopathy (TMA) is a subgroup of MAHA. Specific TMA syndromes include thrombotic thrombocytopenic purpura, complement-mediated TMA (CM-TMA), and Shiga toxin–mediated hemolytic uremic syndrome. Isolated thrombocytopenia is characteristic of immune thrombocytopenia; however, concomitant cytopenias are frequent in critically ill patients, making the diagnosis difficult. Immune thrombocytopenia with large vessel thrombosis is a feature of heparin-induced thrombocytopenia and antiphospholipid antibody syndrome. In addition, thrombocytopenia is common with macrophage activation, which is characteristic of hemophagocytic lymphohistiocytosis. While thrombocytopenia in ill patients can be driven by hypoproliferative processes such as myelosuppression and/or bone marrow failure, this review will focus on consumptive thrombocytopenia due to immune and nonimmune causes.

LEARNING OBJECTIVES

- To understand the mechanisms that contribute to consumptive thrombocytopenia in ill pediatric patients
- To know how to distinguish between different etiologies for consumptive thrombocytopenia

Approach to consumptive thrombocytopenia

Thrombocytopenia in ill children is often multifactorial, and the pathophysiology may be overlapping. Identification of specific etiologies for consumptive thrombocytopenias can be challenging. Consumptive thrombocytopenia may be immune mediated, either due antibodies (immune thrombocytopenia [ITP]) or secondary to T-cell activation and/or phagocytosis (eg, hemophagocytic lymphohistiocytosis [HLH]). Microangiopathic hemolytic anemia (MAHA) is a common nonimmune mechanism of thrombocytopenia that can arise from overlapping syndromes of disseminated intravascular coagulation (DIC) and thrombotic microangiopathies (TMAs). Specific characteristics of these disorders and can help distinguish between diagnoses:

- Schistocytes—MAHA, TMA
- Renal dysfunction/hypertension/proteinuria—complement mediated TMA (CM-TMA), hemolytic uremic syndrome (HUS)
- Severe coagulopathy—DIC
- Young age—inherited genetic mutations ADAM metallopeptidase with thrombospondin type 1 motif 13 [ADAMTS13]—thrombotic thrombocytopenic purpura [TTP]; complement regulatory factors—TMA; effector T cell function or perforin trafficking—HLH)
- Significant hyperferritinemia—HLH
- Bloody diarrhea—Shiga toxin–mediated HUS (ST-HUS)
- Severe and isolated thrombocytopenia—ITP
- Thrombosis— heparin-induced thrombocytopenia (HIT; with heparin exposure), antiphospholipid antibody syndrome (APLS)

When evaluating an ill child with thrombocytopenia, applying diagnostic tools and systematically assessing diagnostic criteria can aid the identification of specific syndromes. Table 1 highlights shared and distinct clinical and laboratory features of these syndromes.

CLINICAL CASE #1

A 15-year-old male presented a left ilio-femoral deep vein thrombus (DVT) (Figure 1A). Therapeutic anticoagulation with unfractionated heparin (UFH) was initiated, and he

Table 1. Comparison of syndromes with microangiopathic hemolytic anemia (MAHA) with thrombocytopenia

Features	DIC	Thrombotic microangiopathies (TMAs)			HLH
		TTP	ST-HUS	CM-TMA	
Etiology	Tissue factor–mediated thrombin activation	ADAMTS13 deficiency	Shiga toxin–induced endothelial injury	Complement activation; deficiency of complement inhibitors	CD8 T-cell activation
Pathology	Systemic microvascular thrombosis	Systemic microvascular thrombosis	Renal microvascular thrombosis	Renal microvascular thrombosis	INF-γ-mediated macrophage activation
Acquired causes	Infection, malignancy, trauma, vascular tumors, circuits	Autoantibodies to ADAMTS13; autoimmune disease	Shiga toxin from *E. coli*	Autoantibodies to complement proteins, HSCT, drug, pregnancy	Malignancy, autoimmune disease, rheumatologic disease
Genetic variants	Neonatal purpura fulminans: *PROC*	*ADAMTS13*	None	Complement regulatory proteins: *CFH, CD46, CFI, C3, CFB, THBD*	Perforin trafficking and effector T-cell function: *PRF1, UNC13D, STX11, STXBP2, Rab27A, SH2D1A, BIRC4, ITK*
Renal involvement **Other organ involvement**	Variable Multiorgan dysfunction	Infrequent Brain	Frequent; AKI, proteinuria, HTN Brain	Frequent; AKI, proteinuria, HTN Lung, gastrointestinal system, brain, serositis	Variable Multiorgan dysfunction, hepatosplenomegaly,
Laboratory screening	ADAMTS13 nl, low; sC5b-9 nl; **D dimer very high;** ferritin high	**ADAMTS13, very low;** sC5b-9 nl D-dimer nl, high Ferritin nl, high	ADAMTS13 nl; **sC5b-9 high;** D-dimer nl, high; ferritin nl, high	ADAMTS13 nl; **sC5b-9 high;** D-dimer nl; Ferritin nl, high	ADAMTS13 nl; sC5b-9 nl; D dimer, high; **ferritin, very high; sCD25, very high; CXCL9, very high**
Diagnosis	ISTH DIC score ≥5	ADAMTS13<10%	*E. coli* 0157:H7 in stool	TMA diagnostic criteria[39]	HLH diagnostic criteria[38]
Treatment	Treat primary cause	Plasma exchange; immunosuppression	Supportive; anti-complement considered with neurologic symptoms	Anti-complement therapy	Immunosuppression; Anti-T cell therapy; HSCT

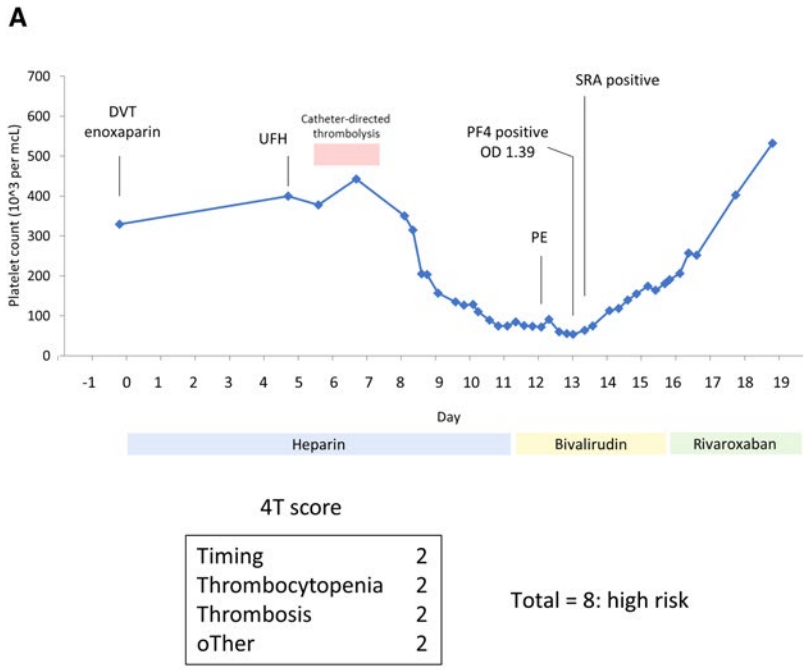
ADAMTS13, ADAM metallopeptidase with thrombospondin type 1 motif 13; CM-HUS, complement-mediated hemolytic uremic syndrome; DIC, disseminated intravascular coagulation; *E. coli*, *Escherichia coli*; HLH, hemophagocytic lymphohistiocytosis; HSCT, hematopoietic stem cell transplant; HTN, hypertension; ISTH, International Society on Thrombosis and Haemostasis; nl, normal; ST-HUS, Shiga toxin–mediated hemolytic uremic syndrome; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura.

underwent catheter-directed thrombolysis. Seven days after initiation of UFH, he developed increased leg swelling and shortness of breath; imaging showed progression of the lower extremity DVT and a pulmonary embolus (PE). Progressive thrombocytopenia was also noted with a platelet count of 358 000 per mm^3 on admission that dropped to a nadir of 54 000 per mm^3. A 4T (Thrombocytopenia, Timing, Thrombosis, and oTher etiologies) score showed a high probability of heparin-induced thrombocytopenia (HIT) (Figure 1B). UFH was discontinued, and he was transitioned to the direct thrombin inhibitor (DTI) bivalirudin. A platelet factor 4 (PF4) immunoassay was strongly positive, and confirmatory serotonin release assay (SRA) was also positive. He continues long-term anticoagulation with rivaroxaban.

Heparin-induced thrombocytopenia

This patient presented with worsening thrombosis and new onset thrombocytopenia during heparin exposure. The development of thrombocytopenia with venous or arterial thrombosis should raise the suspicion of HIT. Heparin-induced thrombocytopenia is a prothrombotic, drug-associated immune thrombocytopenia with significant morbidity and risk of mortality.[1] It is characterized by the development of platelet activating IgG antibodies that target platelet factor 4 (PF4)/heparin complexes. The antibodies typically develop within 5 to 10 days of heparin exposure, which correlate with onset of thrombocytopenia (Figure 2). Paradoxically, HIT is associated with venous and arterial thrombosis; bleeding is uncommon. Heparin use and thrombocytopenia are common in critically ill patients; however, the confirmed diagnosis of HIT is rare. HIT is more common with UFH but can be seen with low molecular weight heparin (LMWH). The rate of HIT in adults[2] varies from 0.1% to 7%; in children,[3] the rate is lower at 0.046%, although the reported incidence from smaller studies varies.[4,5] Clinical evaluation and appropriate laboratory testing are important to prevent overdiagnosis and unnecessary exposure to alternative anticoagulants.[6]

The diagnosis of HIT is made clinically and supported with laboratory testing. The 4T is a validated predictive scoring system that determines the pretest probability of HIT. This score incorporates 4 Ts to characterize the likelihood of HIT (Figure 1B).[7] A low 4T score has a high negative predictive value in both adults and children and can be used to rule out HIT.[3,4,8]



4T score

Timing	2
Thrombocytopenia	2
Thrombosis	2
oTher	2

Total = 8: high risk

B 4T score for pretest probability of HIT

Variable		Points
Thrombocytopenia	50% decrease AND 20-100 K/mcL nadir	2
	30%-50% decrease AND 10-20 K/mcL nadir	1
	<30% decrease OR < 10 K/mcL nadir	0
Timing	Onset 5-10 days OR onset < 1 day (if heparin exposure within 100 days)	2
	Onset >10 days; timing or heparin exposure not clear	1
	Onset ≤ 4 days (without prior exposure)	0
Thrombosis	New thrombosis OR skin necrosis; acute systemic reaction	2
	Progressive or recurrent thrombosis; erythematous skin lesions; suspected thrombosis	1
	None	0
oTher causes for thrombocytopenia	No other cause	2
	Possible other cause	1
	Definitive other cause	0
Score of 0-3 indicates low probability; 4-5, intermediate probability; and 6-8, high probability		

Figure 1. Clinical case 1: heparin induced thrombocytopenia (HIT). (A) Time course for platelet count (blue) with heparin exposure demonstrates characteristic pattern of HIT. The platelet factor 4 (PF4) immunoassay was strongly positive (OD = 1.39; cutoff for positivity OD ≥0.4), and the serotonin release assay (SRA) was positive. Platelet count recovered after discontinuation of heparin and transition to a direct thrombin inhibitor, bivalirudin, and then rivaroxaban. (B) 4T score for pretest probability of HIT. The patient scored a high probability of HIT with a score of 8. DVT, deep vein thrombos; OD, optical density; PE, pulmonary embolism; UFH, unfractionated heparin.

Recognition of characteristic patterns of thrombocytopenia with heparin exposure is key to the diagnosis; the onset of thrombocytopenia accompanies antibody formation (5-10 days), unless there was previous exposure to heparin (rapid-onset HIT) (Figure 2A, B). Examples of other conditions with heparin exposure that can be confused as HIT, such as cardiopulmonary bypass and extracorporeal membrane oxygenation, are illustrated in Figure 2C and D.

Laboratory testing for HIT antibodies is needed to support the diagnosis and include immunoassays and functional assays. ELISA-based immunoassays for PF4/heparin complexes are widely available with rapid turnaround time for results. Only a small fraction of patients that test positive by immunoassays will have a clinical diagnosis of HIT. A positive immunoassay should be followed up with functional testing, such as performing the serotonin release assay (SRA). In adults, a negative PF4 ELISA assay (OD value <0.4) has a high negative predictive value; thus, a negative result is useful to rule out HIT. The utility of the PF4 assay has been evaluated in children, and a low titer similarly has a high negative predictive value for HIT.[3,4]

However, the PF4 immunoassays has a high false positive rate and should only be sent with an intermediate or high 4T score.[2] If the immunoassay returns positive, then the SRA should be performed to confirm the diagnosis. If the 4T score is high, then heparin should be discontinued and patients should be transitioned to a nonheparin anticoagulant, such as intravenous bivalirudin or argatroban, subcutaneous fondaparinux, or a direct oral anticoagulant (DOAC).[2] DOACs have been used exclusively to treat adult patients with HIT. In children, the DOACs rivaroxaban and dabigatran have recently been approved by the US Food and Drug Administration for treatment of thrombosis; however, the experience with their use in HIT is limited. For patients with an intermediate-risk 4T score, the degree of PF4 positivity can be useful to assess the likelihood of HIT; higher OD values are associated with increased risk of HIT.[9,10] Functional SRA testing is used to confirm or rule out HIT in these indeterminate cases; however, the turnaround time for results may be several days, as this testing is typically available only through reference laboratories. Recently, a nonradioactive platelet-activation assay, the PF4-dependent P-selectin expression assay, has shown comparable accuracy to SRA for confirming HIT. This test is technically simple to perform and may facilitate the timely diagnosis of HIT.[11]

Immune thrombocytopenia due to secondary causes in ill children

Most children with ITP are clinically well and do not need hospitalization. However, some will present with other significant symptoms associated with underlying diagnoses or triggers. Heparin-induced thrombocytopenia is an uncommon drug-associated immune thrombocytopenia with significant morbidity and requires recognition, appropriate testing, and specific management. The finding of large vessel venous or arterial *thrombosis with thrombocytopenia* after heparin exposure, as illustrated in case 1, is a hallmark of HIT. The development of thrombosis

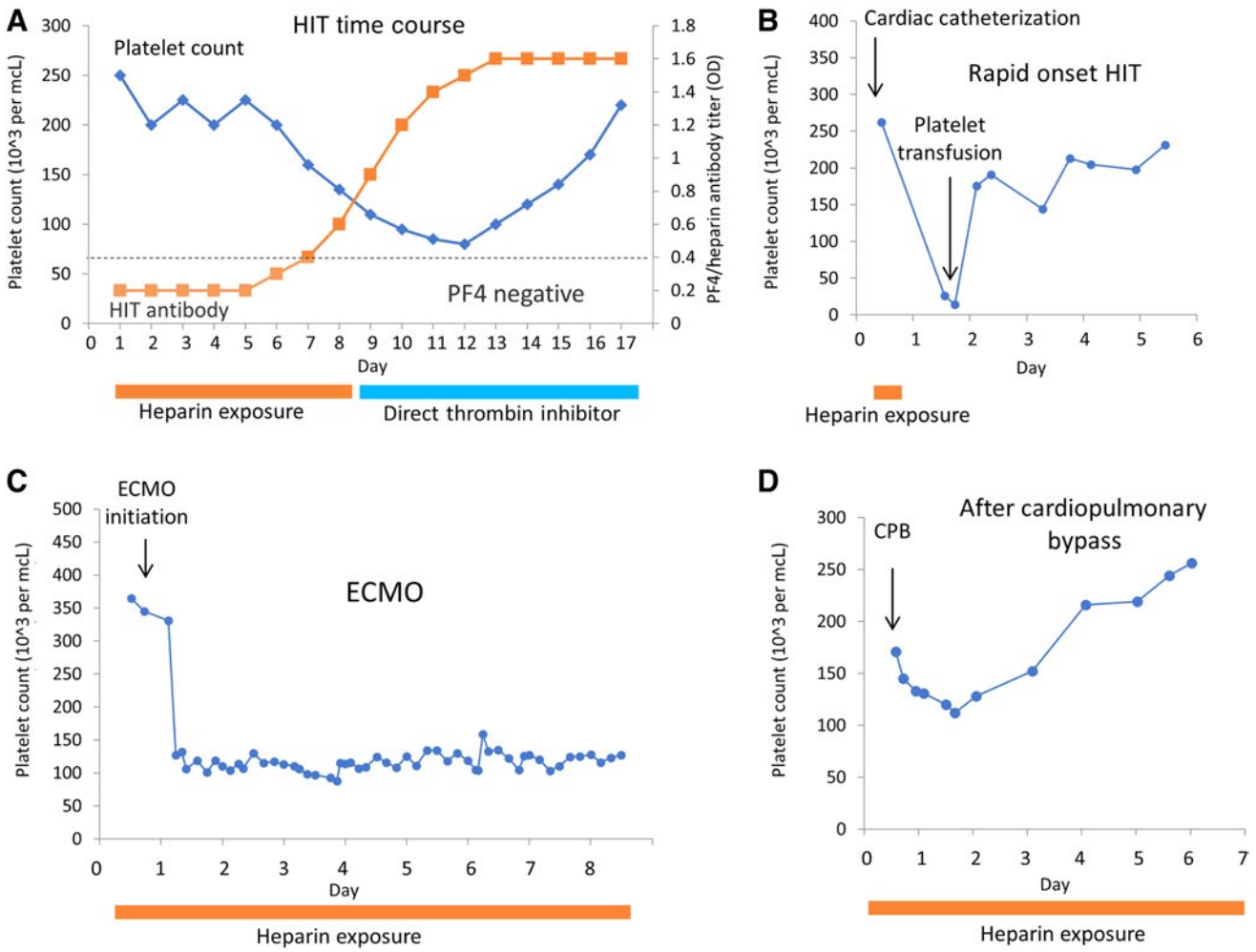


Figure 2. Patterns of thrombocytopenia for HIT and conditions confused for HIT. (A) Time course for drop in platelets (blue) in response to increase in HIT antibody titer (orange squares). Recovery of platelet count with discontinuation of heparin and transition to direct thrombin inhibitor. (B) Rapid onset HIT. Patient was previously treated with heparin and developed rapid onset of thrombocytopenia with heparin exposure. (C) Thrombocytopenia with extracorporeal membrane oxygenation (ECMO)—not HIT. Rapid and sustained thrombocytopenia is seen with initiation of ECMO due to platelet consumption within the circuit. (D) Thrombocytopenia after cardiopulmonary bypass (CPB)—not HIT. Platelet drop within 1–3 days after CPB, followed by recovery.

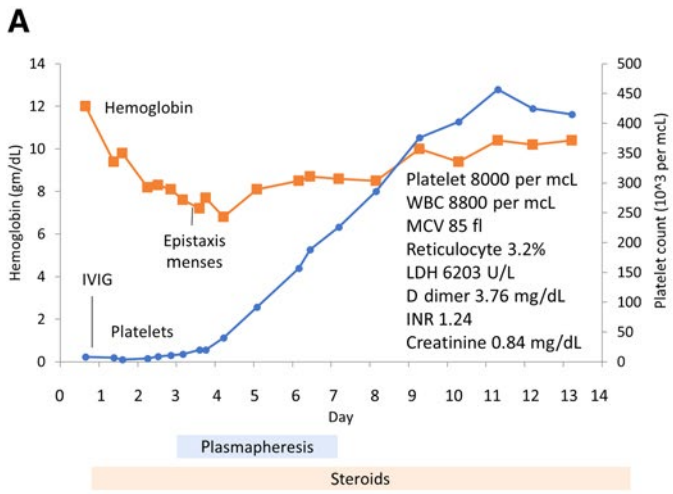
with ITP can also be seen with APLS. Other clinical conditions that may be associated ITP should prompt the provider to consider associated diagnoses. *Multilineage cytopenias* can be caused by both hypoproliferative and consumptive processes. Immune thrombocytopenia with autoimmune hemolytic anemia and/or immune neutropenia was previously described as Evans syndrome. Many individuals with this syndrome of multilineage immune cytopenias have acquired autoimmune diseases or underlying inherited immunoregulatory disorders.[12]

CLINICAL CASE #2

A 14-year-old female presented with fatigue and headaches and was diagnosed with mononucleosis. Laboratory evaluation showed severe and isolated thrombocytopenia. Urinalysis was positive for hemoglobin, and she was treated presumptively for ITP with intravenous immunoglobulin (IVIG) (Figure 3A). The platelet count did not improve, and she developed worsening anemia with increased markers of hemolysis; schistocytes were identified on peripheral blood smear. The diagnosis of TTP was considered, and a prediction score for TTP (PLASMIC score, Figure 3B) showed a high probability of TTP. She was started on steroids and plasmapheresis; subsequently, her thrombocytopenia improved. Her ADAMTS13 activity was undetectable with high titer inhibitor, consistent with acquired TTP due to an autoantibody.

Thrombotic thrombocytopenic purpura

This patient presented with isolated thrombocytopenia suggestive of ITP. She subsequently developed MAHA without renal dysfunction, and TTP was suspected. TTP is caused by a deficiency of the von Willebrand factor–cleaving protease, ADAMTS13. ADAMTS13 cleaves ultra large von Willebrand factor (ULVWF) into smaller multimers; in the absence of ADAMTS13, these ULVWF can bind to platelets and accumulate in the



B PLASMIC score for pretest probability of TTP

Variable	Point
Platelet count <30K per mcL	1
HemoLysis (reticulocyte >2.5%; haptoglobin undetectable; indirect bilirubin >2 mg/dL)	1
No active cancer (treated within last year)	1
No history of solid organ or stem cell transplant	1
MCV < 90 fL	1
INR < 1.5	1
Creatinine < 2.0 mg/dL	1
Risk for ADAMTS13 deficiency (<10%) Score of 0-4 indicates low risk; 5-6, intermediate risk; and 6-7, high risk	

PLASMIC score = 7: high risk

Figure 3. Clinical case 3: thrombotic thrombocytopenic purpura (TTP). (A) Patient presented with severe and selective thrombocytopenia (blue). Platelets did not improve after intravenous immunoglobulin (IVIG) treatment for presumptive immune thrombocytopenia (ITP). Patient developed a progressive decrease in hemoglobin (orange), and markers of hemolysis consistent with a microangiopathic anemia. Platelet count normalized with plasmapheresis and steroids. INR, international normalized ratio; LDH, lactate dehydrogenase; MCV, mean corpuscular hemoglobin; WBC, white blood cells. (B) PLASMIC score was high, compatible with TTP.

microvasculature, resulting in accelerated platelet clearance. In contrast to other TMAs, TTP is not typically associated with acute kidney injury. The incidence in adults is 2.88 cases per million, while in children the incidence is 0.09 cases per million.[13] In children, TTP may be acquired due to inhibitory antibodies or may be hereditary secondary to biallelic mutations in the *ADAMTS13* gene. Hereditary TTP commonly presents with neonatal hyperbilirubinemia with hemolytic anemia and thrombocytopenia; the median age of diagnosis is 5.5 years.[14] It can be misdiagnosed as ITP or neonatal alloimmune thrombocytopenia. Acquired TTP is more common in females and is associated with autoimmune diseases such as systemic lupus erythematosus. Neurologic symptoms are frequent in patients with hereditary and acquired TTP and can range from headaches and lethargy (as seen in this patient) to stroke.[14]

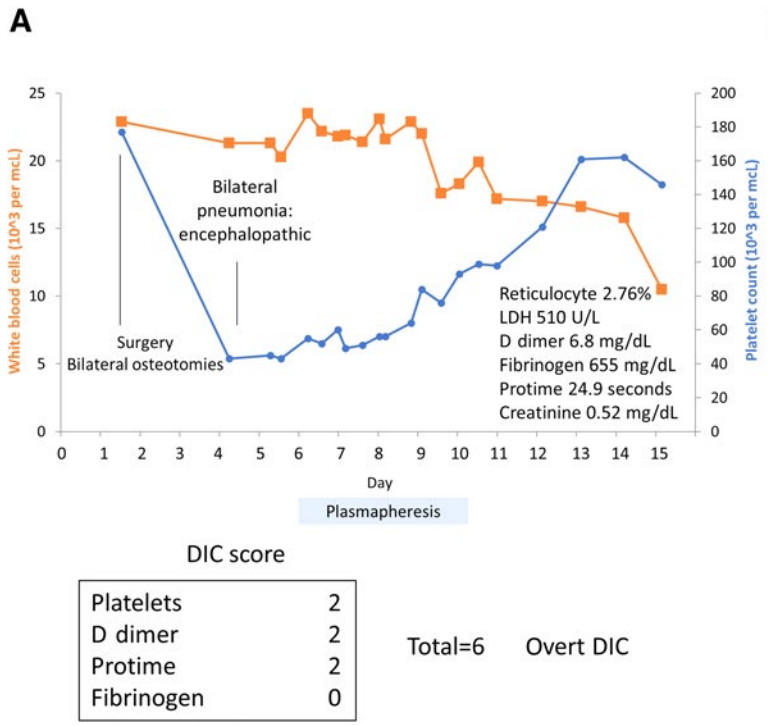
The PLASMIC score (Figure 3B) was high in this patient, identifying this patient at high risk for TTP and severely low ADAMTS13 activity (<10%).[15] This clinical prediction tool can assist treatment decision-making, given the variable availability and turnaround times for ADAMTS13 results. The PLASMIC score uses creatine for renal function assessment; however, creatine varies with age in the pediatric population. Recently, the PLASMICkid score was proposed with similar criteria as the PLASMIC score but uses estimated glomerular filtration rate to account for the changing normal creatinine levels in children.[16] This score has been evaluated in a single institution and requires validation in a larger cohort.

Hereditary TTP is treated with fresh frozen plasma infusions, whereas acquired TTP is managed with both plasmapheresis and immunosuppression with steroids and rituximab.[17] A humanized monoclonal antibody to von Willebrand factor, caplacizumab, has been developed and prevents interaction of the platelets with ULVWF. It has been shown to be effective in improving thrombocytopenia in acquired-TTP treatment.[18,19] Experience with caplacizumab in the pediatric population is limited; however, successful outcomes have been published in case reports and case series.[20]

Other thrombotic microangiopathies

While the main driver of TTP is an ADAMTS13 deficiency, other TMAs are triggered by endothelial injury and characterized by complement dysregulation. The most common TMAs encountered in the pediatric population include CM-TMA and ST-HUS or other infections. Other etiologies include drug-induced TMA, metabolic TMA (associated with cobalamin deficiency), vasculitis-associated TMA (associated with systemic lupus erythematosus, APLS, and anti-neutrophil cytoplasmic antibodies) and hypertension-associated TMA.[21,22]

These disorders are characterized by MAHA with microvascular thrombosis typically involving the renal vasculature with acute kidney injury; however, organ involvement can be widespread. ST-HUS is associated with bloody diarrhea and is caused by Shiga toxin–induced endothelial injury. ST-HUS and other infection-associated HUSs are typically managed with supportive care. CM-TMA describes a group of inherited and acquired TMAs caused by genetic mutations in complement genes or autoantibodies to complement regulatory proteins. Complement activation leads to endothelial damage with subsequent end-organ damage. In patients who present with features typical of HUS without bloody diarrhea, hereditary HUS should be considered. This condition is due to mutations in genes that encode complement proteins (Table 1). Another increasingly recognized type of CM-TMA is hematopoietic stem



B ISTH criteria for DIC

Parameter		Points
Platelet count	<50 000 per mcL 50 000 to <100 000 per mcL ≥100 000 per mcL	2 1 0
Elevated levels of fibrin-related markers (D dimers, fibrin split products)	Severe increase Moderate increase No increase	2 1 0
Prolonged protime	6 seconds 3 to < 6 seconds <3 seconds	2 1 0
Fibrinogen level	< 100 mg/dL ≥ 100 mg/dL	1 0
If ≥5: compatible with overt DIC; repeat score daily If <5:suggestive (not affirmative) for non overt DIC; repeat next 1-2 days		

Figure 4. Clinical case 3: disseminated intravascular coagulation (DIC). (A) Patient with pneumonia develops a rapid drop in platelet count (blue) and increased white count (orange) with microangiopathic hemolytic anemia and high D dimer. Thrombotic thrombocytopenic purpura (TTP) was considered given neurologic symptoms, and plasmapheresis was initiated. The patient recovered with antibiotic treatment for pneumonia and sepsis. (B) International Society on Thrombosis and Haemostasis (ISTH) disseminated intravascular coagulation (DIC) score high, compatible with overt DIC.

cell transplant (HSCT) associated TMA (TA-TMA). TA-TMA is a severe complication of HSCT triggered by endothelial injury. Genetic variants for complement regulatory factors as well as autoantibodies are described in this population.[23,24] Biopsies show characteristic histologic changes, but biopsy is not required for CM-TMA diagnosis. Evaluation of complement activity (C3, C4, CH50, sC5b-9) can aid in the diagnosis, with elevations of sC5b-9 supportive of the diagnosis (Table 1). If there is there is high suspicion of CM-TMA, then anticomplement therapy (eg, eculizumab, an anti-C5 antibody) should be initiated.[25-27]

CLINICAL CASE #3

A 16-year old male with cerebral palsy was hospitalized after tendon release surgery. Postoperatively, he developed fever and pneumonia. Additional findings included leukocytosis with anemia, thrombocytopenia, and coagulopathy (Figure 4A). Schistocytes were noted on peripheral blood smear. The DIC score was 6, consistent with diagnosis of DIC (Figure 4B). He was also encephalopathic; renal function was normal. A PLASMIC score showed an intermediate probability of TTP. Because of the unexplained encephalopathy, he underwent plasmapheresis, which was discontinued when the ADAMTS13 level returned as normal. He improved with antibiotics for pneumonia and supportive care.

Disseminated intravascular coagulation

This patient presented with an acute illness associated with precipitous drop in platelet count. The findings of schistocytes on smear with markers of hemolysis are consistent with MAHA. The most common etiology for MAHA is DIC, although other thrombotic microangiopathies, such as TTP, should be considered. Significant elevations of the INR and/or depression of fibrinogen, as seen in this patient, are more typical of DIC rather than TTP and other TMAs (Table 1). These diagnoses may overlap and may be difficult to distinguish; because of unexplained mental status changes, he was empirically treated for TTP until the ADAMTS13 activity returned to normal.

DIC is an acquired condition that causes extensive activation of the coagulation system. DIC is not a specific disease, but a physiologic process in response to an illness or injury that has become pathologic. It is precipitated by numerous conditions, such as sepsis, trauma, malignancy, mechanical circuits, and vascular tumors; the common trigger is tissue factor–mediated thrombin generation.[28] Accompanying this process is an inability to dampen thrombin activity due to a decrease in the natural anticoagulants antithrombin, protein C, and protein S. The fibrinolytic pathway can also be inhibited with an increase in plasminogen activator inhibitor 1. Dysregulated thrombin activation results in consumption of prothrombotic factors with fibrin deposition in small vessels and platelet trapping.[29] Depletion of platelets and coagulation factors predispose a person to bleeding, and microthrombi contribute to progressive organ dysfunction.[30] While DIC is typically an acquired condition, biallelic

mutations in *PROC*, the gene encoding protein C, manifests as DIC with purpura fulminans at birth.[31]

Several scoring systems for diagnosis and assessing severity of DIC were harmonized by the International Society on Thrombosis and Haemostasis.[32] Increased severity is characterized by worsening thrombocytopenia, prolongation of prothrombin time, reduction in fibrinogen levels, and elevation of fibrin-split products and D dimer. A score of ≥5 is compatible with overt DIC, and <5 is suggestive of nonovert DIC.[32,33] In children, age-dependent coagulation factor changes may affect the performance of DIC scores; however, several studies have demonstrated their utility to predict morbidity and mortality in the pediatric population.[34-36] Treatment of DIC is focused on treating the underlying condition. Replacement of coagulation factors is usually not indicated in the absence of active bleeding.[37]

Hemophagocytic lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (reviewed by Kumar) may present with DIC[38] and thrombocytopenia and manifest with clinical and laboratory features that overlap with other TMAs.[39] Clinical and laboratory features that distinguish HLH from DIC and other TMAs are summarized in Table 1.

Summary

Mechanisms underlying consumptive thrombocytopenia include antibody-mediated destruction, phagocytosis, and microangiopathy. Appropriate and prompt diagnosis is important, as disease-specific therapies may be lifesaving. Heparin-induced thrombocytopenia is critical to recognize and treat, but appropriate diagnostic tools and laboratory interpretation are essential to prevent overdiagnosis. Microangiopathic hemolytic anemia syndromes have considerable overlap and may be difficult to distinguish. Management of DIC is directed at treating underlying etiologies. However, TTP requires specific management with plasmapheresis, and CM-TMA should be managed with anticomplement therapy. Hemophagocytic lymphohistiocytosis may have overlapping features with these syndromes and is treated with immunosuppression. Distinguishing these diagnoses can be challenging; a systematic approach to identification of specific etiologies can aid diagnosis and is critical to implement appropriate treatment.

Conflict-of-interest disclosure

Rohith Jesudas: no competing financial interests to declare.

Clifford M. Takemoto: research funding from GBT, Forma Therapeutics, and Daiichi Sankyo; Novartis Data Safety Monitoring Committee.

Off-label drug use

Rohith Jesudas: enoxaparin, bivalirudin, eculizamab, caplacizumab.
Clifford M. Takemoto: enoxaparin, bivalirudin, eculizamab, caplacizumab.

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How to classify risk based on clinical and molecular modeling: integrating molecular markers in the risk assessment of myelodysplastic syndrome

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Myelodysplastic syndrome (MDS), also known as "myelodysplastic neoplasm," is a heterogeneous group of clonal myeloid neoplasms that typically affects older adults. The clinical phenotype, symptoms, and complications relate to the depth of cytopenia and progression to acute myeloid leukemia (AML). The diagnosis of MDS relies on morphologic criteria, such as evidence of dysplasia, disordered maturation, and increasing blast counts, which separate the disease into histologic subtypes with different probabilities for progression to AML. The treatment of MDS is often risk-adapted depending on the prognostic profile of each patient's disease. There has been a coevolution of diagnostic and prognostic systems for MDS developed over the past 40 years, both of which have now incorporated molecular markers. The new International Prognostic Scoring System-Molecular (IPSS-M) improves partitioning of patients compared to prior versions with resultant upgrading of 34% of patients into higher-risk groups due to the presence of mutations. The new IPSS-M also more accurately distinguishes intermediate-risk patients separating them into two tiers. The two new diagnostic classifications include MDS defined by mutations in *SF3B1* and *TP53*, though there are differences in diagnostic criteria. Future efforts to refine MDS prognostication could investigate the interface between MDS and clonal cytopenia of undetermined significance, expand access to genomic testing, obtain results in a less invasive manner, and develop treatment-response predictors and dynamic risk models.

LEARNING OBJECTIVES

- Compare the foundational risk-assessment models on which the IPSS-M builds
- Examine the clinical implications of the IPSS-M and new disease subclassification systems
- Describe future avenues for improving MDS risk stratification, prognostication, and patient outcomes

Introduction

Myelodysplastic syndrome, also known as myelodysplastic neoplasm (both abbreviated MDS), is a clonal myeloid neoplasm that typically affects older adults with the main symptoms and complications related to the depth of cytopenia and progression to acute myeloid leukemia (AML). There are both lower-risk and higher-risk MDS, with the higher-risk group being more likely to transform into AML. Treatment of MDS is often risk-adapted and relies on the prognostic profile of the disease. While there are several treatment options, the only curative therapy is allogeneic hematopoietic stem-cell transplantation (HSCT).

Similar to most cancers, the diagnosis, prognosis, and treatment of MDS have long been intertwined facets of disease management. The pathologic criteria for MDS captures the morphologic evidence of dysplasia, disordered maturation, and increasing blast counts separating the disease into low- and high-grade subtypes. The available genetic data are extensive, permitting a deeper understanding of the disease and enabling integrated risk-assessment models. Coupling the pathological and clinical features with genomic data, today's prognostic models are more individualized.[1] When treatments can vary from supportive care to hematopoiesis stimulating agents and to lower

intensity therapies, including immunosuppressive or immunomodulatory agents, and higher intensity therapies, including hypomethylating agents (HMA), induction chemotherapy, and HSCT, as well as newer targeted agents (in clinical trials), a uniform description and assignment of prognosis becomes increasingly important. In 2022, the clinical management of patients with MDS was transformed whereby genomic data became fully integrated into international prognostic and diagnostic standards—the International Prognostic Scoring System-Molecular (IPSS-M),[2] the World Health Organization (WHO) Classification of Haematolymphoid Tumours (WHO 5th ed.),[3] and the International Consensus Classification (ICC) of hematologic malignancies.[4] The historical context and the clinical implications of the IPSS-M and new disease classifications will be discussed, as well as unmet needs in MDS risk assessment.

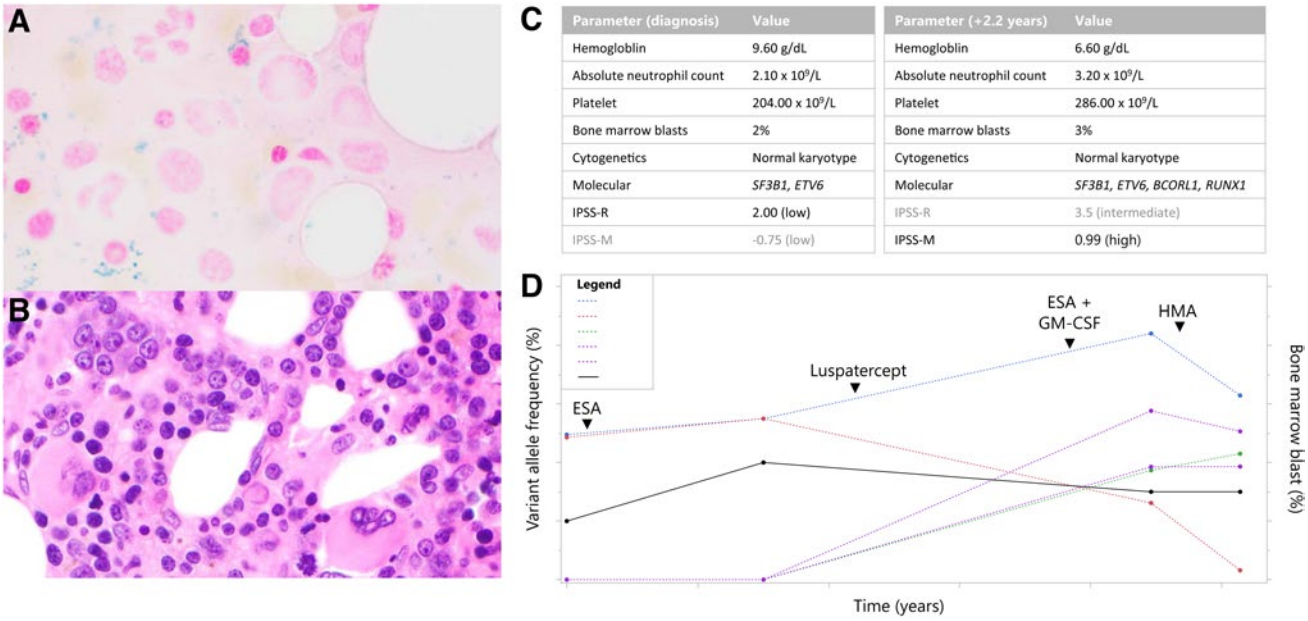
CLINICAL CASE

A 66-year-old woman presented in 2020 with abnormal blood counts, including a white blood cell count of 4.2×10^9/L, absolute neutrophil count (ANC) of 2.10×10^9/L, hemoglobin of 8.9 g/dL, mean corpuscular volume of 111.00 fL, platelet count of 204.00×10^9/L, and serum erythropoietin level of <200 mIU/mL (Figure 1). A bone marrow biopsy showed 2% blasts, erythroid and megakaryocytic dysplasia, and 30% ring sideroblasts. Cytogenetic studies revealed a normal karyotype. Next-generation sequencing (NGS) demonstrated two mutations (*ETV6*, 24% variant allele frequency [VAF]; *SF3B1*, 25% VAF). Based on the 2016 WHO revised 4th edition, a diagnosis of MDS with ring sideroblasts and multilineage dysplasia (MDS-RS-MLD) was rendered. Based on the 2012 revised IPSS (IPSS-R), this profile resulted in a score of 2, which corresponded to lower-risk MDS with a median overall survival (OS) of 9 years.

Continued refinement of MDS risk assessment

From the original French-American-British[5] subclassification of MDS (1982) and the Bournemouth[6] risk-assessment model (1985), there has long been a coevolution of diagnostic and prognostic systems for MDS (Figure 2A). As our understanding of the biology of MDS has improved,[7-9] so has the precision around which diagnostic and prognostic categories are assigned, leading to ever-increasing disease subtypes and risk groups. Since approximately 90% of patients with MDS have a driver mutation compared to 41% with a cytogenetic alteration,[2] somatic mutation testing is particularly informative. Similar to the impact that cytogenetic studies had on risk stratification of MDS in the 1990s, molecular studies have now changed the way patients diagnosed with MDS are managed. The new IPSS-M improves the accuracy of MDS prognosis by building on the foundational knowledge from prior MDS risk models.

Since the 1980, there have been 19 selected studies that propose a new risk-assessment model, modify, and/or validate a prior model for MDS prognosis. Factors that have been



Parameter (diagnosis)	Value
Hemoglobin	9.60 g/dL
Absolute neutrophil count	2.10 x 10^9/L
Platelet	204.00 x 10^9/L
Bone marrow blasts	2%
Cytogenetics	Normal karyotype
Molecular	*SF3B1, ETV6*
IPSS-R	2.00 (low)
IPSS-M	-0.75 (low)

Parameter (+2.2 years)	Value
Hemoglobin	6.60 g/dL
Absolute neutrophil count	3.20 x 10^9/L
Platelet	286.00 x 10^9/L
Bone marrow blasts	3%
Cytogenetics	Normal karyotype
Molecular	*SF3B1, ETV6, BCORL1, RUNX1*
IPSS-R	3.5 (intermediate)
IPSS-M	0.99 (high)

Figure 1. Clinical case findings at diagnosis and follow-up. (A) Diagnostic bone marrow iron stain (100×) showing abundant ring sideroblasts and multilineage dysplasia leading to a diagnosis of MDS with multilineage dysplasia and ring sideroblasts (MDS-MLD-RS). (B) Bone marrow biopsy (40×) 2.6 years after diagnosis obtained after initiation of azacytidine showing persistent multilineage dysplasia, erythroid hyperplasia, and new fibrosis (grade 1–2). (C) Clinical and laboratory parameters at diagnosis when the IPSS-M was not in use and at 2.2 years when the IPSS-M was in use upgrading the risk category to high, based on molecular findings. (D) Clinical course as illustrated by the bone marrow blast count, mutation profile obtained at each of the bone marrow biopsies (time 0, 0.8, 2.2, and 2.6 years), and new therapies initiated. ESA, erythropoiesis-stimulating agent; GM-CSF, granulocyte-macrophage colony-stimulating factor; HMA, hypomethylating agent.

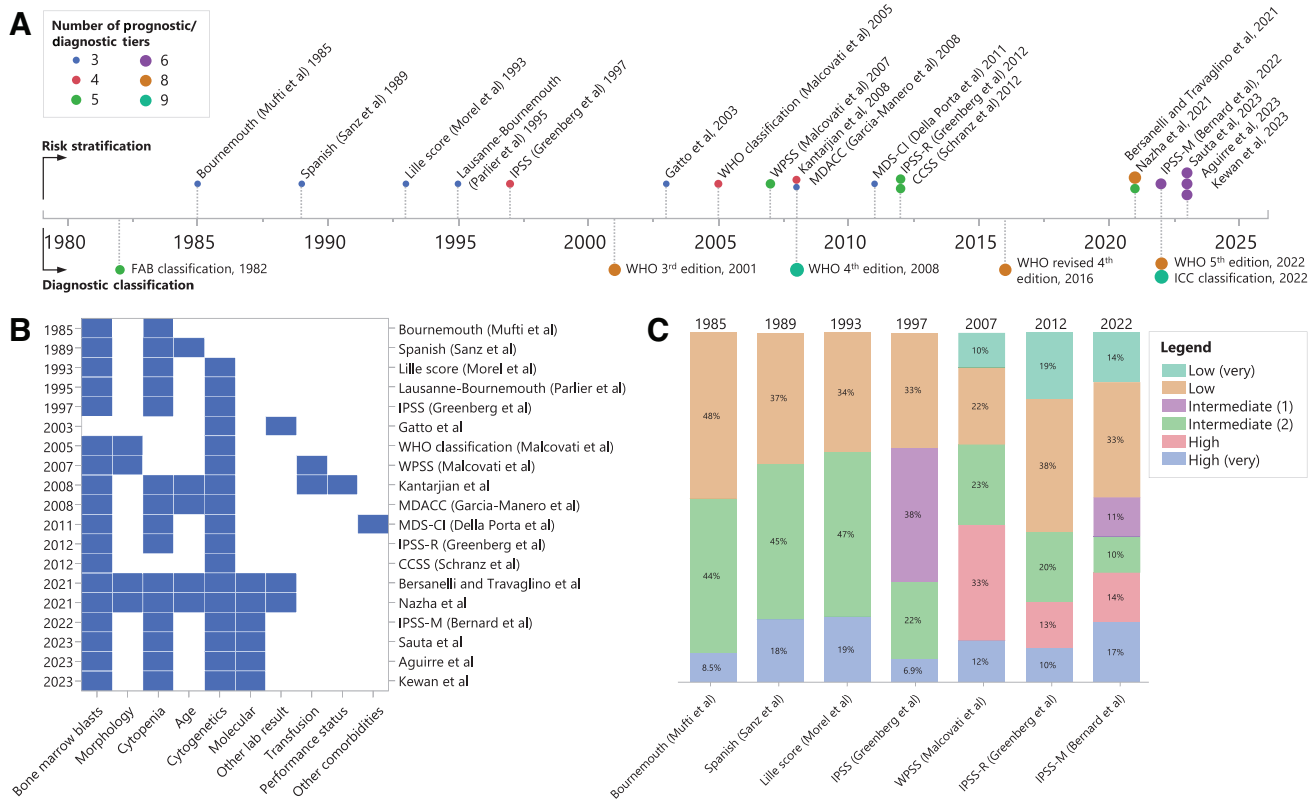


Figure 2. Historical perspective of MDS prognostic models and diagnostic subclassifications. (A) Risk stratification studies and MDS subclassifications from 1982 to present. Colored dots represent number of prognostic or diagnostic tiers used. (B) Select risk stratification studies showing different clinical, pathologic, and laboratory parameters deemed most prognostic. The other lab result is beta-2 microglobulin (Gatto et al[24]), neutrophils, ferritin, LDH (Bersanelli et al[25]), and absolute lymphocyte/neutrophil/monocyte counts (Nazha et al[26]). (C) Select prognostic models and the separation of patients into risk groups. When a single intermediate-risk group is used, this is arbitrarily set as "Intermediate (2)." When a single high-risk group is used, this is arbitrarily set as "High (very)."

consistently identified as prognostic include bone marrow blast count (18/19, 95%),[2,6,10-23] cytogenetic abnormalities (17/19, 89%),[2,11-24] and degree of cytopenia (15/19, 79%)[2,6,10-13,16-19,21-23,25,26] with molecular profiling becoming important in recent years (Figure 2B).[2,21-23,25,26] Interestingly, the MDS subtype, which often correlates with prognosis,[6,13] has only been incorporated into four prognostic models.[14,15,25,26] This may be related to the fact that the degree of dysplasia, while important for diagnosis, is not always externally consistent or prognostic. Over time, the number of risk stratifications has increased from 3 to 6 (Figure 2C), with the largest group being the low-risk group in the IPSS-M. Compared to prior systems, the proportion of low-risk and very-low-risk groups represents the largest fraction, the intermediate-risk group (low and high) remains relatively constant, and the very-high-risk group has increased modestly in the IPSS-M. The new categories improve the prediction of prognosis when comparing survival, hazard ratios (HR), and Harrell's concordance-indices (c-index).

Clinical implications of evolving prognostic and diagnostic systems

The introduction of any new risk-assessment model will lead to a reassortment of patients. The IPSS-R uses cytogenetics risk groups, bone marrow blast count, hemoglobin, platelet count, and ANC to assign points for each categorical variable, with the sum corresponding to five risk groups. The IPSS-M, though not the first to incorporate molecular markers,[25,26] is the first to formulate an integrated model on a continuous scale that can be directly compared to the IPSS-R. The IPSS-M uses the IPSS-R cytogenetics risk groups, bone marrow blast count, hemoglobin, platelet count (capped at 250×10⁹/L), and the presence/absence of mutations in 31 genes (16 main genes and 15 additional genes) without ANC to assign points on a continuum that corresponds to six risk groups. Although more driver mutations correlate with inferior survival, as previously demonstrated,[7,25,26] the total number of mutations was not as informative as individual mutations. Despite the importance of gene mutations, the IPSS-M can accommodate missing data. Data for 15 genes (all of the main genes except *KRAS*) will maintain 70-80% accuracy, but fewer than 10 genes is not advisable.[21]

Given the weight that adverse cytogenetics and high blast counts provide, the additional molecular markers have a limited ability to reduce an already high-risk score, but they have a high likelihood of upgrading scores. If the same patients were scored using the IPSS-R and IPSS-M, 31-69% (54% overall) of the categorizations remain unchanged (Figure 3A), whereas 34%

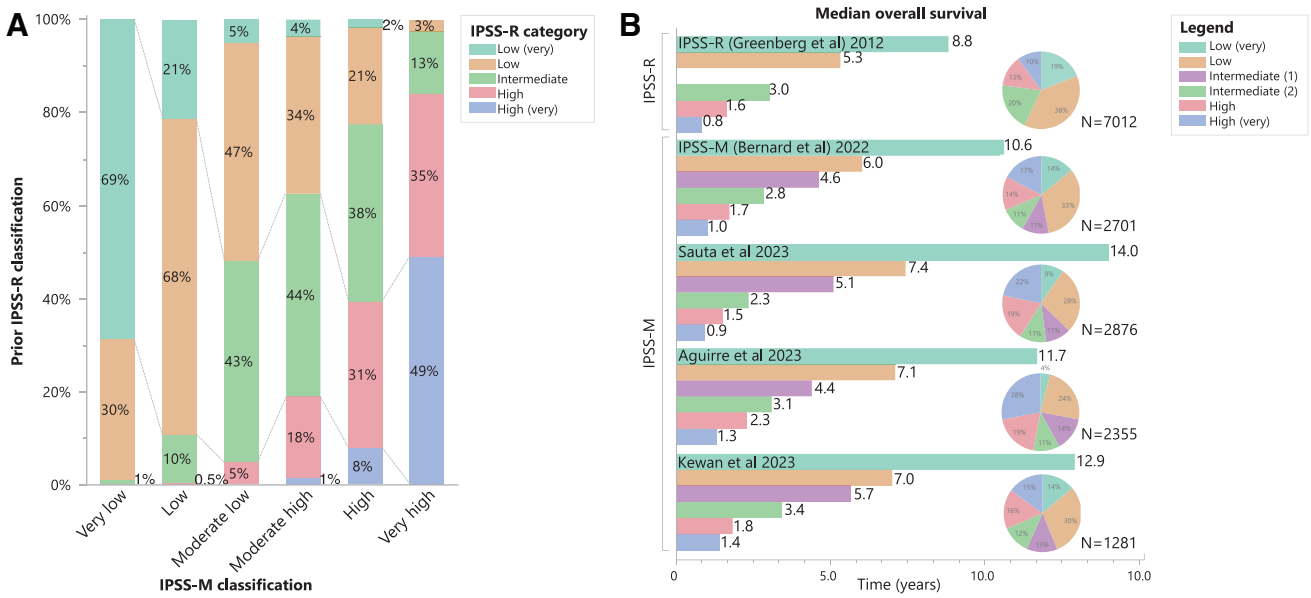


Figure 3. Clinical implications of changing risk stratifications using IPSS-M. (A) IPSS-M risk groups and the equivalent risk group using the prior IPSS-R criteria. Dashed lines outline unchanged risk classes based on either model. Adapted from Bernard et al.[2] (B) Comparison of median overall survival based on IPSS-R and IPSS-M in the indicated studies. Pie charts show groupings of patients into the different IPSS-M risk categories.

of the patients would be upgraded and 12% would be downgraded.[2] With the exception of IPSS-M very-low-risk patients, the majority of the reassignments using IPSS-M represent upgrades. For instance, 52% and 37% of the moderate-low and moderate-high, respectively, IPSS-M patients were previously lower risk. Similarly, 61% and 51% of the high and very-high IPSS-M patients had a lower IPSS-R assignment. This trend for upgrading the risk group according to IPSS-M has been confirmed by multiple studies.[21-23]

To determine whether the IPSS-M model translates into more distinct outcomes, median OS can be compared (Figure 3B).[2,19,21-23] Acknowledging that the study populations are different, the OS of the high-risk and very-high-risk patients are comparable. The most notable change is seen when a two-tiered intermediate group is introduced. Whereas a single IPSS-R intermediate group has a median OS of 3 years, the two-tiered IPSS-M intermediate groups now show survival differences ranging from 1.3-2.8 years. The importance of separating the intermediate group is additionally supported by the calculated HRs (IPSS-R intermediate 2 vs IPSS-M moderate-low 1.5 and IPSS-M moderate-high 2.5).[2] Another difference of the IPSS-M is the OS in the very-low-risk and low-risk groups. Whereas the IPSS-R very-low risk group shows a 3.5-

Table 1. Median survival comparison using IPSS-R and IPSS-M in the same cohort of MDS patients

Risk	IPSS	n (%)	Overall survival			Time to AML in 25%		
			Median (years)	95% CI (lower)	95% CI (upper)	Median (years)	95% CI (lower)	95% CI (upper)
Very low	R	293 (10.2)	12.9	12.9	NR	NR	NR	NR
	M	275 (9.6)	NR	10.5	NR	11.4	11.4	NR
Low	R	806 (28.0)	7.3	6.2	8.5	11.4	9.3	NR
	M	797 (27.7)	7.4	6.2	9.6	12.5	10.3	NR
Intermediate	R	610 (21.2)	3.5	3.2	4.8	4.3	3.7	6.2
Moderate Low	M	306 (10.6)	4.8	3.6	5.7	5.1	4.1	NR
Moderate High	M	319 (11.1)	2.3	1.9	3.2	2.7	1.9	4.7
High	R	595 (20.7)	1.6	1.3	1.8	2.5	1.9	3.3
	M	555 (19.3)	1.4	1.3	1.7	2.2	1.7	3.3
Very high	R	572 (19.9)	0.8	0.7	0.8	1.4	1.2	1.8
	M	624 (21.7)	0.8	0.8	1.0	1.7	1.2	2.0

M, IPSS-M; R, IPSS-R; NR, not reached.
Adapted from Sauta et al.[21]

year improvement in OS when compared to the low-risk group, the IPSS-M very-low-risk group gains 4.6-6.6 years over the low-risk group. If the IPSS-R and IPSS-M strata are compared within the same population, the main improvement is again demonstrated in the two-tiered intermediate group (Table 1).[21] With all these changes, the IPSS-M improves the c-index by 5 percentage points.[2,21-23,27] As an extension of improved risk stratification, there is the possibility of improving the selection of risk-adapted therapies. The IPSS-M has been shown to be a superior predictor of post-HSCT outcomes than the IPSS-R,[21] which may be related to high-risk mutational profiles, such as bi-allelic *TP53*.[2,28] With regard to HMA in higher-risk patients, the IPSS-M risk groups do not appear to correlate with the probably of overall response,[21] though bi-allelic *TP53* mutations continue to be a strong predictor of worse outcomes when receiving HMA or lenalidomide.[2]

As the prognostic models are now including somatic mutations, so are the diagnostic classifications. Since 1982, there have been six iterations of classification systems beginning with 5 to 8-9 present-day MDS subtypes (Figure 2A). While genetically defined entities are well-known in AML, until 2022, the only genetically defined MDS was MDS associated with isolated del(5q), which was first introduced in 2001. In 2022, the WHO 5th edition and ICC classifications both introduced two MDS subtypes defined by gene mutations (Table 2). Both systems now accept mutations in certain genes as evidence of AML with myelodysplasia-related changes, whereas this was only previously afforded to specific chromosomal abnormalities. The WHO has also recognized a set of genes mutated in clonal cytopenia of undetermined significance (CCUS), while the ICC also recognizes *UBA1*-mutated "VEXAS" syndrome as a related clonal cytopenia.

With regard to genetically defined MDS entities, both systems now recognize *SF3B1*[8,29] and *TP53*[2,28,30] mutations as specific disease subtypes. While the mutation requirements are subtly different, the main distinction between the diagnostic criteria is morphologic evidence of dysplasia. The WHO maintains dysplasia (10% of cells in at least one lineage) as a diagnostic criterion, but the ICC does not as long as the genomic profile is appropriate. While most patients will have a concordant diagnosis by either system, occasionally these differences may lead to a diagnosis of CCUS in one instance and a diagnosis of MDS in another, particularly for *SF3B1*-mutated cases with low blast counts. It is still unknown at this time whether patients diagnosed with CCUS in this context should be managed as presumptive MDS. It is also unknown if future disease classification systems will continue to uphold the requirement for morphologic dysplasia and if new mutation-defined subtypes will emerge.

Future opportunities for improving prognosis of MDS

Despite more comprehensive and accurate prognostication provided by the IPSS-M, there are still challenges. The IPSS-M is not designed to be used dynamically, though other models have been shown to be useful for repeat assessment.[15,26,31] The predictive power of the IPSS-M to guide the selection of therapies outside of HSCT and in patients without multiple *TP53* mutations remains unknown. The treatment implications for patients in IPSS-M-upgraded risk groups, previously lower-risk MDS according to IPSS-R, continues to be an open question. The response rates achieved in different IPSS-M risk groups when treated with higher-intensity therapies, such as HMA, and novel therapies will no doubt be an area of active investigation in the coming years. Complementary to these challenges, future avenues for improving MDS risk-modeling follow three main themes.

Clonal hematopoiesis was first reported 10 years ago[32,33] and is now recognized as a risk factor for developing hematologic malignancies,[34] particularly in the context of cytopenias.[35,36] CCUS is a recognized entity in both the WHO 5th edition and ICC classification systems, and some patients with CCUS may benefit from treatments for MDS.[37] It is known that idiopathic (non-clonal) cytopenia, even if accompanied by dysplasia, has a much lower risk of progression to MDS[35,36] and should not be diagnosed or treated as MDS. It is yet unknown if genetically defined MDS entities should be classified as CCUS in the absence of dysplasia. Continued efforts should be made to identify patients with high-risk CCUS that may progress imminently to MDS.[36] A related consideration is that future prognostic models may have to contend with differences in diagnostic criteria for CCUS and MDS, which may affect enrollment in prospective studies and outcomes studies related to the risk of progression to MDS/AML and the risk of cardiovascular disease. The clinical implications of differing diagnostic criteria would also be important areas of investigation, as well as efforts to harmonize the diagnostic classifications, especially as pertains to MDS and CCUS.

Another area of potential improvement in MDS prognostication is to increase testing of highly prognostic and predictive molecular targets and more routinely obtain these results from peripheral blood. While the availability of molecular diagnostics has improved, resource-limited settings are often excluded due to access and financial toxicity. Even when NGS is available, a subset of the markers is rarely tested. The diagnostic field should strive to improve the detection of markers that are difficult to identify by routine NGS, such as MLL (*KMT2A*)-PTD and *TP53* copy-neutral loss-of-heterozygosity. Germline predisposition to myeloid neoplasms is increasingly being recognized as an important aspect of clinical outcome. As paired-normal sequencing studies and dedicated germline predisposition testing become more accessible, this should enhance recognition of patients and families carrying germline risk mutations and enable ongoing research related to the screening and management of these individuals. While conventional cytogenetic studies are rarely informative in peripheral blood, whole genome sequencing[38] and targeted NGS[39] are both high yield. These types of peripheral blood studies could provide a genomic karyotype and mutational profile without the need for a bone marrow biopsy and may be useful in the initial evaluation of cytopenia and for treatment monitoring.

The last area for improvement involves dynamic risk-assessment models and treatment response predictors. Studies that profile risk parameters over time, including failure to respond to certain lines of therapy or the emergence of new clonal abnormalities, would be very useful. With regard to treatment response predictors, measurable residual disease (MRD) indicators have long been used in lymphoblastic leukemia to guide therapies. However, MRD markers for MDS are problematic to develop since most mutations are found in the entire myeloid compartment. One way to circumvent this may be monitoring blast-specific genomic abnormalities in plasma cell-free DNA,[40] which is enriched for DNA from cells with rapid

Table 2. **Diagnostic and prognostic significance of gene mutations**

Gene	IPSS-M main gene	IPSS-M residual gene	MDS defining (ICC)*	MDS subtype		AML with MRC		WHO 5th key mutations		Total categories
				ICC	WHO 5th	ICC	WHO 5th	CCUS	Other mutations	
SF3B1	■		■	■	■	■	■	■		7
TP53	■		■	■	■	■	■	■		
ASXL1	■					■	■	■		4
BCOR		■				■	■	■		
EZH2	■					■	■		■	
SRSF2	■					■	■	■		
STAG2		■				■	■		■	
U2AF1						■	■	■		
RUNX1						■		■		3
ZRSR2						■	■		■	
BCORL1		■						■		2
CBL	■							■		
CEBPA		■							■	
DNMT3A	■							■		
ETV6	■								■	
GATA2		■							■	
GNB1		■						■		
IDH1		■						■		
IDH2	■							■		
KRAS	■							■		
NRAS	■							■		
PHF6		■							■	
PPM1D		■						■		
PTPN11		■						■		
SETBP1		■							■	
WT1		■							■	
BRAF									■	1
BRCC3								■		
CALR									■	
CREBBP									■	
CSF1R									■	
CSF3R									■	
CTCF								■		
CUX1									■	
ETNK1		■								
FLT3	■									
GNAS								■		
JAK2								■		
JAK3									■	
KDM6A									■	
KIT									■	
KMT2A									■	

Table 2. Diagnostic and prognostic significance of gene mutations (*Continued*)

Gene	IPSS-M main gene	IPSS-M residual gene	MDS defining (ICC)*	MDS subtype		AML with MRC		WHO 5th key mutations		Total categories
				ICC	WHO 5th	ICC	WHO 5th	CCUS	Other mutations	
MLL (KMT2A)-PTD										
MPL										
MYD88										
NF1										
NOTCH1										
NPM1										
PIGA										
PRPF40B										
PRPF8										
PTEN										
RAD21										
SF1										
SF3A1										
SMC1A										
SMC3										
STAT3										
TET2										
U2AF2										
ZBTB33										

Genes are ranked from highest diagnostic and prognostic relevance to lowest with the number of categories implicated.

*Based on the ICC classification, a diagnosis of MDS with *SF3B1* may be made if the *SF3B1* mutation is present at ≥10% VAF, even if there is no known/appreciable dysplasia. A diagnosis of MDS with mutated *TP53* may be made without known/appreciable dysplasia if blasts are less than 10% and one of the following is true: two or more *TP53* mutations are each at ≥10% VAF, one *TP53* mutation is at ≥50% VAF, or one *TP53* mutation is ≥10% VAF combined with either chromosome 17p deletion/copy-neutral loss-of-heterozygosity or a complex karyotype. A diagnosis of MDS/AML with mutated *TP53* may be made without known/appreciable dysplasia if blasts are 10–19% and there is one *TP53* mutation ≥10% VAF. The presence f del(5q), –7 or del(7q), or a complex karyotype will also be sufficient to render a diagnosis of MDS without known/appreciable dysplasia. The presence of these mutations or cytogenetic abnormalities without evidence of dysplasia will not be sufficient for a diagnosis of MDS based on the WHO 5th classification.

MRC, myelodysplasia-related changes.

turnover. Future studies could also evaluate sensitizing and resistant molecular profiles to disease-modifying therapies and identify markers of treatment failure.

CLINICAL CASE (continued)

Since the patient was symptomatic to her anemia, an erythropoiesis-stimulating agent was started. The response waned after 18 months, and the patient became transfusion-dependent, despite interval treatment with luspatercept (Figure 1). A bone marrow biopsy performed in 2022 showed similar findings to the prior marrow 2 years before, but NGS revealed the emergence of new mutations—a *BCORL1* mutation (19% VAF) and two *RUNX1* mutations (29% and 20% VAF). In accordance with the new diagnostic classifications, the pathologic diagnosis was changed from MDS-RS-MLD to MDS with mutated *SF3B1* (ICC classification), and MDS with low blasts and *SF3B1* mutation (WHO 5th classification). Although neither the IPSS-R or IPSS-M are dynamic in nature, the patient's disease profile in 2022 would be associated with intermediate-risk MDS (IPSS-R, median OS 3 years) and higher-risk MDS (IPSS-M, median OS 2 years). The absence of treatment response and the evidence of clonal progression raised the possibility of off-label lenalidomide, Imetelstat in the setting of a clinical trial, and higher-intensity therapies. Ultimately, an HMA was initiated with plans for curative intent therapy with a future HSCT based on shared decision-making and the goals of the patient.

Conclusions

Enormous strides have been made over the last 40 years in the diagnosis, prognosis, and treatment of patients with MDS. Risk-assessment models, especially those arising from international collaborations, have been able to guide more consistent patient therapies and more informed clinical trials. These prognostic models are now more comprehensive than ever before incorporating somatic mutations. Despite these advances, there are still avenues for refining the approaches to risk assessment of

MDS. The clinical and scientific community will no doubt partner together in these future endeavors to improve patient outcomes.

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Conflict-of-interest disclosure

Rena R. Xian: no competing financial interests to declare.

Off-label drug use

Rena R. Xian declares no off-label drug use.

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Next-generation therapy for lower-risk MDS

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Myelodysplastic syndromes (MDS) are malignant myeloid neoplasms characterized by ineffective clonal hematopoiesis leading to peripheral blood cytopenia and a variable risk of transformation to acute myeloid leukemia. In lower-risk (LR) MDS, as defined by prognostic scoring systems recently updated with the addition of a mutation profile, therapeutic options aim to reduce cytopenia, mainly anemia. Although options for reducing the transfusion burden have recently been improved, erythropoiesis-stimulating agents (ESAs), lenalidomide, hypomethylating agents, and, more recently, luspatercept have shown efficacy in rarely more than 50% of patients with a duration of response often far inferior to the patient's life expectancy. Nevertheless, several new therapies are currently under investigation aiming at improving cytopenia in patients with LR-MDS, mostly by targeting different biological pathways. Targeting ligands of the transforming growth factor β pathway has led to the approval of luspatercept in LR-MDS with ring sideroblasts or *SF3B1* mutation, potentially replacing first-line ESAs in this population. Here, we also discuss the evolving standard of care for the treatment of LR-MDS and explore some of the most promising next-generation agents under investigation.

LEARNING OBJECTIVES

- Learn about the role of molecular-driven biology in next-generation therapies for myelodysplastic syndromes
- Review therapies for lower-risk myelodysplastic syndromes that are currently in development
- Understand how pathologic inflammatory pathways in myelodysplastic syndromes can be targeted by novel therapies

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal malignant myeloid malignancies characterized by ineffective hematopoiesis, leading to peripheral blood cytopenia, and a variable risk of transformation to acute myelogenous leukemia.[1] As 80% of patients with MDS are over 60 years of age, existing scoring systems based on cytopenia, bone marrow blast percentage, and somatic oncogenetic events are used to identify the risk of MDS progression in order to define therapeutic goals in this elderly and sometimes frail population.[2] About two-thirds of patients with MDS will present with lower-risk (LR) disease at diagnosis.[3] Even if hematopoietic stem cell transplantation (HSCT) is the only curative option, most patients with MDS are ineligible because of age or comorbidities, and the main approach for patients with LR-MDS still aims at improving cytopenia (mainly anemia) and its complications.

For many years, the therapeutic strategy for these patients was very limited, relying solely on transfusion, HSCT, and erythropoiesis-stimulating agents (ESAs). Given the recent progress made in the understanding of the pathophysiology of LR-MDS and the results of clinical trials recently published, we could now propose an updated algorithm for the management of these patients, shown in Figure 1. Through a few practical examples, we will discuss these different new therapeutic options.

CLINICAL CASE 1

A 79-year-old man with a history of type 2 diabetes and hypertension was referred to the hematology department after a routine blood test revealed anemia. The complete blood count showed hemoglobin (Hb) of 6.5 g/dL, mean corpuscular volume of 85 fL, a white blood cell count of 4.3×10^9/L, an absolute neutrophil count of 2.7×10^9/L, and platelet count of 152×10^9/L. B12, folate, and iron levels were normal, and erythropoietin was 110 U/L. A bone marrow (BM) aspirate showed marked dysplastic changes in 25% of erythroid cells, 3% of blasts, and 15% of ring sideroblasts, and next-generation sequencing (NGS) revealed an isolated *SF3B1* mutation with 25% variant allele frequency (VAF). Cytogenetics were normal. The patient received a

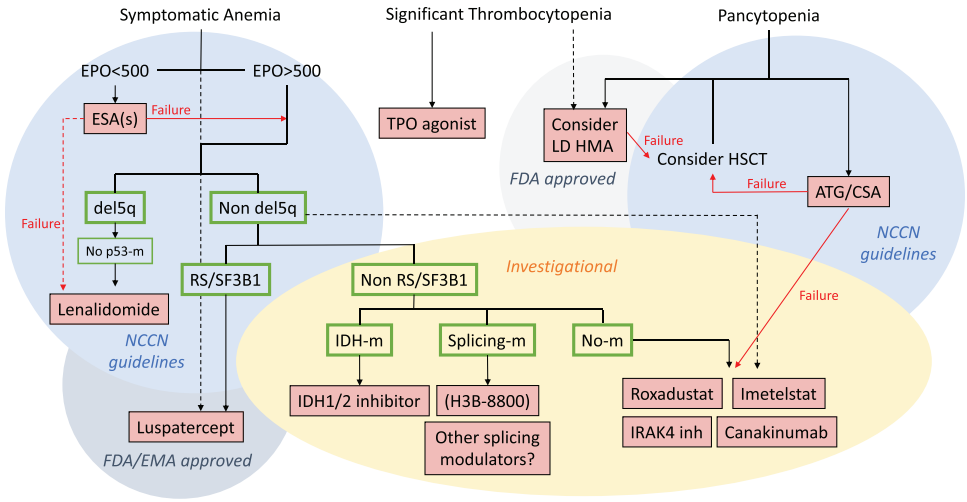

Figure 1. Treatment algorithm for lower-risk MDS—IPSS-R ≤3.5. CSA, cyclosporine; EMA, European Medicines Agency; FDA, Food and Drug Administration; m, mutated; NCCN, National Comprehensive Cancer Network; TPO, thrombopoietin.[19]

transfusion for anemia at diagnosis and ESA, epoetin (EPO)–α 450 IU/kg/wk. His Hb increased to 11.5 g/dL by the second month of EPO, and he became transfusion independent (TI). Two years later, he relapsed while still on EPO with isolated anemia and received a transfusion again. The repeated aspiration of the BM was similar to the one performed at diagnosis. Subcutaneous luspatercept (starting dose 1 mg/kg every 3 weeks) with transfusion support was started, and he reached transfusion independency by the fifth dose of luspatercept.

For all patients with MDS, existing scoring systems define multiple risk categories, but these are reduced to what is used clinically—lower- and higher-risk MDS—to guide treatment options. Even if most of the available drugs for MDS was approved according to the classical International Prognostic Scoring System (IPSS) classification published in 1998, the International Working Group (IWG) for prognosis in MDS was revised in 2012 (IPSS-R) and even more recently in 2022 (IPSS-M), taking into consideration somatic gene mutations,[2] representing a valuable tool for individual risk assessment and treatment decisions.[4]

In lower-risk MDS, defined by IPSS-R ≤3.5 or IPSS-M (moderate low, low, and very low), available therapeutic options are mainly limited to supportive care with transfusions, ESAs, lenalidomide, hypomethylating agents (HMAs, azacitidine or decitabine), and, more recently, luspatercept (Luspa) (Table 1).

Anemia is the most common cytopenia in LR-MDS and is present in almost 90% of the cases. Symptoms related to anemia deeply impact the quality of life of these patients but can also lead to worsening of cardiopulmonary function or cognitive decline. Anemia management was first based on red blood cell (RBC) transfusion, but RBC transfusion dependency (TD) is associated with decreased quality of life and iron overload. ESA was the first-line agent used for the treatment of anemia in patients with LR-MDS, being more effective among patients with serum erythropoietin (sEPO) levels ≤500 U/L and limited transfusion burden, leading to an overall response rate of 30% to 45% and an 18- to 24-month median duration of response.[5] Thus, in the recent years, many efforts have been made to improve the response rate of these patients as a first- or second-line therapy.

Targeting the transforming growth factor β pathway

SF3B1-mutated MDS are a distinct MDS subtype, as initially proposed by the IWG for the prognosis of MDS[14] and now fully defined in the recent World Health Organization 2022 classification of MDS,[1] largely overlapping MDS with ring sideroblasts (MDS-RS or with *SF3B1* mutation).

Luspa is a recombinant fusion protein derived from human activin receptor type IIb linked to a portion of immunoglobulin G. Transforming growth factor β (TGF-β) signaling is mediated by a regulatory circuit of inhibitory and activating SMAD proteins that can inhibit the proliferation of hematopoietic stem cells (HSCs) while increasing erythroid differentiation, altogether leading to dysplastic erythropoiesis and reduced erythroid output with anemia phenotypes.[15]

The MEDALIST trial was a phase 3, randomized, double-blind, placebo-controlled study, assessing the efficacy of Luspa vs placebo in LR patients with MDS-RS refractory/intolerant or ineligible for ESA (EPO level >200 U/L) and RBC-TD.[12] In total, 153 patients received Luspa at the starting dose of 1 mg/kg subcutaneously every 21 days, while 71 patients received a placebo. According to the longer-term analysis of this study[16] and applying the new IWG 2019 response criteria,[17] the primary end point of RBC-TI ≥8 weeks was achieved in 69 (45.1%) patients in the Luspa arm vs 12 (15.8%) in the placebo arm (P<.0001); RBC-TI ≥16 weeks was achieved in 43 (28.1%) in the Luspa arm and 5 (6.6%) in the placebo arm (P=.0001).[16] One limitation of this study is that there were potential differences in the criteria for response assessment between this trial and previous studies. Importantly, the drug was well tolerated, and there was no evidence for disease progression on therapy.

There are several other clinical trials evaluating Luspa in non–MDS-RS as well as in combination with other agents, as a first- or second-line therapy. The interim efficacy analysis of the COMMANDS study, comparing in a frontline randomized trial Luspa to ESA (NCT: 03682536) in 301 TD non-del5q ESA-naive patients

Table 1. Available therapies in LR-MDS

Agent	Mechanism of action	Population	Identifier/Trial Name	Phase	Status	Ref.
Epoietin alfa	ESA	LR-MDS with anemia	NCT01381809 EPOANE3021	3	EMA approved	5
Darbepoietin alfa	ESA	LR-MDS with anemia	NCT00095264	2	Off-label	6
Lenalidomide	Immune regulatory agent	LR-MDS del5(q)	NCT00179621	3	FDA/EMA approved	7
		LR-MDS without del(5q)	NCT01029262	3	Off-label	8
Azacitidine	HMA	MDS with pancytopenia	NCT01720225	2/3	FDA approved	9
Decitabine	HMA	MDS with pancytopenia	NCT01720225	2/3	FDA approved	9
Eltrombopag	TPO mimetic	MDS without excess blasts, with thrombocytopenia or pancytopenia	NCT02928419 EQoL-MDS	2	Off-label	10
ATG/CSA	Immunosuppressive	LR-MDS with pancytopenia	Retrospective study	NA	Off-label	11
Luspatercept	TGF-β inhibitor	MDS-RS or with *SF3B1* mutation	NCT02631070MEDALIST	3	FDA/EMA approved	12
		LR-MDS with anemia	NCT:03682536 COMMANDS	3	Off-label	13

CSA, cyclosporine A; EMA, European Medicines Agency; HMA, hypomethylating agent; NA, not applicable; TPO, thrombopoietin.

with MDS-LR, was recently published.[13] TI for at least 12 weeks with a concurrent mean hemoglobin increase of at least 1.5 g/dL was achieved in 86 (59%) patients in the Luspa group compared to 48 patients (31%) in the EPO group (P<.0001) with a significantly better duration of response with Luspa (P=.005). Most patients enrolled in this study had RS-MDS, and it should be noted that the responses observed in patients without RS did not differ between the Luspa and ESA arms. Despite some manageable suspected Luspa-related events (fatigue, asthenia, nausea, dyspnea, hypertension, and headache), these results suggest that treating transfusion-dependent patients with LR-MDS with Luspa as a first-line therapy might be beneficial, at least among patients with RS.

Moreover, other new drugs targeting the TGF-β pathway are under investigation (Table 2), including KER-050, a therapeutic protein designed to increase not only red blood cells but also platelets by inhibiting the signaling of a subset of the TGF-β family of proteins to promote hematopoiesis (phase 2, NCT04419649).

CLINICAL CASE 1 (continued)

After 6 months, the patient lost his response to Luspa, and NGS revealed the acquisition of an *IDH1* mutation with 15% VAF, while the patient still had LR-MDS.

Over the past decade, genomic technologies have led to a better understanding of the genetic events underlying the onset and progression of MDS and how they functionally contribute to specific aspects of the disease pathophysiology. These studies have revealed that MDS is driven by a multistep acquisition of genetic alterations that affect a recurrent set of genes, which promote the self-renewal of mutant HSCs and lead to their clonal expansion over their normal counterparts.[18] New agents targeting altered signaling pathways that induce mutant HSC clonal advantage of specific genetic alterations in MDS are currently under investigation, and the trend toward a more individualized, molecularly driven approach to patient care is likely going to increase. (Figure 1 and Table 2).

Somatic mutation-driven therapies: Isocitrate dehydrogenase and spliceosome inhibitors

Isocitrate dehydrogenase (*IDH*) mutations are gain-of-function mutations, leading to an hypermethylated phenotype, disrupting TET2 function, and leading to an impaired hematopoietic differentiation. *IDH1* or *IDH2* mutations are detected in about 10% of patients with MDS. Following US Food and Drug Administration approval in acute myelogenous leukemia, IDH1/2 inhibitors ivosidenib, olutasidenib, and enasidenib (ENA) are currently developed in higher-risk patients with MDS, but some clinical trials also evaluate their efficacy in LR-MDS (Table 2).[20,21] Of note, in a preclinical study, ENA was shown to increase the erythroid differentiation of the hematopoietic stem and progenitor cells without myeloid differentiation, suggesting an erythroid-specific differentiation effect independent of its effect on mutant and wild-type *IDH2*.[22] Based on this preclinical rationale, ENA is under investigation in anemic patients with LR-MDS without *IDH2* mutation (NCT05282459).

MDS cells with splicing factor mutations rely on the wild-type allele for splicing, and the preferential inhibition of the wild-type allele results in lethality of the cells. H3B-8800 is an oral small-molecule splicing modulator, preferentially targeting the sF3b complex, and in preclinical models, including xenograft leukemia models with or without core spliceosome mutations, it has broad antitumor activity.[23] This drug was evaluated in a phase 1, open-label, first-in-human study in patients with myeloid malignancies (n=84) and splicing factor mutations (NCT02841540).[24] Unfortunately, no complete or partial responses meeting IWG criteria were observed; however, RBC transfusion-free intervals >56 days were observed in 9 patients who were transfusion dependent at study entry (15%). Given the high frequency of splicing mutations in MDS, additional splicing inhibitors are also undergoing preclinical assessments.

Table 2. Emerging therapy in LR-MDS

Agent	Mechanism of action	Phase	Population	Identifier	Ref.
Ivosidenib	IDH1 inhibitor	2	Treatment-naive HR-MDS R/R (HMA) HR-MDS R/R (ESA) LR-MDS with anemia All with IDH1m	NCT03503409	20
Enasidenib	IDH2 inhibitor	2	Treatment-naive HR-MDS R/R (HMA) HR-MDS R/R (ESA) LR-MDS with anemia All with IDH2m	NCT03744390	21
		2, with AZA	MDS, excess blats, AML, CMML with IDH2m	NCT03383575	
Olutasidenib (FT-2102)	IDH1 inhibitor	2, with/without AZA/L-DAC	SMD and AML with IDH1m	NCT02719574	
H3B-8800	Splicing modulator	1	SMD, AML, CMML	NCT02841540	24
Roxadustat	HIF inhibitor	3	LR-MDS with anemia, low transfusion burden	NCT03263091	29
		2/3	LR-MDS with anemia	NCT03263091	
Imetelstat	Telomerase inhibitor	2/3	R/R (ESA) LR-MDS	NCT02598661	25
KER-050	TGF-β inhibitor	2	R/R (ESA) LR-MDS	NCT04419649	
Canakinumab	Il-1β inhibitor	1/2, with darbepoietin	R/R (ESA) LR-MDS	NCT04798339	
		2	R/R (ESA) LR-MDS	NCT05237713	
		2	R/R (ESA/HMA) LR-MDS/CMML	NCT04239157	
Emavusertib (CA-4948)	IRAK4 inhibitor	2	Treatment-naive and R/R (ESA) LR-MDS	NCT05178342	
BMS-986253	IL-8 inhibitor	1/2, with/without DEC/cedazuridine	R/R (HMA) HR-MDS R/R (ESA/LEN/Luspa) LR-MDS	NCT05148234	
SX-682	CXCR1 and CXCR2 inhibitor	1	R/R (ESA/LEN) LR-MDS	NCT04245397	
Tomaralimab	TLR2 inhibitor	1/2	R/R (ESA/LEN) LR-MDS	NCT02363491	

AML, acute myelogenous leukemia; AZA, azacitidine; CMML, chronic myelomonocytic leukemia; DEC, decitabine; HIF, hypoxia-inducible factor; L-DAC, low-dose aracytine; LEN, lenalidomide; MDS, myelodysplastic syndrome; R/R, relapse/refractory.

Targeting telomerase activity

As in vitro studies have shown increased telomerase activity compared with controls in MDS cells and that the expression of human telomerase reverse may drive the neoplastic clonal cell expansion, imetelstat, a novel telomerase inhibitor, has been developed in MDS. This is a potent, first-in-class, competitive inhibitor of telomerase enzymatic activity that specifically targets the RNA template of human telomerase. In a phase 2 study that included 57 patients with heavily TD LR-MDS (61% MDS-RS), imetelstat induced durable TI in 37% (8-week TI), with a median TI duration of 65 weeks.[25] Nevertheless, profound myelosuppression was the main side effect of this drug. Results of the phase 3 double-blind placebo-controlled iMerge trial evaluating imetelstat in RBC-TD, ESA-relapsed/refractory LR-MDS were presented at American Society of Clinical Oncology and EHA in 2023.[26] The primary end point was met, 47 patients (39.8%) vs. 9 patients (15.0%) (P<.001) achieving 8-week TI, with a significantly longer duration of TI with imetelstat compared to placebo (51.6 vs 13.3 weeks, P<.01). TI rate was also significantly higher with imetelstat vs placebo across subgroups, including patients without RS. No new safety signals were identified and similar rates of grade ≥3 bleeding and infections were observed on imetelstat and placebo. These results support imetelstat's benefit to a heavily TD LR-MDS patient population, and it is very likely that this drug will join the therapeutic armamentarium of LR-MDS in the coming years.

Targeting the hypoxia-inducible factor pathway

The hypoxia-inducible factor pathway has been implicated in the regulation of hematopoiesis. Roxadustat is an oral hypoxia-inducible factor–prolyl hydroxylase inhibitor. It has been shown to increase hemoglobin and EPO levels as well as reduce hepcidin in patients with chronic kidney disease in phase 3 trials.[27,28] In MDS, roxadustat has been studied in a phase 3, double-blind, placebo-controlled study (MATTERHORN) evaluating the efficacy of roxadustat to treat low transfusion burden anemia in LR-MDS (NCT03263091). Interim results of 24 enrolled patients have shown 8-week and 20-week RBC-TI of 38% and 17%, respectively, with efficacy across MDS subtypes and baseline EPO levels.[29] However, a press release recently reported that the MATTERHORN study did not met its primary efficacy end point (47.5% for roxadustat compared to 33.3% for placebo; P=.217).[30] Another phase 2/3 trial is currently evaluating roxadustat in patients with LR-MDS with anemia (not only low transfusion burden anemia) in China (NCT03263091). While these results are disappointing, it remains important to continue the investigation of low-toxicity oral treatments that can improve quality of life in these patients.

CLINICAL CASE 2

A 65-year-old woman was admitted to our hospital for fatigue, dyspnea, and pancytopenia (Hb, 6.3 g/dL; absolute neutrophil

count, 0.8×10⁹/L; platelets, 23×10⁹/L). A BM biopsy specimen showed a hypocellular marrow with dysgranulopoiesis and erythroid dysplasia and a normal reticulin staining pattern. Karyotype was normal. NGS panel analysis identified an isolated *TET2* (VAF 12%) somatic mutation. She received anti–thymocyte globulin (ATG) plus oral cyclosporine with transfusion support and reached complete remission within 8 weeks following treatment. Sixteen months later, she relapsed with mild pancytopenia, BM was still hypocellular, karyotype was normal, but there was clonal evolution with the acquisition of another *TET2* (VAF 5%) in addition to the prior one (VAF 15%) and an additional *EZH2* mutation (VAF 13%). She was recused for HSCT due to comorbidities and subsequently received HMA therapy without response.

In eligible patients with severe hypoplastic MDS, HSCT should be considered as soon as possible. For those who are not candidates, and for some patients in whom the clinical picture can parallel bone marrow failure phenotype with pancytopenia and hypocellular marrow, anti–T-cell immunosuppressive therapy or HMA therapy is often considered (Figure 1).

In a large retrospective analysis of 207 patients with MDS treated with immunosuppressive therapy, horse ATG plus cyclosporine was more effective than rabbit ATG, and the highest rate of RBC-TI was achieved among patients with hypocellular BM.[11] Moreover, eltrombopag, a thrombopoietin agonist might be also effective as a single agent in patients with LR-MDS with a predominating thrombocytopenia,[10] but thrombopoietin agonist should be avoided in patients with excess blasts, and this drug is not approved in this indication (Table 1).

Low-dose HMA-based regimen

Although in Europe, HMA use is restricted to patients with higher-risk MDS, low-dose HMA (5-day regimen) is commonly used in the United States for patients with LR-MDS with multilineage cytopenia or as second-line therapy. The 5-year follow-up of attenuated dosing schedules of lower-dose HMA (azacitidine or decitabine, daily×3 days in every 28-day cycle) in patients with LR-MDS was recently published.[9] Among the 113 evaluable patients, the overall response rate was 60% with 36% achieving complete response and 18% hematological improvement, with no survival difference between those who received azacitidine or decitabine (median overall survival of 33 months). These results suggest the use of lower-dose HMA in this frail population. Indeed, the 3-day regimen is currently evaluated in a phase 2 randomized study comparing azacitidine and decitabine when given on a shorter than standard dosing schedule in patients with TD LR-MDS (NCT02269280).

Targeting inflammatory signaling

Emerging data demonstrate that inflammation can lead to a selective outgrowth of aberrant stem cells while inhibiting healthy hematopoiesis, resulting in worsening of cytopenia in MDS.[31] Moreover, clonal hematopoiesis mutations, especially in the *TET2* gene, can by themselves lead to a proinflammatory state by making macrophages more proliferative and secretory.[32]

For this reason, several agents are under investigation targeting immune/inflammatory pathways (Table 2). One critical target is IRAK4, which hyperactivates NF-κB. IRAK4 can be found in longer and shorter isoforms, and *U2AF1* and *SF3B1* mutations can lead to altered exon inclusion, leading to preferential production of a longer isoform (IRAK4-L). This longer isoform results in a maximal activation of innate immune signaling pathways.[33] Preclinical studies have shown that inhibition of IRAK4-L by pharmacologic and genetic means can suppress leukemic proliferation, and clinical trials are now evaluating the efficacy of IRAK4 inhibitors (CA-4948, emavusertib) in LR-MDS (NCT05178342).

Among other pathways, a phase 2 trial of canakinumab, an interleukin 1β–blocking monoclonal antibody that is well tolerated in other inflammatory conditions, has recently opened for inclusion (NCT04239157). Interestingly, an exploratory analysis suggested that the presence of clonal hematopoiesis predicts for cardiovascular benefit with canakinumab.[34] Two other trials evaluating canakinumab in MDS are under way, including one in association with ESA (NCT 04798339, NCT 05237713).

A number of additional agents are being studied for LR-MDS targeting the concept of inflammation and innate immunity (Table 2, Figure 1).

Conclusion

The genetic and biological heterogeneity of MDS provides significant challenges in developing new clinical therapeutics, maybe due to the lack of good preclinical in vitro/in vivo model. However, emerging data in lower-risk MDS pathobiology, including the role of the TGF-β pathway, telomerase inhibition, and inflammation, have led to a recent increase in next-generation therapies for these patients. In particular, recent reports from luspatercept and imetelstat trials suggest an alternative to the current standard-of-care treatment for anemia in patients with lower-risk myelodysplastic syndromes with or without ring sideroblasts who require RBC transfusions.

Conflict-of-interest disclosure

Marie Sébert: honoraria: Abbvie, Servier, BMS, Gilead, Jazz Pharmaceuticals.

Off-label drug use

Marie Sébert: There is nothing to disclose.

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Frontline treatment options for higher-risk MDS: can we move past azacitidine?

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Although remarkable international efforts have been ongoing for over 17 years to improve upon azacitidine, representing the standard of care therapy for higher-risk myelodysplastic neoplasms (MDS), there still has not been a positive randomized trial in comparison to azacitidine. Real-world data from numerous trials have shown similar results with a median overall survival of 14–18 months, a 40%-50% overall response rate, and a complete remission rate close to 20%. Despite these outcomes, 6 randomized controlled trials have failed to improve outcomes in this patient population, although relevant issues in some of these studies included improper dose adjustments of the hypomethylating agent, lack of placebo-controlled studies, and lack of overall survival (OS) as a primary endpoint, among others. Critical updates in MDS management include the development of molecular prognostication models (eg, the molecular international prognostic scoring system), updates in classification systems highlighting significant overlap in patients with MDS-increased blasts and acute myeloid leukemia (most relevant to *TP53* mutations), and refinement of response criteria. Although these paradigm-shifting studies have had great impact in MDS management, the current ongoing randomized phase 3 trials were initiated prior, and prognostic stratification remains via the revised international prognostic scoring system) and with bone marrow blast counts of <20%. Notably, azacitidine + venetoclax, azacitidine + sabatolimab, and azacitidine + magrolimab have shown exciting results in large, single-arm studies and have completed accrual in placebo-controlled, double-blind studies with OS as a primary endpoint. We all eagerly await the results of these studies.

LEARNING OBJECTIVES

- To understand major updates in HR-MDS diagnosis, prognosis, and response evaluation
- To understand potential reasons for past failures to improve upon azacitidine monotherapy
- To review the cutting-edge landscape of novel therapies that ideally will change the standard of care for HR-MDS

CLINICAL CASE

A 59-year-old male presents with severe pancytopenia with absolute neutrophil count (ANC) of 0.4 k/μL, hemoglobin of 7.5 g/dL, and platelets of 35 k/μL. Bone marrow aspirate shows 7% myeloblasts with trilineage dysplasia. Cytogenetics showed complex karyotype with monosomy and deletion 17p. Next-generation sequencing (NGS) shows a *TP53* mutation (mt) with a variant allele frequency (VAF) of 56%. The patient has symptomatic anemia but has excellent performance status and no comorbidities. The patient presents to an academic medical center for discussion of potential treatment options and wishes to focus on curative intent.

Introduction

The treatment paradigm for patients with higher-risk myelodysplastic neoplasms (HR-MDS) has remained largely unchanged for nearly 2 decades, with hypomethylating agent (HMA) therapy and allogeneic hematopoetic stem cell transplantation (HSCT) representing the standard-of-care (SOC) therapies to improve overall survival (OS). Tremendous advancements have been made in the prognostic discrimination of patients with HR-MDS with the inclusion of molecular data, although key clinical questions remain in the clinical implementation of these prognostic systems. Similarly, revised diagnostic classifications have further blurred the lines between HR-MDS and acute myeloid leukemia (AML). Although in the long-term these initiatives will ideally increase therapeutic options for our patients, the dichotomies in these systems have brought forth clinical challenges. In addition, although monumental efforts have been undertaken to improve upon azacitidine, we still currently have not had a positive randomized trial in HR-MDS. We believe the horizon remains bright. This review focuses on the key updates in defining the HR-MDS population,

understanding response assessment, and comprehensive discussion on past failed trials with lessons learned to ideally lead to future new SOC therapies.

Updated classifications and risk stratification for MDS

A revised classification was published in 2022 as part of the 5th edition of the World Health Organization (WHO) classification.[1] In the same year, the International Consensus Classification (ICC) of Myeloid Neoplasms 2022 was published.[2] The similarity and differences between WHO 2022 and ICC 2022 are shown in Figure 1. The details on these 2 classification systems have been expertly reviewed elsewhere.[3] Herein, we briefly discuss how these new classifications and their differences might affect the SOC, clinical trial design/enrollment, efficacy evaluation, and response interpretation, as well as regulatory aspects of novel agent approval in patients with MDS. First, both WHO 2022 and ICC 2022 have incorporated the genetic aspects into MDS classification, including the mutations in *SF3B1*, biallelic *TP53* mutations, and deletion of 5q. Biallelic status, as defined by the WHO, includes *TP53* VAF ≥50%, 2 or more *TP53*mt, and/or *TP53*mt with deletion 17/17p abnormalities. Additionally, >90% of patients with biallelic *TP53*mt have complex karyotypes. Although comprehensive allelic status determination requires an assay to evaluate copy number status (eg, genomic hybridization or single nucleotide polymorphism arrays), a majority of patients can have determination with just standard cytogenetic and NGS evaluation. Separately, the WHO 2022 added 2 novel disease entities by morphology, including hypoplastic MDS and MDS with fibrosis, which are absent in the ICC 2022. These 2 unique entities have important clinical implications: hypoplastic MDS is associated with increased responsiveness to immunosuppressive therapy, and MDS with fibrosis is associated with a worse clinical prognosis.

On the other hand, ICC 2022 introduced a novel entity, MDS/AML, defined by 10%-19% blasts in the peripheral blood and/or bone marrow in the absence of AML-defining genetic abnormalities. The advantage of this new MDS/AML subtype is that it may facilitate the enrollment of patients with MDS 10%-19% blasts to either MDS or AML trials and thereby might speed up drug approval for patients with MDS. From a regula-

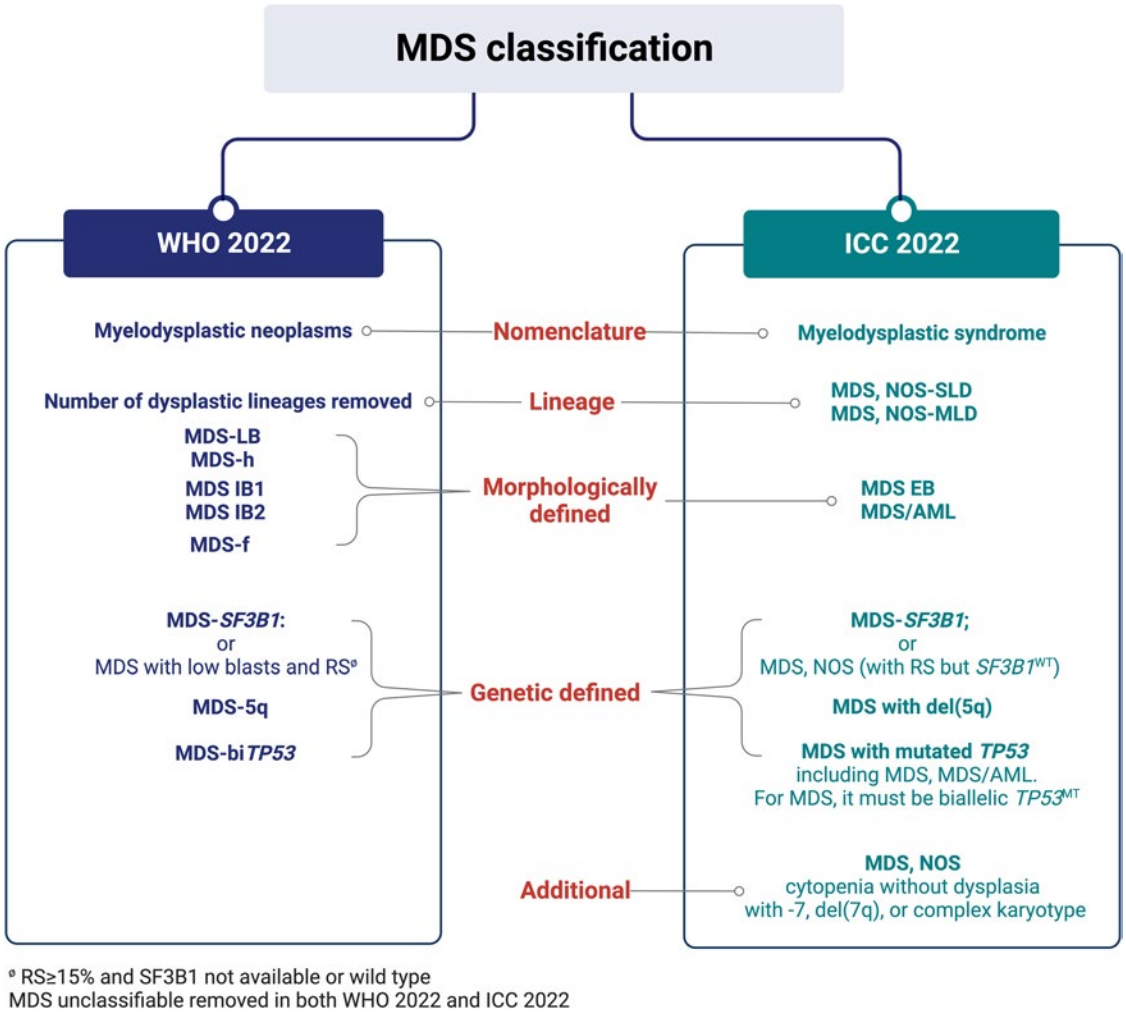

Figure 1. MDS classification comparison between WHO 2022 and ICC 2022. Bi, biallelic; f, fibrosis; h, hypocellular; IB, increased blasts; LB, low blasts; NOS-MLD, not otherwise specified with multi-lineage dysplasia; NOS-SLD, not otherwise specified with single-lineage dysplasia; NOS-MLD RS, ringed sideroblasts.

tory perspective, patients with MDS/AML defined by ICC 2022 may benefit from novel therapies approved in AML. However, we need to keep in mind that patients with MDS are relatively older than patients with AML and have decreased reserves for functional hematopoiesis, which may lead to an increased risk for cytopenia complications, including infection. As such, patients with MDS treated with AML-like therapy may therefore suffer from the risk of overtreatment and toxicities as was evidenced by the requirement of a reduced schedule of venetoclax in patients with MDS (see below). In addition, differences exist in the response criteria between the HR-MDS International Working Group (IWG) 2023 (see below) and AML European LeukemiaNet (ELN) 2022 guidelines. Whether the response to therapy should be assessed based on HR-MDS IWG 2023 or AML ELN 2022 needs further investigation.[4,5]

Recently, the molecular international prognostic scoring system (IPSS-M) was developed and incorporated molecular data, including the allelic state of *TP53*. The IPSS-M now has 6 categories from very low to very high, and the performance of IPSS-M has been recently validated.[6-8] Although clearly this model improves the risk prognostic performance in OS and AML transformation, many questions remain regarding how the IPSS-M should be incorporated into clinical practice, including clinical trial design. Notably, there has not been an HR-MDS study that has incorporated the IPSS-M, although this will likely occur in the near future.

Response criteria in MDS

Ultimate approval of therapeutic agents is partially dependent upon response criteria. In particular, the definition of complete remission (CR) is a critical criterion as it has been shown to be a robust predictor of outcomes in patients with MDS.[9] The IWG originally instituted consensus response criteria for MDS in 2000, with a major update in 2006, which has served as the response system in all pivotal trials on MDS to date.[10] However, these criteria have been subject to critique in HR-MDS, particularly around the stringency of CR calling (ie, blasts <5%, ANC >1.0 × 10^9/L, platelets ≥100 × 10^9/L, hemoglobin >11 g/dL), as well as the impact of marrow CR with or without hematologic improvement (HI).

As an example in 2 prospective clinical trials utilizing CPX-351 as frontline treatment in patients with HR-MDS, the CR rate improved from the low 20s to >50% when ELN response criteria were used instead of IWG 2006 criteria.[11,12] In a large multicenter study evaluating the impact of complete remission with hematologic recovery (CRh) (ie, blasts <5%, ANC >0.5×10^9/L, platelets ≥50×10^9/L) in patients with MDS, there was no difference in OS in patients who achieved CR vs CRh (23 and 25 months, respectively), which were confirmed in multivariable analysis accounting for HSCT.[13] Ultimately, there was a recent consensus proposal in 2023 for revised IWG criteria with key updates shown in Figure 2.[4] Notably, for CR, the hemoglobin threshold has been decreased to ≥10 g/dL with the removal of marrow CR as a response (with caveat of consideration for patients bridged to HSCT). CR with limited count recovery (CR$_L$) and CRh are provisional entities focused on marrow responses in combination with hematopoietic recovery that require prospective validation. Additionally, the blood counts required for responses should occur temporarily around disease assessment (ideally within 2-4 weeks). For CR$_L$, there are additional delineations based on unilineage or bilineage response. Overall response rates (ORR) would include the composite CR response as defined previously in addition to partial remission and HI. Notably, the panel also recognized the importance of serial molecular annotation with the goal to ultimately defining measurable residual disease (MRD) negativity. Multiple studies have demonstrated that achieving NGS or flow negativity is associated with improved OS.[14-17] However, a majority of these studies have not utilized NGS with a sensitivity for robustly capturing MRD as can be done with error-corrected sequencing using molecular barcodes or duplex sequencing. Notably, in 1 key study using MRD NGS via error-corrected sequencing, the risk of progression after allogeneic HSCT was predicted based on analysis at day +30.[14] It is critical for multimodal assessment of MRD to occur in future clinical trials, which may help optimize selection of therapies for patients.

Similarly, clearance of *TP53* VAF to <5% has now been shown in multiple studies to predict improved OS.[16-18] Two recent prospective clinical trials highlighted that clearance of *TP53* VAF may be the best predictor of improved outcomes to HSCT.[19,20] These data are particularly relevant given controversies in the field about the utilization of HSCT in this molecular subset. However, long-term survivors are seen in patients with *TP53* mutant MDS/AML ranging between 20% and 30%; therefore, perhaps repeat NGS and cytogenetic evaluation after therapy and pre-HSCT could best decipher which patients should ultimately

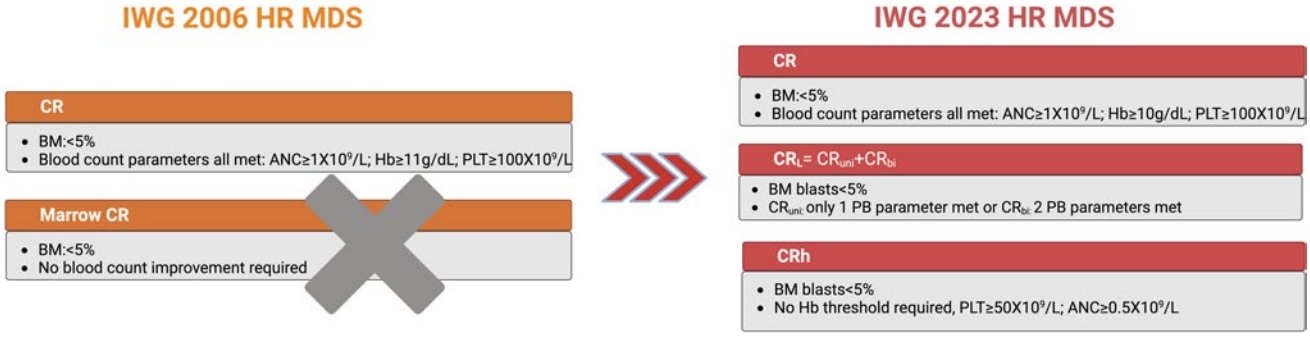

Figure 2. Comparison between IWG 2006 vs 2023 HR MDS response criteria. BM, bone marrow; CR$_L$, CR with limited count recovery; CRh, CR with partial hematologic recovery; CR$_{bi}$, CR with bilineage; CR$_{uni}$, CR with unilineage; Hb, hemoglobin; PB, peripheral blood; PLT, platelet.

move forward with HSCT.[21,22] This is particularly relevant given clear prospective data supporting allo-HSCT in patients that are fit, up to the age of 75 years.[23]

Azacitidine, the unbeaten standard of care for HR-MDS

There has been only 1 positive randomized control trial in patients with HR-MDS, which was azacitidine vs conventional care regimens. This trial showed an improved OS of 24.5 vs 15 months.[24] Unfortunately, real-world data, including current prospective clinical trials, have shown inferior outcomes, with median OS ranging from 14 to 19 months.[25] In addition, many randomized clinical trials (RCTs) comparing novel therapies vs azacitidine have failed (Table 1).[19,20,26-31] As it is critical to learn from past failures, we delve into some of the challenges in improving the SOC and flaws in clinical trial design to ideally help guide future studies in this patient population.

The SWOG S1117 study was a 3-arm randomized study of either azacitidine + lenalidomide, azacitidine + vorinostat, or azacitidine monotherapy. The azacitidine + lenalidomide arm was supported strongly by a prior phase 2 study.[32] Although there was a trend for improved ORR, the trial failed to reach its primary endpoint and also had no improvement in key secondary endpoints. The decreased RR and worse outcomes than the earlier phase 2 study were possibly related to non–protocol-defined dose modifications.[28] Notably, lenalidomide dose reduction was associated with worse OS. Overall, the vorinostat arm did worse than single-agent azacitidine, which is consistent with another RCT with a histone deacetylase inhibitor (ie, entinostat).[29]

The azacitidine + durvalumab study represents the first RCT in HR-MDS to evaluate the combination of immune checkpoint blockade with durvalumab, a PD-L1 inhibitor, to build upon the paradigm shift in management of solid malignancies and some prior data suggesting improved response rates with HMA immune checkpoint combination.[30,33] The ORR (primary endpoint) was 61.9% in the combination arm vs 47.6% with no difference in OS.[30] Notably in patient samples, PD-L1 was increased on granulocytes/monocytes but was not increased on bone marrow blasts, with conflicting prior reports about expression levels in the setting of HMA therapy.[34,35]

Until this time, no RCT had an event-driven endpoint as the primary endpoint of the study. The PANTHER trial was a phase 3 RCT of azacitidine and pevonedistat, a selective inhibitor of NEDD8-activating enzyme, vs azacitidine alone. The trial was supported by a previous phase 2 RCT with a near doubling of the CR rate and improved event-free survival (EFS) in the HR-MDS cohort.[27,36] Notably, the EFS was not positive in the total intention-to-treat (ITT) population, which included patients with oligoblastic AML and CMML. Despite these data, the PANTHER trial enrolled the identical population as the phase 2 trial and had a primary endpoint of EFS. In the ITT analysis, there were no significant differences between the 2 arms regarding EFS and OS (Table 1).[27]

Two parallel phase 2 studies evaluated the combination of the p53 reactivator APR-246 (eprenetapopt) + azacitidine with high CR rates between 44% and 47%.[19,20] This led to the first phase 3 study for patients with mutant *TP53,* although unfortunately the study was negative, with the only reported data by press release and by update on clinical trials.gov (CR rate of 34.6% vs 22.4% by ITT; *P*=0.13).

Last, the phase 2 RCT of sabatolimab+HMA vs HMA alone was recently presented.[31] Sabatolimab is a novel immunotherapy targeting TIM-3. The study had co-primary endpoints of CR and PFS. The CR rate was no different between arms, 21.5% vs 17.7%, *P*=0.769. Although the PFS was not statistically different (11.1 vs 8.5 months; *P*=0.102), the PFS curves separated after 9 months, supporting a potential immune mechanism, and there were subsets of patients that had improved outcomes (ie, <10% blasts or patients without very-high-risk disease). Importantly, the OS curves were essentially superimposable, although notably the median follow-up was only 17 months and thus unclear if there would be late separation similar to the PFS results.

Together, there have been many challenges in prospective randomized trials for HR-MDS encompassing heterogeneous patient populations, response metrics/timing of response, dose adjustments/reductions, and, at times, lack of strong preclinical/translational rationale for the combination. Additionally, the impact of salvage off-label therapies (eg, venetoclax) and increasing utilization of allo-HSCT with clear improved OS in patients up to the age of 75 have added additional complicating factors.[23,37]

A bright horizon for patients with HR-MDS?

As described, there has not been a phase 3 RCT with a primary endpoint of OS, but now there are 3 ongoing studies that have completed accrual, all of which have either a sole primary endpoint or a co-primary endpoint of OS (Figure 3). Notably the STIMULUS-MDS2 phase 3 MDS study is a double-blind RCT study in intermediate to very-high-risk MDS or CMML-2 evaluating the combination of sabatolimab + azacitidine vs azacitidine alone with a sole primary endpoint of OS. Secondary to the negative readout of the phase 2 RCT as described,[31] the current ongoing studies are focused on the evaluation of lower-risk MDS. However, based on sabatolimab's safety profile, additional novel combinations are allowed with this agent.

As the innate immune system plays a significant role in cancer immune surveillance, unleashing key effectors cells of innate immunity, specifically macrophages, represents an attractive therapeutic modality. The antitumor activity of macrophages is tightly regulated by a balance of prophagocytic ("eat-me"; eg, calreticulin) and antiphagocytic signals ("don't eat-me," ie, CD47). CD47 is widely overexpressed on cancer cells, including HR-MDS subsets with overexpression on myeloblasts and leukemia stem cell populations.[38] Importantly, azacitidine leads to robust upregulation of the pro-"eat me" signal calreticulin in myeloid cell lines and animal models.[39] The most mature anti-CD47 agent is magrolimab, which has completed a large phase 1b expansion the study was published.[15] The trial showed a CR rate of 32.6% (40% in *TP53* mutant cohort) with a median duration of CR of 11.1 months and a median OS not reached at a median follow-up of 17.1 months. Importantly, around 60% of *TP53* wild-type patients were alive at data cutoff vs a median OS of 16.3 months in the *TP53* mutant group. Additionally, 36% of patients were bridged to HSCT with a 1-year survival of 91%. Last, MRD negativity, as previously discussed, was achieved in 23% and predicted for improved outcomes. Responses were seen across molecular cohorts, although questions remain if there is enhanced efficacy in patients with *TP53* mutations. The phase 3 ENHANCE study is a 520-patient, double-blind, placebo-controlled study with co-primary endpoints of CR and OS.

Table 1. Randomized trials in higher-risk MDS

Trial name	Phase	Investigational arm	Control arm*	Patient population	Eligibility	Primary endpoint	Results of primary endpoint	Secondary endpoint	Reference #
SWOG S1117	2	azacitidine + lenalidomide (10 mg/day days 1–21)	azacitidine	HR-MDS/CMML	Blasts ≥5%; IPSS ≥1.5	↑ ORR 20% (CR/PR/HI)	49% vs 38% ($P = 0.16$)	No improvement in OS	[27]
SWOG S1117	2	azacitidine + vorinostat (300 mg twice daily on days 3–9)	azacitidine	HR-MDS/CMML	Blasts ≥5%; IPSS ≥1.5	↑ ORR 20% (CR/PR/HI)	27% vs 38%; ($P = 0.16$)	11.6 versus 16.7 months ($p = 0.74$)	[27]
E1905 Study	2	azacitidine + entinostat (4 mg/m^2/day on days 3 and 10)	azacitidine	Therapy-related MDS/AML	Any IPSS	CR, PR, or trilineage HI	17% vs 46%	OS versus 13 months	[28]
FUSION-AML-001 (MDS Cohort)	2	azacitidine + durvalumab (1500 mg IV q 4 weeks)	azacitidine	Int to very high MDS	IPSS-R int to very high	ORR (CR, mCR, HI)	61.9% vs 47.6% ($P = 0.18$)	No Increase PDL1 on BM Blasts	[29]
SUPPORT	3	azacitidine + eltrombopag (200 mg/day, up to 300 mg/day)	azacitidine	Int to HR-MDS	int-1, int-2, high IPSS	Platelet transfusion-free interval	16% vs 31% ($P = 0.001$)	ORR 20% vs 35%	[25]
NCT02610777	2	azacitidine + pevonedistat (20 mg/m^2 IV days 1,3,5)	azacitidine	HR-MDS/CMML/ oligoblastic AML	IPSS-R int to very high	OS	21.8 vs 19.0 months ($P = 0.334$)	EFS 20.2 vs 14.8 months ($p = 0.045$) for HR-MDS	[34]
NCT03745716	3	azacitidine + eprenetapopt (4.5 g IV days 1–4)	azacitidine	*TP53* mutant HR-MDS	IPSS-R int to very high	CR	34.6% vs 22.4%; $P = 0.13$	NA	NA
PANTHER	3	azacitidine + pevonedistat (20 mg/m^2 IV days 1,3,5)	azacitidine	HR-MDS/CMML/ oligoblastic AML	IPSS-R int to very high	EFS	17.7 months vs 15.7 months ($P = 0.447$)	OS 21.6 vs17.5 (0.293) in HR-MDS	[26]
STIMULUS-MDS1	2	azacitidine/decitabine + sabatolimab (400 mg day 8 and 22)	azacitidine/ decitabine	Int to very high MDS	IPSS-R int to very high	CR and PFS	PFS 11.1 vs 8.5 months ($P = 0.102$); CR 21.5% vs 17.7% ($P = 0.769$)	Lower risk and <10% blasts with improved PFS	[30]
STIMULUS-MDS2	3	azacitidine + sabatolimab (800 mg day 8)	azacitidine	Int to very high MDS/CMML-2	IPSS-R int to very high	OS			
ENHANCE	3	azacitidine + magrolimab (priming/loading over C1–2; C3+30 mg/kg days 1 and 15)	azacitidine	Int to very high MDS	IPSS-R int to very high	CR and OS			
VERONA	3	azacitidine + venetoclax (400 mg days 1–14)	azacitidine	Int to very high MDS; excludes t-MDS	IPSS-R int to very high	OS			

*Azacitidine 75 mg/m^2 in all studies with exception of E19905 study, which used 50 mg/m^2×10 days).

BM, bone marrow; CMML, chronic myelomonocytic leukemia; HI, hematologic improvement; Int, intermediate; IPSS-R, revised International Prognostic Scoring System; mCR, marrow CR; PR, partial remission.

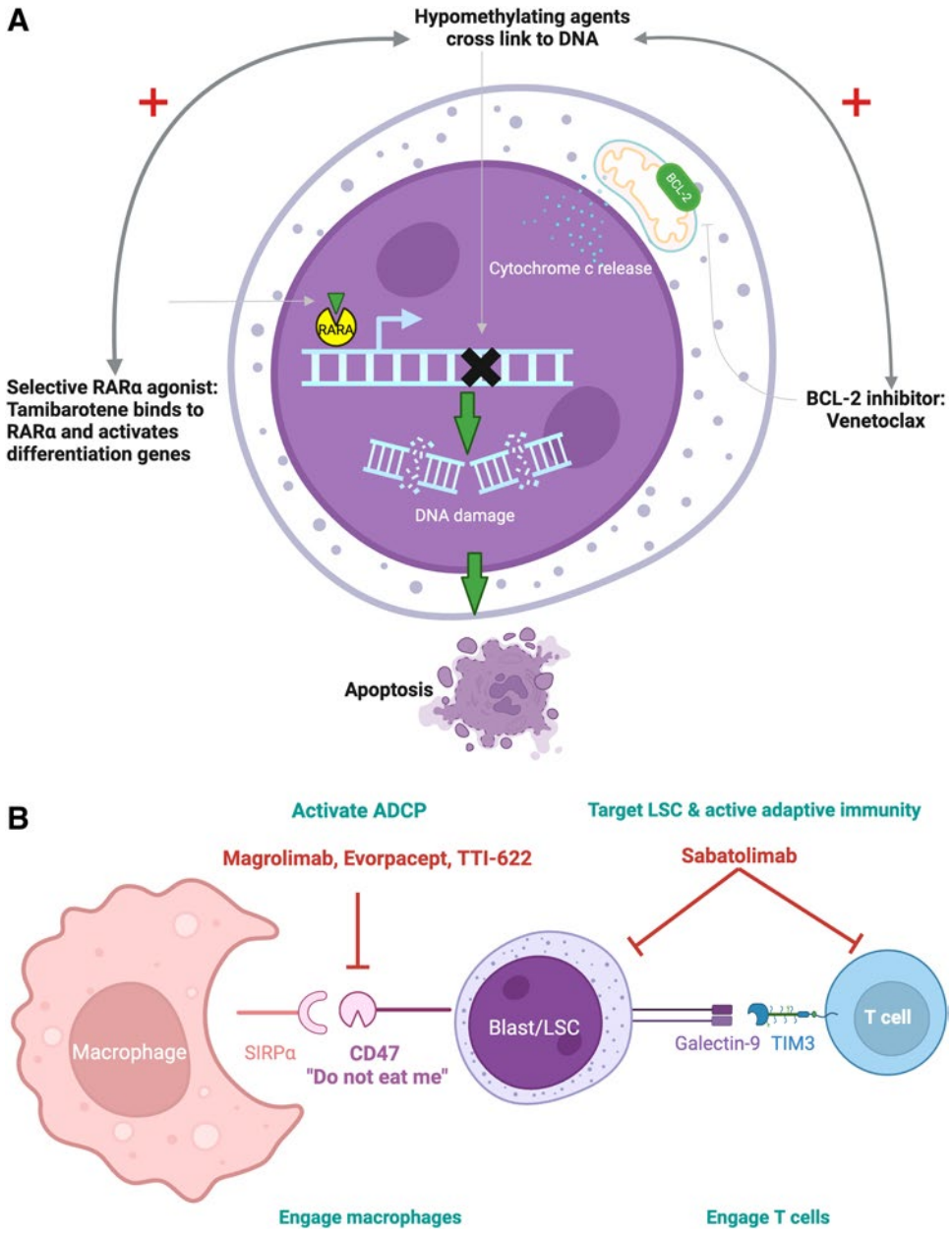

Figure 3. Novel therapy in higher-risk myelodysplastic neoplasms (HR-MDS). (A) Targeted therapy in patients with RARα overexpressing cells with tamibarotene (SY-1425), a selective RARa agonist and blockade of the anti-apoptotic protein BCL-2 via the BH3 mimetic venetoclax; ultimately leading to apoptosis induction with the combination of hypomethylating agents. (B) Novel immune myeloid therapy targeting both the underlying leukemic stem cell (LSC)/blast as well as activating both innate and adaptive immunity. Magrolimab, and other inhibitors of the CD47/SIRPα axis, allowing for activation of antibody dependent cellular phagocytosis (ADCP) and likely subsequent adaptive immune activation via increased antigen presentation. Sabatolimab blocks the galectin-9/TIM-3 pathway leading to direct targeting of the LSC as well as T-cell activation and potentially augmentation of ADCP.

Unfortunately, as of July 2023, there was a press release that magrolimab will be discontinued for patients with MDS, based on futility. Critical questions remain from this study, including the molecular demographics and outcomes in *TP53* mutant vs wild-type populations, among others. Notably, there are 2 additional phase 3 studies in AML evaluating the doublet for *TP53* mutant AML (ENHANCE-2; NCT04778397) and a triplet with venetoclax in all-comer elderly AML (ENHANCE-3; NCT05079230).

Last, the selective BCL-2 inhibitor venetoclax, which has led to a paradigm change in the management of elderly patients with AML,[40] has undergone evaluation in patients with HR-MDS. Specifically, a large phase 1b expansion study with the combination of azacitidine with venetoclax, at a reduced 14-day schedule secondary to cytopenia toxicity, had a CR rate of 40% (16% for patients with *TP53* mutations) and particularly favorable OS for patients who achieved CR/mCR. These data are supported

by additional studies, including recent real-world data in both HMA-naïve and HMA-failure settings, particularly for patients that can be bridged to HSCT.[41-43] These data support the ongoing placebo-controlled VERONA study with a primary endpoint of OS. Although this trial is for all molecular subsets, growing data support lack of improved efficacy in the patient population with *TP53* mutations.[44] Importantly, there are growing data supporting synergy of venetoclax with multiple agents, including with magrolimab.

Ideally, the previously mentioned phase 3 trials will lead to new approvals in HR-MDS, although clearly we are all awaiting the first breakthrough in this patient population; we have now suffered again a major setback with the negative phase 3 magrolimab study. Major questions will arise as far as best sequencing and questions of triplet combinations or sequenced doublets will be of high value. In addition, the only actively accruing phase 3 trial is with SY-1425 (tamibarotene) for RARA-overexpressing HR-MDS (~30%-50% of patients) with a primary endpoint of CR (Figure 3). Additionally, there are multiple trials incorporating oral HMA therapy (particularly oral decitabine/cedazuridine) with the novel agents described previously in efforts to decrease the burden of clinic visits required by parenteral HMA therapy.

CLINICAL CASE (follow-up)

As the patient was identified to have multi-hit *TP53*-mutant MDS, the patient was presented a clinical trial option with a novel HMA combination regimen. In addition, the patient was referred for HSCT consultation with the plan to bridge to transplant when *TP53* VAF was <5%.

Conflict-of-interest disclosure

David A. Sallman: research funding, Aprea; advisory board and steering committee member, Gilead.
Zhuoer Xie: no competing financial interests to declare.

Off-label drug use

David A. Sallman: Venetoclax in MDS.
Zhuoer Xie: Venetoclax in MDS.

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EVIDENCE-BASED MINIREVIEW

Mutational screening to improve the transplantation decision-making process in MDS

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LEARNING OBJECTIVES

- Discuss the clinical benefit of allogeneic hematopoietic stem cell transplantation (HSCT) in older patients with MDS
- Describe the clinical relevance of genomic profiling in MDS
- Provide evidence for the utility of genomic information to define eligibility for and the optimal timing of transplantation

CLINICAL CASE

Two patients aged 72 and 71 years, referred to as patient A and patient B, respectively, were admitted to our institution in March 2022. Both presented with blood cytopenia and were diagnosed with myelodysplastic syndrome (MDS) with multilineage dysplasia according to World Health Organization 2016 criteria. Bone marrow blasts were less than 5%, and karyotype was normal in both cases. Mutation screening revealed an isolated *TP53* gene mutation in patient A and mutations in *TET2* and *SRSF2* genes in patient B. Accordingly, both patients were classified as intermediate Revised International Prognostic Scoring System (IPSS-R) risk and as moderate-low risk according to the Molecular IPSS (IPSS-M) score. After 6 months, patient A acquired a 7q deletion and an additional *TP53* gene mutation, thereby identifying an MDS with biallelic *TP53* inactivation. These molecular changes did not affect IPSS-R risk assessment; however, the patient was shifted to a very high risk of disease evolution by IPSS-M. In contrast, patient B had a stable disease without acquiring additional genomic lesions (Table 1).

Advances in MDS risk assessment

MDSs have extreme clinical heterogeneity, and a risk-adapted treatment strategy is needed.[1] The IPSS-R is used to assess disease-related risk based on the percentage of marrow blasts, blood cytopenias, and specific clonal cytogenetic abnormalities. While IPSS-R is an excellent tool for clinical decision-making, this scoring system may fail to capture reliable prognostic information at an individual patient level.[1]

MDS development is driven by mutations on genes involved in RNA splicing, DNA methylation, chromatin modification and signal transduction; the identification of these driver genomic lesions can provide valuable information on disease evolution, treatment response, and outcome prediction.[2,3] As a result, conventional MDS classifications and prognostic systems are being complemented by the introduction of genomic features that better capture clinical-pathological entities and predict clinical outcome. Recently, a clinical-molecular prognostic model (IPSS-M) was developed using hematologic parameters, cytogenetic abnormalities, and the mutations of 31 MDS-related genes. IPSS-M improved prognostic discrimination across all clinical end points compared to IPSS-R.[4]

At diagnosis, more than 70% of MDS patients belong to very low, low, and intermediate IPSS-R risk,[1] and only a minority of these patients experience leukemic evolution. These individuals are commonly referred to as "low-risk" MDS, in which the treatment goal would be to improve cytopenias and quality of life. In contrast, patients with an advanced disease stage ("high-risk" MDS) require therapeutic interventions to prevent disease evolution and prolong survival. Despite recent therapeutic progress, hematopoietic stem cell transplantation (HSCT) is the only potentially curative treatment for MDS. However, the effectiveness of transplantation is considerably limited by morbidity and mortality, and therefore an accurate patient selection is needed.[5] Several factors must be considered in the decision process of how to select MDS patients as

Table 1. Clinical and molecular characteristics of patient A and patient B and treatment decisions

	Time of diagnosis		After 6 months of follow-up	
	Patient A	***Patient B***	***Patient A***	***Patient B***
Age	72y	71y	72y	72y
Performance status by KS	>80%	>80%	>80%	>80%
Comorbidities by HCT-CI	Low	Low	Low	Low
ANC × 10^9/L	0.78	1.5	1	1.8
Hb g/dL	8.2	8	8.1	8.3
PLT × 10^9/L	135	95	167	91
% of marrow blasts	4	3	4	3
Cytogenetics by IPSS-R	Normal karyotype (good risk)	Normal karyotype (good risk)	Isolated del(7q) (intermediate risk)	Normal karyotype (good risk)
Gene mutations (VAF)	*TP53* (5%)	*TET2* (43%), *SRSF2* (31%)	Additional *TP53* (4%)	*TET2* (42%), *SRSF2* (32%)
IPSS-R risk	Intermediate	Intermediate	Intermediate	Intermediate
IPSS-M risk	Moderate low	Moderate low	Very high	Moderate low
Treatment decision	Watch and wait; consider ESA if moderate to severe anemia persists	Watch and wait; consider ESA if moderate to severe anemia persists	HSCT up-front	To continue ESA

ANC, absolute neutrophil count; ESA, erythropoiesis stimulating agents; Hb, hemoglobin; HCT-CI, HSCT-specific comorbidity index; IPSS-M, Molecular International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System; KS, Karnofsky scale; PLT, platelet; VAF, variant allele frequency.

suitable candidates for HSCT: these consist of age, performance status, comorbidity, disease stage, and status after nontransplant interventions.[5] The complexity of this decision is further highlighted by the fact that many eligible MDS patients do not receive appropriate assessment or consideration for transplantation, particularly outside academic centers (Table 2).

Addressing several critical questions is crucial to increase the implementation of HSCT as a curative option in MDS.

Benefit of reduced-intensity conditioning regimen in elderly patients with MDS

Given that most patients are over 60 years old, it is important to define whether reduced-intensity conditioning (RIC) can provide a valuable clinical benefit in this specific patient population. The rationale for using RIC before HSCT is to shift from high-dose chemotherapy that is aimed at maximizing cytotoxic leukemia killing to a more immune-mediated effect by harvesting the graft-versus-tumor effect to eradicate the disease.[5] The European Society for Blood and Marrow Transplantation conducted a prospective study (RICMAC trial) comparing RIC with a myeloablative conditioning regimen (MAC) in 129 patients with MDS or acute leukemia from MDS. The 2 conditioning regimens showed comparable 2-year relapse-free and overall survival rates (62% and 76%, respectively, after RIC and 58% and 63%, respectively, after MAC; $P = .58$ and $P = .08$). The only risk factor for relapse in a multivariable analysis was advanced disease status.[6] Based on this evidence, RIC can be offered as an alternative to a MAC regimen in MDS patients, especially in those subjects without advanced disease and/or high-risk cytogenetics (recommendation grade 2A).

Benefit of HSCT vs hypomethylating agents

The second relevant question is whether HSCT can improve the survival of older MDS patients in comparison with nontransplant strategies (hypomethylating agents [HMAs]).

This issue was addressed by 2 prospective studies using a treatment biologic-assignment. The VidazaAllo study enrolled 190 patients receiving 4 to 6 cycles of HMAs followed by human leukocyte antigen (HLA)–matched HSCT or by continuous HMAs if no donor was identified. Some methodological considerations regarding immortal time bias for HCST in the study should be taken into account (patients must survive long enough to receive a transplant; that is, they are immortal until they receive a transplant. Since the clock to estimate patient survival starts long before transplant, this can lead to a bias in favor of HCST). Despite these concerns, the analysis on 108 out of 190 patients revealed that after a 2-year period HSCT led to an improvement in event-free survival (though not necessarily overall survival) when compared to continuous HMAs (event-free and overall survival were 34% and 50% after HSCT and 0% and 32% after continuous HMAs treatment, respectively; $P < .0001$ and $P = .12$).[7] Another study, the CIBMTR 1102 trial, compared reduced-intensity HSCT to HMAs or best supportive care in 384 high-risk MDS patients aged 50 to 75. Subjects were assigned to the donor vs no-donor arms according to the availability of a matched donor within 90 days of study registration. After 3 years, the overall survival rate in the donor arm was 47.9% compared with 26.6% in the no-donor arm ($P = .0001$), and the leukemia-free survival was also greater in the donor arm (35.8% vs 20.6%; $P = .003$).[8] Overall, available evidence suggests that HSCT should be considered as a reasonable treatment option for high-risk older MDS patients with HLA-matched donors (recommendation grade 1B).

HSCT from mismatched HLA-related donor

An HLA-matched donor is available for fewer than 50% of elderly patients. The use of HLA-mismatched related donors (especially HLA haploidentical-related donors) significantly increased in the last few years, taking advantage of substantial improvements such as the administration of posttransplant cyclophosphamide

Table 2. Prognostic risk factors relevant for HSCT eligibility, for outcome after HSCT, and for optimal timing of the procedure

Prognostic risk factor	Tools to measure risk factors in patients with MDS	Outcome after HSCT in patients with MDS
Patient-related features		
Age	Calendar, age-adjusted IPSS-R	Worse outcome in elderly patients
Performance status	KS	KS >80% associated with better outcome
Comorbidities	HCT-CI	Low CI associated with better outcome
Disease-related features		
% of marrow blasts	IPSS-R	<5% blasts associated with better outcome
Cytogenetics	IPSS-R	Poor-risk cytogenetics and monosomal karyotype associated with higher risk of relapse
Gene mutations	IPSS-M	IPSS-M high/very high risk (often including *TP53* mutations) associated with poor outcome and high risk of relapse
Disease status after treatment interventions		
ESA-lenalidomide failure	IWG criteria	No direct impact reported
HMAs-ICT failure	IWG criteria	HSCT is the best available treatment after HMAs-ICT failure, but response status is a prognostic factor
Prognostic risk factor	**Tools to measure risk factors in patients with MDS**	**Impact on timing of HSCT**
Disease-related risk (without molecular information)	IPSS-R	Immediate transplantation is associated with maximal life expectancy in patients with early disease (IPSS-R ≤ 3.5), while for those with higher risk delayed transplantation offers optimal survival benefit.
Disease-related risk (including molecular information)	IPSS-M	Patients with higher risk according to IPSS-M should be considered for HSCT earlier than the conventional scoring system (IPSS-R) would dictate.

ESA, erythropoiesis stimulating agent; HCT-CI, HSCT-specific comorbidity index; ICT, intensive chemotherapy; IPSS-M, Molecular International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System; IWG, International Working Group; KS, Karnofsky scale.

as a graft-versus-host disease prophylaxis. Recently, the EBMT reported the outcome of 228 MDS patients transplanted from a mismatched HLA-related donor. One-third of patients were alive and free of disease 3 years after HSCT, and the use of posttransplant cyclophosphamide was found to improve the effectiveness of the procedure, suggesting that haploidentical HSCT is a suitable option for MDS patients lacking an HLA-matched donor (recommendation grade 2C).[9]

Impact of genomic screening on the optimal timing of HSCT

Finally, a crucial issue is to define the optimal timing of HSCT, which would be a disease stage that provides the best life expectancy accounting for both pretransplantation and posttransplantation survival. Patients at early stages may experience long periods with stable disease after diagnosis, and the risks of morbidity/mortality related to HSCT would be unacceptably high for many of them.[1,5] However, a number of studies have shown that advanced disease stage at transplantation is associated with inferior overall survival.[5] Previous decision analyses concluded that delayed transplantation is associated with maximal life expectancy in patients with a lower IPSS-R risk (≤3.5 score points), while for those with high/very high IPSS-R risk immediate transplantation offers the optimal survival benefit.[10] Although these studies provided clinicians with useful information, there are still areas of uncertainty that affect decision-making.

A precise risk score is essential to improve personalized medicine strategies for MDS. The accurate definition of the probability of leukemic evolution is particularly important for lower-risk patients, in whom treatment approaches, including HSCT, may be addressed in a refined manner.[1,5]

The IPSS-M reflects the relevance that molecular characterization can provide on clinical outcomes. Importantly, in the original study in the groups with very low, low, and intermediate IPSS-R risk, 20% of patients were reclassified into a less favorable prognostic category, with over 90% having one or more mutated IPSS-M genes.[4] Therefore, the clinical implementation of IPSS-M is expected to result in a more effective selection of candidates to disease-modifying therapies (including HSCT) among patients with early-stage disease. Accordingly, patients with higher risk according to IPSS-M should be considered for transplantation earlier than the conventional scoring system (IPSS-R) would dictate (Table 2).

Genomic features also impact the posttransplantation prognosis of MDS. *TP53* mutations, especially when combined with complex karyotype, resulted in very poor outcomes after HSCT.[3,4,11] Recently, it was observed that IPSS-M significantly improved prediction of the probability of survival with respect to IPSS-R in MDS treated with HSCT. In particular, IPSS-M was able to efficiently capture the probability of relapse, potentially refining the choice of the optimal conditioning regiment at individual patient level and improving the identification of patients who can be considered for preemptive treatments of disease recurrence.[12]

While prospective studies are needed and the optimal pre-HSCT therapy at individual patient level remains to be clarified (especially in high-risk MDS), genomic screening improves MDS

prognostication and may result in a more effective selection of candidates for HSCT and a better definition of the optimal timing of the procedure (recommendation grade 2C).

CLINICAL CASE (continued)

Both patients required only sporadic red blood cell transfusions and had fewer than 5% bone marrow blasts. However, different therapeutic decisions were made based on their genomic profile. Patient A underwent up-front HSCT from an HLA-haploidentical family donor after RIC, followed by posttransplant cyclophosphamide. Meanwhile, patient B continued to receive regular follow-up and erythropoiesis stimulating agents. As of October 2023, patient A is in good general condition with full donor chimerism; patient B still presents mild anemia without transfusion dependence and no evidence of disease progression. Although a formal validation of the clinical value of a dynamic IPSS-M assessment is still pending, the use of the molecular score may provide a proof of concept to objectively measure (in an easily understandable way for clinicians) the change in patent prognosis when a modification of the genomic profile occurs.

Conflict-of-interest disclosure

Alessia Campagna: no competing financial interests to declare.
Matteo G. Della Porta: no competing financial interests to declare.

Off-label drug use

Alessia Campagna: Nothing to disclose.
Matteo G. Della Porta: Nothing to disclose.

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Long-term follow-up of CD19-CAR T-cell therapy in children and young adults with B-ALL

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The tremendous successes of CD19-directed CAR T cells in children and young adults with B-cell acute lymphoblastic leukemia (B-ALL) has led to the more widespread use of this important treatment modality. With an ability to induce remission and potentially lead to long-term survival in patients with multiply relapsed/chemotherapy refractory disease, more children are now receiving this therapy with the hope of inducing a long-term durable remission (with or without consolidative hematopoietic cell transplantation). While overcoming the acute toxicities was critical to its broad implementation, the emerging utilization requires close evaluation of subacute and delayed toxicities alongside a consideration of late effects and issues related to survivorship following CAR T cells. In this underexplored area of toxicity monitoring, this article reviews the current state of the art in relationship to delayed toxicities while highlighting areas of future research in the study of late effects in children and young adults receiving CAR T cells.

LEARNING OBJECTIVES

- Review the current landscape of subacute/delayed toxicities following CAR T-cell therapy
- Identify approaches to evaluation and management of delayed toxicities following CAR T cells
- Recognize the need for study of late effects in long-term survivors following CAR T-cell therapy

Introduction

The advent of CD19-targeted chimeric antigen receptor (CAR) T cell therapy is changing the approach to the management of relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL) in pediatric patients. Over the past decade, early clinical studies have established a remarkable initial efficacy profile that led to FDA approval of tisagenlecleucel for pediatric B-ALL.[1] Cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS) have been recognized as potentially severe acute toxicities of CAR T-cell therapy. Standardized grading systems, consistent monitoring, and informative correlative studies have led to improved management strategies for these acute toxicities and supported the integration of CAR T-cell therapies into standard of care.[2] In contrast, there is still limited knowledge of longer-term toxicities after CD19-CAR T-cell therapy.

As the field continues to evaluate where CAR T-cell therapy should fit in current treatment paradigms, investigating beyond the acute toxicities of these novel therapies will be critical in making informed treatment decisions. Based primarily on the experience with CAR T-cell therapy in B-ALL, this review focuses on describing the current landscape of subacute/delayed toxicities and late effects following CAR T cells in children and young adults. (Figure 1)

CLINICAL CASE 1

A 19-year-old man with relapsed/refractory B-ALL is referred for CD19-CAR T-cell therapy. He was initially diagnosed at age 15 and relapsed after completing therapy. Reinduction therapy induced a second remission, but he subsequently experienced a second bone marrow relapse. He was then referred for CAR T-cell therapy, with 50% leukemic burden in bone marrow prior to infusion. He was treated with a single infusion of tisagenlecleucel after lymphodepletion with fludarabine and cyclophosphamide. During his acute CAR T-cell treatment course, he developed grade 3 CRS, which was fully reversible with a single dose of tocilizumab. He had no evidence of ICANS. At day 30 after CAR T-cell infusion, bone marrow studies demonstrated MRD-negative remission, and he had B-cell aplasia with hypogammaglobulinemia. However,

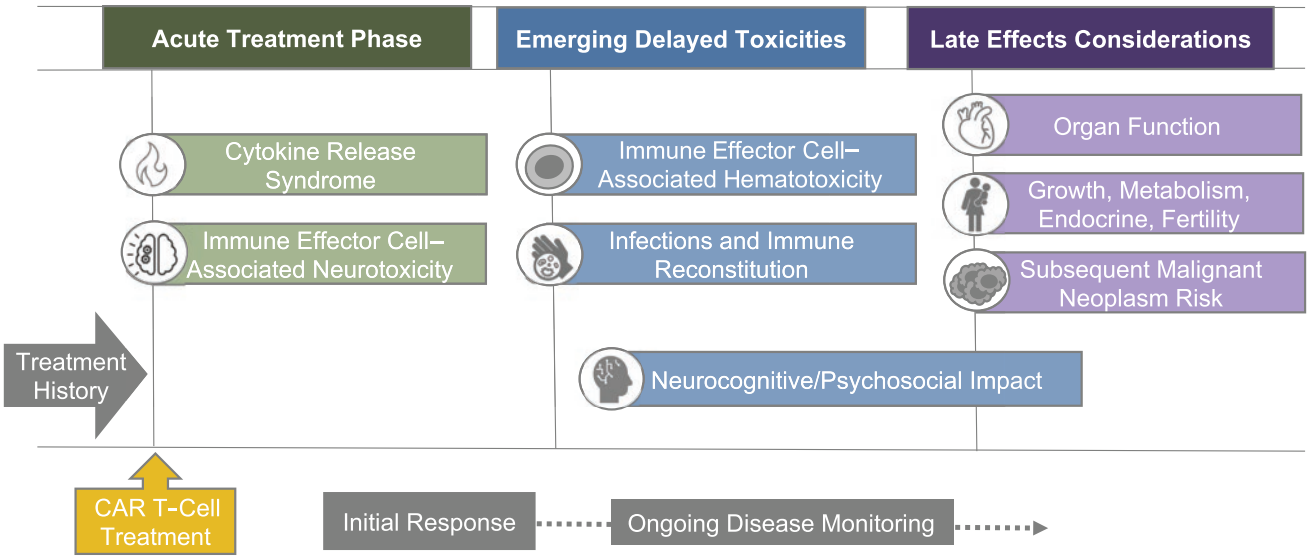

Figure 1. General approach to follow-up after CAR T-cell infusion. CAR, chimeric antigen receptor.

Table 1. Delayed and subacute CAR T-cell toxicities (≥ 30 days post infusion)

Toxicity	Presentation	Risk factors or alternate etiologies
Immune effector cell–associated hematotoxicity	Generalized cytopenias (anemia, thrombocytopenia, neutropenia) with bone marrow hypocellularity and/or transfusion dependence Bimodal pattern of presentation	CRS severity Medication effects Viral or other infection Disease relapse Delayed IEC-HS
Immune reconstitution	B-cell aplasia Persistent hypogammaglobulinemia Recurrent infections (particularly sino-pulmonary) Vaccination responses (prior titers)	On-target, off-tumor targeting
Neurocognitive function	Difficult to assess without formal testing, which would need to be done prospectively. Changes may be subtle and not consistent across domains.	ICANS, severity and association with long-term outcomes unknown
Other end organs	Organ specific (eg, persistent cardiopulmonary compromise)	CRS severity and acute impact on end-organ function during event Site of extramedullary disease

CAR, chimeric antigen receptor; CRS, cytokine release syndrome; ICANS, immune effector cell–associated neurotoxicity syndrome; IEC-HS, immune effector cell–associated hemophagocytic lymphohistiocytosis-like syndrome.

he also had cytopenias with decreased bone marrow cellularity (5–10%), an absolute neutrophil count of 250 cells/μL, and platelet and red blood cell transfusion dependence. At 3 months, his repeat bone marrow confirms ongoing remission, but he remains with severe neutropenia although transfusion requirements are starting to decrease. He has not had any serious infections during this period.

Delayed toxicities of CAR T-cell therapy

Navigating the management of acute CAR T-cell–related toxicities such as CRS, ICANS, and more recently immune effector cell–associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS)[3] has been imperative in the ability to broadly use these novel immunotherapies. However, implications from these inflammatory conditions, or the treatment thereof, can impact the manifestations of delayed toxicities that occur beyond 30 days following CAR T-cell infusion (Table 1). Emerging experience has revealed bone marrow dysfunction, immune reconstitution, and neurologic impact as key areas of interest for delayed toxicities.[4]

Bone marrow dysfunction

Newly termed as immune effector cell–associated hematotoxicity (ICAHT),[5] there is an increasing appreciation that prolonged cytopenias are a delayed CAR T-cell–associated toxicity, particularly in those with severe CRS.[6] Based primarily on literature from adults with lymphoma receiving CAR T cells, hematologic recovery after lymphodepletion and CD19-CAR T-cell therapy generally follows a bimodal distribution.[7,8] While most patients

recover neutrophil, platelet, and red blood cell counts within the first month after CAR T-cell therapy, early clinical studies have reported that 40%–50% of patients have persistent grade 3–4 neutropenia or thrombocytopenia 30 days after CAR T-cell infusion.[9] While some patients recover spontaneously, up to 15% have persistent severe cytopenias beyond 3 months.[10] While prior treatment, disease burden, and baseline inflammatory status are thought to predispose to early cytopenias, risk factors for delayed cytopenias in pediatric and young adult CAR T-cell recipients have not been well described.[9]

In addition to providing transfusion support, patients with persistent cytopenias should be evaluated for any contributing destructive or consumptive etiologies.[11,12] While there are no standard definitions for bone marrow dysfunction after CAR T-cell therapy, patients meeting criteria for aplastic anemia in at least two of three cell lines or with single lineage involvement and evidence of bone marrow hypoproduction may be suspected of abnormal marrow function. While growth factors are generally used with caution after infusion due to potential for exacerbating inflammatory side effects, the benefit of granulocyte-colony stimulating factor may outweigh this risk in patients with prolonged neutropenia, particularly with active infections.[12] For recipients of prior hematopoietic cell transplant (HCT), administration of a CD34+ hematopoietic progenitor cell boost from the prior HCT donor may improve cytopenias.[13,14] Further investigation is needed to understand the etiology of prolonged bone marrow dysfunction observed in a subset of patients after CD19-CAR T-cell therapy, as this is beyond the expected recovery duration from lymphodepleting chemotherapy and not explained by direct on-target off-tumor effects. Particularly, with increasing utilization of alternative CAR T-cell constructs, monitoring for these delayed cytopenias across new trials will remain critical. Future directions seek to develop consensus grading and management approaches.[8] The impact on quality of life in patients with persistent cytopenia and utilization of health care resources are other areas of ongoing research.[11]

Immune reconstitution

With CD19-CAR T-cell therapy, B-cell aplasia is an expected on-target off-tumor effect and can serve as a surrogate marker of CAR T-cell persistence. The duration of B-cell aplasia is variable, ranging from weeks to years.[15] While sustained CAR T-cell persistence is valuable for relapse prevention, B-cell aplasia and hypogammaglobulinemia produce a humoral immune defect. While immune globulin supplementation is discontinued in some adult patients in the absence of recurrent infections despite persistent hypogammaglobulinemia, this approach has not been evaluated in pediatrics.[16] Because immune reserve is dependent on plasma cell mass, which increases with age, the adult experience cannot be directly extrapolated to pediatrics.[17] In addition to immune globulin support, prophylactic antimicrobial agents are considered for patients undergoing CAR T-cell therapy. In general, Pneumocystis jiroveci pneumonia (PJP) and herpes viral prophylaxis are recommended at a minimum until CD4+ lymphocyte counts are greater than 200/μL, though optimal duration is not well defined.[12,18,19] Practices for additional antifungal and antibacterial prophylaxis are variable and may include considerations for duration of neutropenia.

Current recommendations are to continue immune globulin supplementation in pediatric patients unless there is evidence of *de novo* production.[12,20] With this supportive care practice, the limited initial experience of late infections is low, with mild upper respiratory infections occurring most frequently.[21] Ongoing immune globulin supplementation limits the ability to assess potential vaccine response after CD19-CAR T-cell therapy. While live vaccinations should be avoided due to safety considerations in patients without immune recovery, further investigation is needed to determine whether there is any clinical benefit for attempting other re-vaccination in patients with indefinite B-cell aplasia.[16,22]

Neuropsychiatric and neurocognitive impact

In the acute setting, ICANS can have variable presentations, ranging from headache and confusion to seizures and somnolence.[23-25] While the most obvious symptoms of ICANS typically resolve within the first month, patients have not been routinely assessed for the persistence of more subtle neurocognitive changes. In quality-of-life measures, patients report that CAR T-cell therapy carries a notable symptom burden in the acute phase but improves over time after therapy.[26] However, in adult cohorts, CAR T-cell recipients report an increased incidence of neuropsychiatric symptoms compared with the general population[27] and concerns for persistence of some cognitive delay, despite generalized improvements.[28] While history of ICANS is identified as a potential risk factor for ongoing neurocognitive and neuropsychiatric effects, these have also been identified in patients who did not experience ICANS.[27,28]

Routine neurocognitive assessments and evaluation for persistent or delayed-onset neurologic toxicities incorporating patient-reported outcomes will be required to better profile the neurologic and psychosocial impact of CAR T-cell therapy. Identifying factors such as persistent anxiety, stress, or depression related to the CAR T-cell treatment experience that impact social function will be necessary to provide optimal psychosocial support to patients and families. This evaluation is complex in a cohort historically exposed to other potentially neurotoxic therapies with delayed-onset symptoms, including intrathecal chemotherapy, radiation, and HCT.[29] Capturing prior treatment exposures will be necessary to isolate which neurocognitive outcomes may be attributed to CAR T-cell therapy and will be vital for decision-making as CAR T cells are increasingly integrated into the care of children with B-ALL.

Other organ toxicities

Additional organ-specific toxicities have been identified in the acute phase after CAR T-cell therapy, particularly cardiac, pulmonary, and renal toxicities in the setting of cytokine release syndrome.[30-33] In the observed experience to date, primarily in adult patients, these effects generally improve with resolution of the acute inflammatory state.[7] With B-ALL, local inflammation at sites of extramedullary disease (eg, pulmonary, periocular) may also be associated with manifestations of unique toxicities.[32,34,35] Accordingly, as approaches in CAR T cells tar geting brain tumors evolve, recognition of tumor inflammation–associated neurotoxicity[36] necessitates both unique monitoring and treatment strategies. Evaluation of novel CAR T-cell targets for a range of malignancies will also require a high index of suspicion for new on-target, off-tumor effects. Further systematic evaluation will be required to determine the delayed toxicities of CAR T-cell therapy on systems

Table 2. Future study of late effects following CAR T-cells

	Recommendations
Long-term monitoring guidelines	At present guidelines specific to CAR T-cell long-term follow-up do not exist. Recommend use of existing guidelines for post HCT (if indicated) or completion of therapy follow-up for specific end-organ monitoring (eg, endocrinopathies, neurocognitive function, cardiac) as related to impact of therapy a patient may have received prior to CAR T-cells. Continue monitoring for B-cell aplasia, hypogammaglobulinemia, and responses to vaccination.
CAR T-cell–associated mutagenesis	To date, CAR T-cell–induced malignancies have not been seen with use of standard approaches to transduction and manufacturing approaches. Continue ongoing monitoring as novel strategies are implemented.
Second malignant neoplasms	Risk is likely not higher with use of CAR T-cells above and beyond what would be anticipated in patients with comparable lines of prior therapy. Close monitoring will be needed as patients receive fewer lines of therapy and get CAR T-cells earlier in the treatment paradigm.
Fertility	The impact of CAR T-cells on fertility is unknown. Systematic studies of patients who go on to father a child/become pregnant and have a live birth are needed. Improved strategies for implementing fertility discussion in the peri CAR T-cell setting are needed (beyond those advising on avoiding pregnancy in the immediate CAR T-cell infusion period).

HCT, hematopoietic cell transplant.

especially relevant to children and young adults, including psychosocial considerations, endocrine, growth, and metabolism, and to evaluate how the long-term risk profile of CAR T-cell therapy compares with other therapeutic options.[4]

CLINICAL CASE 2

A 23-year-old woman with a history of relapsed/refractory B-ALL is now 5 years status post tisagenlecleucel infusion. Her history is notable for a prior myeloablative total body irradiation–based allogeneic HCT from a matched sibling donor. She received CAR T cells for relapsed disease 1 year post HCT. Following infusion, she achieved a complete remission, has not received any subsequent intervention or reinfusions, and remains with B-cell aplasia requiring immunoglobulin replacement. She recently moved to a new state and is establishing care with a survivorship clinic. Her new provider asks her about recommendations for long-term follow-up after CAR T cells.

Late effects of CAR T-cell therapy

As the earliest cohorts of children and young adults who received CAR T-cell therapy for B-ALL are entering into a decade post their initial infusion, there is an emerging need to understand late effects for children and young adults who receive this novel therapy. With the goal of improving long-term durable remissions, extended follow-up from initial studies confirm that CD19-directed CAR T cells may be used as a singular therapy in a subset of patients[37] or as a bridge to HCT for others.[38,39] Experience accumulated over the past decade has generated important insights into clinical factors important for maintaining long-term durable remissions.[40,41] Evolving strategies will likely serve to help differentiate patients in whom CAR T cells will be curative as standalone therapy versus those at highest risk of treatment failure where risk-mitigation strategies to prevent relapse, such as a preemptive consolidative HCT, may be indicated, particularly for an HCT naïve patient.[42] Accordingly, the number of children and young adults who receive CAR T cells will continue to increase, as will the proportion of patients who live into the survivorship phase.

Long-term monitoring following CAR T cells

At present, there are no standard guidelines specific to long-term monitoring in recipients of CAR T cells (Table 2). As patients who are referred for CAR T cells are those with relapsed/refractory disease and have generally received multiple lines of prior therapy (including HCT) or will be receiving HCT, referral to survivorship clinics and/or adopting use of guidelines applicable to monitoring organ-specific toxicities in the post-HCT or completion of therapy setting will be critical until CAR T-cell–specific late toxicities are more well-established.[43,44] Similarly, current recommendations for screening and monitoring neurocognitive function in long-term survivors of B-ALL therapy could be evaluated for use in ongoing follow-up for patients receiving CAR T cells.[45,46]

As recent data have shown that contemporary survivors of standard-risk ALL have reduced late mortality and morbidity,[44] it will be imperative to evaluate whether long-term morbidity and mortality continue to decrease with earlier utilization of CAR T cells prior to receiving multiple lines of salvage therapy and potentially reducing the need for HCT.

CAR T-cell–associated mutagenesis (or lack thereof)

Beyond single CAR T-cell infusions, reinfusion of the same CAR T-cell product[47,48] or use of an alternative CAR T-cell construct[49] for preventing or treating post–CAR T-cell relapse is increasingly being employed. How this utilization, with receipt of multiple doses of genetically modified therapy, impacts long-term outcomes remains to be seen. Reassuringly, extensive data over numerous CAR T-cell trials have shown no evidence of replication competent retrovirus/lentivirus using standard CAR T-cell manufacturing and transduction methodologies.[50,51] However, with technological advances, ongoing monitoring will be needed—as shown in a recent case of CAR T-cell–associated lymphoma using a piggyBac-modified CD19-CAR T-cell construct.[52]

Second malignant neoplasms

In addition to considerations of CAR T-cell–associated malignancies, patients remain at risk of developing second malignant neoplasms based on their prior therapies. The additive impact of CAR T cells in this setting is unknown but reassuring, suggesting that the incremental risk of CAR T cells (and the associated lymphodepletion chemotherapy with fludarabine and cyclophosphamide) on second malignant neoplasms is not higher than what would be expected in patients who are heavily pretreated.[53,54] Earlier incorporation of CAR T cells prior to multiple lines of therapy and/or HCT may improve the risk of second malignancies overall and warrants further study.

Lineage switch, which is an immunophenotypic switch of the underlying genomic clone, as to be differentiated from a second malignant neoplasm, remains problematic—particularly in B-ALL following immunotherapy. While the overall incidence remains unknown, a recent study suggests that it comprises 7.2% of all the relapses seen following CD19-CAR T cells in a pediatric population—all of whom had poor outcomes.[55] As most cases occurred acutely (much earlier than 2 years post infusion), it remains unclear whether patients will remain at risk of lineage switch when they are several years out from CAR T cells.

Fertility following CAR T cells

As children and adolescents move into the phase of cancer survivorship, issues of fertility often move into the forefront. Guidelines for fertility preservation,[56,57] generally implemented prior to initiation of therapy—as feasible and if age appropriate—establish a critical foundation for enhancing long-term quality of life in cancer survivors. In acute leukemia, however, fertility preservation may not be possible prior to initiation of therapy, and concern for residual disease in sanctuary sites like the ovary[58] (eg, for ovarian cryopreservation) remain problematic. Additionally, in individuals undergoing myeloablative HCT with use of TBI or busulfan, gonadal toxicity is substantial, leading to permanent infertility in most patients.[59-61] In the context of CAR T cells in patients with refractory disease who have received multiple lines of prior therapy, potentially including myeloablative HCT, concerns for preexisting infertility and the need to get to CAR T cells urgently often precludes discussions regarding fertility.

Nonetheless, with increasing use of CAR T cells to spare HCT and/or additional chemotherapy, several patients who have had children after using CAR T cells (either fathered a child or became pregnant with a live birth) have been briefly reported.[62] Indeed, as CAR T cells are used earlier, the proportion of patients in whom fertility could be preserved may increase—making it imperative to systematically address fertility issues in the peri–CAR T-cell setting moving forward.

Discussion

The transformative impact of CAR T cells for children and young adults with B-ALL is undisputed. Indeed, those with chemotherapy refractory disease and whose hope of cure was dismal are now surviving. As the CAR T-cell use becomes more prevalent and moves earlier into the treatment paradigm, understanding both the subacute and delayed toxicities, alongside identifying issues unique to CAR T cells in the study of late effects and survivorship, will become paramount. As CAR T cells continue to expand in scope with novel antigen targeting, combinatorial strategies and across different diseases, issues of delayed toxicities and post–CAR T-cell survivorship will increase, particular as the therapeutic index of these novel strategies improves. We outline current considerations and anticipate tremendous growth in the study of delayed toxicities and late effects over the next decade.

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Off-label drug use

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Stem cell transplantation for ALL: you've always got a donor, why not always use it?

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Hematopoietic stem cell transplantation (HSCT) represents a consolidated therapeutic strategy for high-risk pediatric acute lymphoblastic leukemia (ALL), offering the potential for curative treatment. This manuscript delves into the debate around the more universal application of HSCT for pediatric ALL in the modern era, considering the ubiquitous availability of suitable donors. In fact, despite significant advancements in chemotherapy, targeted therapy, and immunotherapy, a subset of pediatric patients with ALL with high-risk features or relapse continue to encounter poor prognostic outcomes. For this subgroup of patients, HSCT often remains the only potentially curative measure, leveraging the graft-versus-leukemia effect for long-term disease control. Nevertheless, the procedure's complexity and associated risks have traditionally curtailed its widespread use. However, the scenario is shifting with improvements in HLA matching, availability of alternative donor sources, less toxic conditioning regimens, and improved supportive care protocols. Concurrently, emerging therapies like CD19+ CAR T cells present new considerations for definitive therapy selection in relapsed/refractory ALL. This article reviews critical current evidence and debates the potential of HSCT as a more universal treatment for ALL, reevaluating traditional treatment stratification in light of the constant availability of stem cell donors.

LEARNING OBJECTIVES

- To understand the current landscape of donor availability for HSCT in pediatric ALL and the reasons why HSCT is not utilized universally
- To weigh the benefits and drawbacks of HSCT in pediatric ALL, including survival outcomes, risk of GvHD, and treatment-related toxicities
- To explore the potential of integrating HSCT with other treatment modalities such as CAR T therapy
- To understand how advances in HSCT have improved accessibility to treatment for pediatric ALL, reducing disparities in care

CLINICAL CASE 1

A 9-year-old male presented with relapsed B-lineage acute lymphoblastic leukemia (ALL), which was first diagnosed when he was 2 years old. He received ALL treatment per BFM-90 in his home country. This patient had his first relapse at the age of 7 and was treated with chemotherapy per BFM-95. He presented to our institution with a second relapse and had both bone marrow (BM) and central nervous system involvement. Cytogenetics testing showed complex chromosomal abnormalities, including additions of unknown material to chromosomes 3, 7, and 10, as well as monosomy 17. He received CD19 CAR T cells (Kymirah) and achieved complete remission by both flow cytometry and next-generation sequencing (NGS). Subsequently, the patient underwent allogeneic hematopoietic stem cell transplantation (HSCT), while still having B-cell aplasia (BCA) and in complete remission (CR) as determined by NGS minimal residual disease (MRD), with rabbit antithymocyte globulin, 1320 cGy of fractionated total body irradiation (TBI), thiotepa 10 mg/kg, fludarabine 160 mg/m^2, followed by TCRαβ/CD19+ B-cell–depleted peripheral blood stem cell graft. The post-HSCT course was complicated by transplant-associated thrombotic-microangiopathy, which subsequently led to renal failure. Six months post-HSCT, he experienced a third leukemia relapse (BM only). As a result, he received institutional CD19/22 CAR T (NCT03233854) but had no response. A

Heme Stanford Actionable Mutation panel performed on leukemic BM cells shortly prior to his death showed pathogenic *TP53* and *NRAS* mutations with high variant allele frequency not identified at the diagnosis sample.

CLINICAL CASE 2

A 20-year-old patient presented with very-high-risk B-lineage ALL first diagnosed at 14 years old. Cytogenetic testing revealed hypodiploidy. He received treatment per COG AALL1131 and achieved CR with negative MRD by flow cytometry at end of the induction cycle. He relapsed 16 months after the end of his chemotherapy treatment (BM only) and was treated with institutional CD19/CD22 CAR T cells (NCT03233854) 1 month after relapse. Foundation One testing on the relapsed BM sample prior to CAR T infusion found CDKN2A/B exon 2–3 loss and RUNX1T1 A471V alteration. He achieved CR2 with CAR T cells but had loss of BCA 3 months later and relapsed 6 months post CAR T. He was then treated with an individualized protocol consisting of inotuzumab, blinatumomab, dexamethasone, and vincristine. Once in remission, he underwent HSCT using a haploidentical sibling BM graft conditioned with TBI 1200 Cy, cyclophosphamide 100 mg/kg, thiotepa 10 mg/kg, and 100 mg/kg of posttransplant cyclophosphamide. Tacrolimus and mycophenolate were administered as GvHD prophylaxis. His transplantation course was complicated by veno-occlusive liver disease with hepatorenal syndrome requiring dialysis, as well as idiopathic pneumonitis syndrome. At the last follow-up (120 days post-HSCT) the patient is alive, disease free, and off steroids and etanercept used to control the pulmonary alloimmune-mediated complication.

Introduction

Pediatric ALL, a rapidly progressive hematologic malignancy, is the most prevalent form of childhood leukemia. Current therapeutic modalities such as chemotherapy, targeted therapy, and immunotherapy have substantially improved overall survival (OS) rates.[1] Despite these advancements, a subset of patients with high-risk features or those who experience a relapse after initial treatment continue to face poor outcomes, with 5-year survival rates approximately at 50%.[2] For this group of patients, allogeneic HSCT often represents the only potentially curative option.[2] HSCT is a long-established standard of care for these patients with long-term favorable OS outcomes.[3] However, its role in the treatment paradigm of ALL remains a topic of ongoing discussion. HSCT leverages the immunologic graft-versus-leukemia effect to eradicate residual leukemic cells, offering the promise of long-term disease control. Even so, HSCT is a complex procedure with significant associated risks, including graft-versus-host disease (GvHD), infections, and transplant-related mortality (TRM), which have traditionally limited its universal application.

In recent years, improvements in donor matching, conditioning regimens, and supportive care, coupled with the advent of alternative donor sources such as haploidentical donors and umbilical cord blood, have expanded the availability of HSCT. Additionally, the recent emergence of CD19+ CAR T cell therapy as viable treatment option for relapsed and refractory (R/R) ALL offers unique considerations for the selection of definitive therapy in these patients. These advances raise a provocative question: if a suitable stem cell donor can always be found for patients with ALL, should HSCT be used more universally in the treatment of this high-risk disease? Here we will explore this question, reviewing the current evidence and providing perspectives on the optimal use of HSCT in the management of R/R ALL (Table 1).

Allogeneic HSCT for pediatric ALL in 2023: an overview

HSCT has an established track record in pediatric ALL, spanning multiple decades and hundreds of pediatric patients. Studies by Pulsipher et al and others show that 5-year event-free survival (EFS) and OS rates post allogeneic HSCT in high-risk pediatric patients with ALL range between 55% and 75%, influenced by factors like conditioning regimen, donor source, and MRD.[4,5] The 2022 Center of International Blood and Marrow Research registry data show a 3-year OS of ~78% in pediatric patients with ALL transplanted with HLA-matched donors (related or unrelated) in CR1, and 68% in CR2. Similarly, excellent results are observed in HSCT using unrelated mismatched donors (68% in CR1, 61% in CR2).[4,6] It is important to note that these survival rates can vary significantly based on several factors, such as the patient's risk stratification, disease status at transplantation, conditioning regimen, donor type, and GvHD prophylaxis.

For instance, while HSCT has been demonstrated capable of achieving durable remission, it is mostly ineffective in patients with detectable disease at the time of HSCT.[7] This observation underscores the importance of effective induction and consolidation therapy for achieving deep MRD negativity prior to proceeding with HSCT in the pediatric population with ALL. A study by Bader et al revealed that MRD positivity ($\geq 10^{-4}$) pre-HSCT was associated with an increased relapse rate and inferior EFS and OS rates when compared to patients who achieved MRD negativity ($<10^{-4}$) before HSCT.[8] Pulsipher et al confirmed that MRD negativity pre-HSCT correlates with improved 5-year EFS in this setting.[9] Thus, therapeutic strategies aiming to maximize the likelihood of achieving MRD negativity pre-HSCT may significantly improve long-term survival in this patient population.

Quality of life post-HSCT is an equally critical aspect. While HSCT can introduce potential complications such as chronic GvHD and long-term organ toxicities, advancements in supportive care and GvHD prophylaxis have led to significant improvements. Although HSCT recipients might experience some long-term effects, most pediatric patients exhibit acceptable to good health-related quality-of-life scores in the years following HSCT compared to patients with other cancers.[10] Collectively, all available data suggest that, while HSCT has shown great benefits in terms of survival outcomes and an improved quality of life, careful patient selection, optimal conditioning regimen, meticulous GvHD prophylaxis, and comprehensive supportive care remain crucial for its success in treating pediatric ALL.

Historically, the use of HSCT has been limited by the availability of matched donors. More recently, the adoption of high-resolution HLA-typing methods has enabled more accurate matching of donors and recipients, leading to better survival and a reduction in the risk of GvHD. Of note, the likelihood of finding an optimal donor varies among racial and ethnic groups, with the probability of identifying an appropriate donor being highest

Table 1. **This table describes key modern studies investigating the optimal use of HSCT in the management of R/R ALL.**

Reference	Design	N; Median age; Range	Indication	Staging	Graft source	Conditioning	GVHD prophylaxis	aGVHD	cGVHD	Relapse	TRM	Survival
Bethge et al (2022)[19]	Prospective	60; 18.5; 1–63	ALL AML MDS MPS MM NM ST	CR1 20% >CR1 22% NR 13%	MMRD	ATG/Flu/TT/Mel	TCRαβ/CD19 depletion	Grade II-IV 10% Grade III-IV 0%	Overall 31% Severe 8%	20%	17%	2 year OS63% DFS 50% GRFS 39%
Pulsipher et al (2022)[17]	Prospective	51; 35; 6–61	ALL AML MDS	CR1 53% CR2 37.3%	MMRD PBSC	MAC-TBI 31%, MAC-Bu 22% RIC-Mel 43% RIC-TLI 4%	TCRαβ/CD19 depletion					2 year OS 75% DFS 75% EFS 69%
Peters et al (2021)[33]	Randomized, controlled, international, multicentered, phase 3	417; 4–21	ALL	CR1 54% CR2 40% CR3 4%	MSD or MD BM, PBSC or Cord	MAC-TBI 50% MAC-Bu 24% Treo 23% Other 3%	MSD-CSA only MD-ATG/MTX/CSA	Grade II-IV TBI 37% Chemo29%	Overall-TBI 16% Overall-Chemo 11%	TBI-12% Chemo-33%	TBI-2% Chemo-9%	2-year OS-TBI 91% OS-chemo 75% GRFS-TBI 72% GRFS-chemo 51%
Ruggeri et al (2021)[16]	Retrospective	180; 9.25	ALL	CR1 24% CR2 45% CR3 12% NR 19%	MMRD BM or PBSC	MAC-TBI 25.6% MAC-Chemo 51.6% RIC 22.78%	PtCy	Grade II-IV 28.3% Grade III-IV 12.4%	Overall 21.9% Extensive 9.5%	CR1 25% CR2 37% CR3 50% NR 70%	19.6%	2 year OS 50.8% LFS 38.5% GRFS 29%
Symons et al (2020)[44]	Prospective, single center, phase 2	96; 42; 1–65	ALL AML MDS Lymphoma	CR1 41% >CR1 18%	MMRDBM	MAC-TBI MAC-Bu	PtCy	Grade II-IV 11% Grade III-IV 4%	Overall 15% Moderate to severe 6%	43%	11%	3 year OS 54% EFS 48%
Bertaina et al (2018)[43]	Retrospective	98: 6.6; 0.1–17.3	AL	CR1 43% CR2 47% Other CR 10%	MMRD PBSC	MAC-TBI 74%	TCRαβ/CD19 depletion	Grade II-IV 16% Grade III-IV 0%	Overall 6%, Extensive 1%	29%	9%	5 year OS 68% LFS 62%
Locatelli et al (2017)[18]	Prospective	80; 9.7; 0.9–20.9	ALL AML	CR1 39% CR2 56%	MMRD PBSC	TBI/TT/Flu TBI/TT/Mel Bu/TT/Flu Bu/Cy/Mel with ATG/Ritux	TCRαβ/CD19 depletion	Grade I-II 30%	Limited 5%	24%	5%	5 year OS 72% DFS 71%

AL, acute leukemia; AML, acute myeloid leukemia; ATG, antithymocyte globulin; Bu, busulfan; DFS, disease-free survival; Flu, fludarabine; GRFS, GVHD relapse-free survival; LFS, leukemia-free survival; MAC, myeloablative conditioning; MD, matched related or unrelated donor; MDS, myelodysplastic syndrome; Mel, melphalan; MM, multiple myeloma; MMRD, mismatched related donor; MPS, myeloproliferative syndrome; MSD, matched sibling donor; NM, nonmalignant disease; NR, not in remission; RIC, reduced intensity conditioning; Treo, treosulfan; TT, thiotepa; ST, solid tumor.

among whites of European descent (75%) and lowest among blacks of South or Central American descent (16%).[11] Thus, it is paramount to develop strategies that allow the use of mismatched donors, which would significantly widen the accessibility of HSCT for every patient in need. One such strategy is the introduction of alternative donor sources, such as haploidentical donors and umbilical cord blood. With haploidentical HSCT, a partially HLA-matched family member can serve as a donor, thus virtually providing a suitable donor for nearly every patient in need. A recent meta-analysis revealed no significant difference in OS between haploidentical, matched, or cord blood transplants. Interestingly, relapse was higher in matched sibling donor transplants, but haploidentical transplants had a higher GvHD incidence, suggesting their safety and a better leukemic control if GvHD is managed. Of note, the meta-analysis predominantly included T-replete haploidentical transplants with specific GvHD prophylaxis (serotherapy, calcineurin inhibitor, and methotrexate or mycophenolate).[12]

The successful implementation of haploidentical (haplo-) HSCT has been largely the result of the development of 2 approaches: the introduction of post-transplant cyclophosphamide (PT-Cy) and the use of ex vivo T-cell depletion strategies.[13,14] The use of PT-Cy after unmanipulated HSCT has shown ability to control severe GvHD in adults.[15] However, long-term outcome data in pediatric patients are still lacking. On behalf of the EBMT, Ruggeri et al analyzed the outcomes of haplo-HSCT with PT-Cy in 180 children with ALL and found that disease status, age at transplantation older than 13, and the use of peripheral blood stem cells (PBSC) were factors associated with decreased OS.[16]

On the flip side, in the Western world, a burst of ex vivo T-cell depletion strategies have been implemented, with the goal of reducing the risk of GvHD mediated by the mismatched alloreactive T cells, while retaining the graft-versus-leukemia effect of effector T cells. A strategy that attracted considerable interest and that is now widely used in both Europe and the US involves the selective elimination of αβ T cells and CD19+ B cells from the graft (αβhaplo-HSCT). This advanced graft manipulation approach enables the transfer of large numbers of donor hematopoietic stem cells and committed hematopoietic progenitors, as well as mature natural killer and γδ T cells to the recipient.[17,18] European studies found risks following αβhaplo-HSCT in pediatric patients with leukemia comparable to HLA-identical sibling or unrelated donor-HSCT, but with lower GvHD rates and improved GvHD-free/relapse-free survival.[19] A 2022 study by Pulsipher et al confirmed low GvHD and TRM rates and suggested the superiority of reduced toxicity preparative regimen in αβhaplo-HSCT settings, challenging the benefits of TBI-based regimens.[17]

Another source of stem cells is umbilical cord blood. Compared to BM or PBSC transplants, UCB transplantation offers advantages such as faster availability, less stringent HLA matching requirements, simpler procurement, and low chronic GvHD rates.[20,21] There is evidence indicating that UCB transplantation may be considered the preferred option in situations at high risk of leukemia relapse, as recently observed in patients with pretransplant persistent MRD.[22]

Is HSCT a universal solution for pediatric R/R ALL? Weighing the pros and cons

As we strive to refine the best therapeutic approach in pediatric R/R ALL, the universal application of HSCT is a subject of continuous debate, especially in light of more recent advancements in cell therapy (ie, CAR T). To fully understand HSCT potential as a standard intervention, it is crucial to weigh the advantages and drawbacks that stem from its broader use in this patient population.

Pros of universal HSCT for pediatric R/R ALL

1. Higher long-term survival rates: several studies indicate HSCT can offer improved survival rates, especially for high-risk or relapsed patients. Leukemia-free survival and OS have been well established in hundreds of patients over decades of collective experience utilizing different donor sources.[4]
2. Potential for cure: HSCT offers the possibility of a definitive cure, rather than the management of disease remission as other therapies.[23]
3. Universal donors' availability: Improvements in HLA-matching techniques have expanded the donor pool, making HSCT a viable option virtually for every patient who needs it.[24]
4. Reduced treatment-related toxicities: Advances in conditioning regimens, supportive care, and strategies to prevent and treat GvHD have helped minimize transplant-associated morbidity and mortality.[25-27]
5. Strategic role of HSCT: HSCT can serve as a powerful alternative or adjunct treatment in patients with refractory disease unable to achieve remission.[5,28]

Cons of universal HSCT for pediatric R/R ALL

1. Risk of GvHD: Despite the implementation of modern prophylactic strategies, the incidence of acute GVHD remains significant, varying greatly depending on the donor sources and contributing to both morbidity and mortality.[26] However, the implementation of graft manipulation strategies remarkably reduced this risk.[29]
2. Risk of relapse: While reduced, the risk of relapse post-HSCT is still present and ranges between 20% and 50%, mostly based on the disease status at the time of HSCT (see also Table 1).[30]
3. Long-term side effects: HSCT recipients may suffer from long-term sequelae, including endocrine, cardiovascular, and psychosocial complications.[31] This risk is mostly associated with the use of myeloablative conditioning regimens. TBI constitutes a key component of myeloablative conditioning for ALL.[32,33] One mitigating strategy is the recent use of volumetric modulated arc therapy TBI, an innovative and precise radiation delivery strategy that has showed favorable toxicity profiles, excellent disease control, and improved organ sparing in children and young adults.[34-36]
4. TRM: Despite tremendous improvements, the risk of TRM remains and accounts for about 5%-20% of patients across donor sources. Infections and severe GvHD still represent the main causes.[37]
5. Cost and resource-intensive: HSCT is a complex procedure requiring significant healthcare resources and can be cost prohibitive in certain settings.[38] Strategizing about cost-effective care models and optimizing resource allocation is crucial to increase the accessibility and affordability of HSCT, ensuring that all children with ALL who could benefit from it can do so.

Look to the future: the expert's perspective

While allogeneic HSCT stands as the current gold standard for pediatric ALL, the revolutionary allure of CAR T-cell therapies

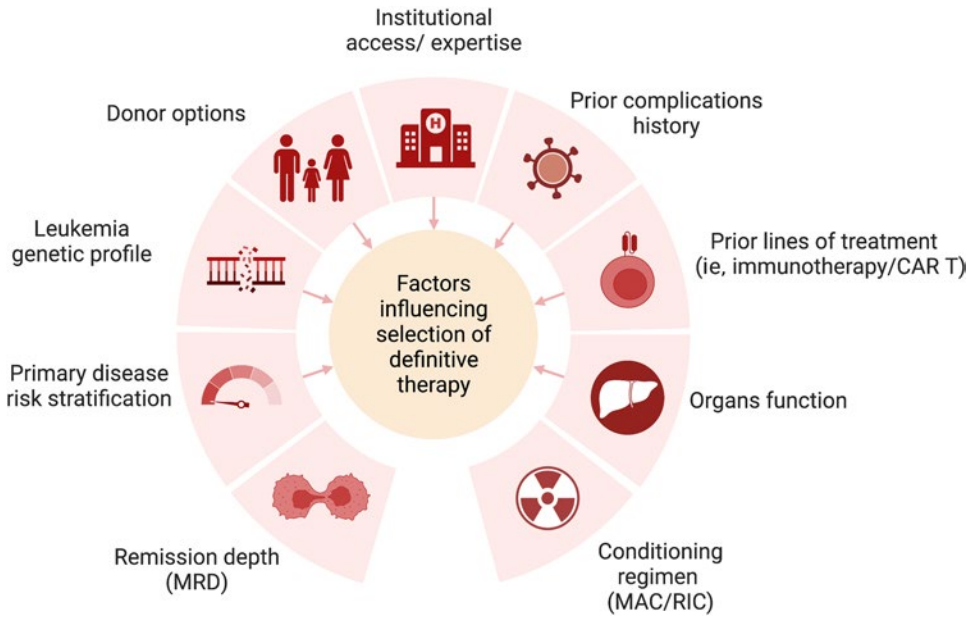

Figure 1. Factors influencing the selection of definitive therapy in R/R pediatric ALL. The chart graphically represents the many factors that contribute to the cost-benefit analysis of the selection of definitive therapy with the highest chance of clinical success in R/R pediatric patients with ALL.

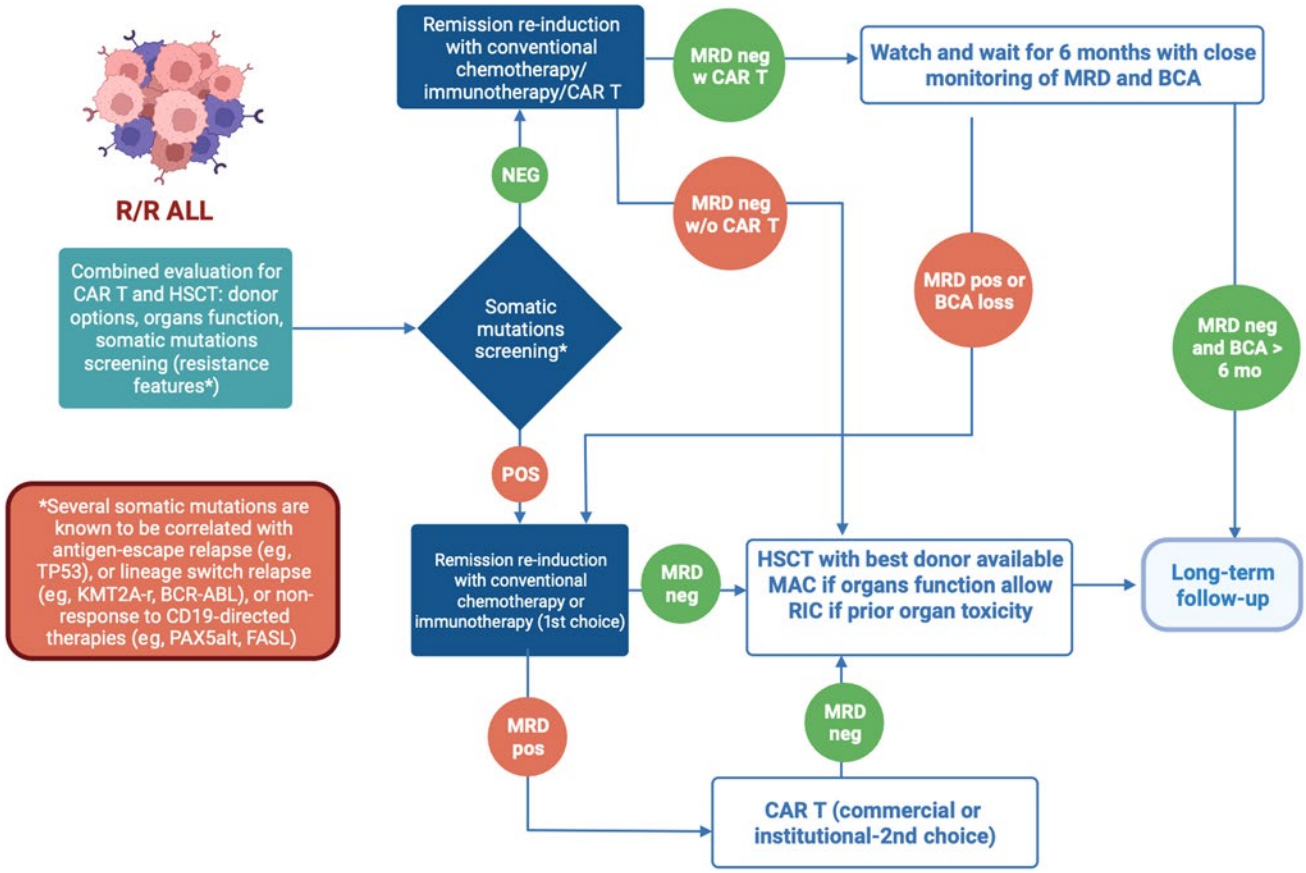

Figure 2. Proposed clinical decision-making flowchart for R/R pediatric ALL. This figure proposes a novel schema for clinical decision making regarding the selection of definitive therapy for R/R pediatric ALL. Given the vast number of considerations that influence the selection of definitive therapy in these difficult cases, this schema provides a framework to guide clinical decision making.

cannot be denied. Boasting impressive remission rates (80% CR MRD at 100 days[39-42]) and a tantalizing glimpse at a potential cure for ALL, CAR T-cell therapies beckon as a game-changing contender in the therapeutic landscape. However, recent data from the pivotal ELIANA trial showed 3-year OS of 63% and relapse-free survival around 50%, suggesting HSCT may be needed for long-term control.[40]

Currently, the convergence of HSCT and immunotherapy represents the most captivating frontier in the field, and we can foresee that the combination of HSCT with innovative cell therapies will lead the wave of future pediatric cancer immunotherapy clinical trials. Identifying biomarkers and other parameters that determine the response to CAR T treatment (ie, anticipate antigen-escape relapse) vs the need for immune and myeloablation followed by HSCT is crucial for successfully pinpointing the need and the optimal timing for HSCT. As described in the clinical case 1, using HSCT as consolidative therapy after CAR T might not always be the right choice. On the other hand, watch and wait for CAR T and/or BCA loss inevitably means significantly increasing the risk of transplant-related toxicity as a result of the need of reinducing leukemia remission (as in the clinical case 2).

In conclusion, in managing pediatric R/R ALL, HSCT and CAR T both present unique strengths and challenges. The selection of treatment must consider a multitude of factors specific to the patient and treatment center, beyond just reported outcomes (Figure 1). To enhance therapeutic decision making, guidelines incorporating disease risk stratification, leukemia genetic profile, ultrasensitive NGS MRD measurement to assess remission depth, organ function, complications history, donor options, conditioning regimens, and cellular therapy access need development. Here we propose a cascade of clinical considerations to decide whether HSCT should be always offered as curative treatment for pediatric R/R ALL or not (Figure 2). While current evidence supporting the use of new diagnostic tools (ie, the role of somatic mutations) for clinical management remains inadequate, their potential to influence treatment outcomes is significant. Determining the optimal treatment for R/R ALL patients continues to be challenging. However, well-designed prospective clinical trials hold promise for more informed decision making, using a range of treatment strategies tailored to the individual patient's needs.

Conflict-of-interest disclosure

David Shyr: no competing financial interests to declare.
Kara L. Davis: no competing financial interests to declare.
Alice Bertaina: no competing financial interests to declare.

Off-label drug use

David Shyr: Nothing to disclose in terms of off-label drug use outside of standard of care practice for SCT.
Kara L. Davis: Nothing to disclose in terms of off-label drug use outside of standard of care practice for SCT.
Alice Bertaina: Nothing to disclose in terms of off-label drug use outside of standard of care practice for SCT.

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Preventing relapse after CD19 CAR T-cell therapy for pediatric ALL: the role of transplant and enhanced CAR T cells

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CD19-specific chimeric antigen receptor (CAR) T-cell therapy has become an integral part of our treatment armamentarium for pediatric patients with relapsed or refractory B-cell acute lymphoblastic leukemia (B-ALL). However, despite initial remission rates of greater than 80%, durable remission occurs in only 40% to 50% of patients. In this review we summarize our current knowledge of the role of consolidative hematopoietic cell transplantation in the management of pediatric patients who achieved a minimal residual disease-negative complete response post CD19 CAR T-cell therapy. In addition, we review approaches to enhance effector function CD19 CAR T cells, focusing on how to improve persistence and prevent the emergence of CD19− B-ALL blasts.

LEARNING OBJECTIVES

- Understand the role of hematopoietic stem cell transplantation post CD19 CAR T-cell therapy
- Explain common mechanisms of ALL recurrence post CD19 CAR T-cell therapy
- Explain approaches to enhance the anti-ALL activity of CD19 CAR T cells

Introduction

CD19-redirected chimeric antigen receptor (CAR) T-cell therapy is an effective therapeutic modality for pediatric patients with relapsed and/or refractory (R/R) acute lymphoblastic leukemia (B-ALL), a patient cohort that historically was largely incurable.[1,2] However, despite initial remission rates of greater than 80% across studies, durable remission occurs in only 40% to 50% of patients. This includes the use of the US Food and Drug Administration–approved product tisagenlecleucel, as well as other CD19-directed CAR T-cell products evaluated in clinical trials.[3-8] While data suggesting risk factors for disease nonresponse or relapse have been reported, including high leukemic disease burden and a history of nonresponse to other CD19-directed therapies, it is currently unknown for which patients stand-alone CD19 CAR T-cell therapy is curative.[8-12]

Outcomes after CAR T-cell relapse are dismal,[13] and it is therefore critical to identify patients at high risk of relapse through close monitoring for CAR T-cell persistence in conjunction with frequent disease evaluations in the first year post CAR T-cell infusion. While many factors have to be taken into consideration when making post–CAR T-cell therapy decisions, a univeral algorithm is yet to be developed. Therefore, a key focus in the field includes efforts to better identify patients at higher risk of disease recurrence post CAR T-cell therapy, as well as investigation of novel treatment approaches aimed to enhance CAR T-cell efficacy.[14]

In this educational review, we present 2 cases highlighting the current challenges and then review the role of hematopoietic cell transplantation (HCT) post CD19 CAR T-cell therapy and approaches to enhance the anti-ALL activity of CD19 CAR T cells. Thus, the broad learning objectives are to i) understand the role of HCT post CD19 CAR T-cell therapy and ii) explain common mechanisms of therapeutic failure and approaches to enhance the anti-ALL activity of CD19 CAR T cells.

CLINICAL CASE 1

A 6-year-old boy with standard-risk B-ALL was treated with standard chemotherapy, achieved remission at the end of induction therapy, and then suffered a relapse during maintenance therapy. He had persistent CD19+ B-ALL after 2 cycles of intensive reinduction chemotherapy. He received CD19-redirected CAR T-cell therapy and

achieved remission, with no detectable clonal cells by next-generation sequencing (NGS) testing. He subsequently proceeded to a consolidative HCT with a matched related donor and remains in remission 2 years post HCT.

CLINICAL CASE 2

An 18-year-old man with primary refractory Philadelphia chromosome–like B-ALL received CD19-redirected CAR T-cell therapy and achieved remission. At 8 months post-CAR infusion, a loss of B-cell aplasia (BCA) was noted. A bone marrow biopsy performed at that time revealed detectable disease by NGS testing, with a rising copy number on a short interval repeat marrow. In the setting of loss of BCA and rising NGS, the patient was considered at risk for impending relapse, received treatment with blinatumomab, proceeded to HCT, and remains in remission without excessive HCT-related toxicity.

HCT post CD19 CAR T-cell therapy: experience to date

Data on the use of consolidative HCT post CD19 CAR T-cell therapy in pediatric patents are limited and come largely from single-center experience using varying CAR T-cell products (Table 1).[3,4,6-8,10,15-17] While at present it is difficult to draw overarching conclusions about the role of consolidative HCT in this patient population, the current experience with HCT post CD19 CAR T-cell therapy can be used to better understand potential predictors of relapse and identify patients who might benefit from HCT.

Tisagenlecleucel (Kymriah) is the only commercially available CAR T-cell product for pediatric patients with R/R CD19+ ALL. It consists of ex vivo activated and expanded autologous T cells genetically modified with a lentiviral vector encoding a CD19 CAR with a 41BB.zeta signaling domain (CD19/41BB). With a follow-up of 38.8 months, the seminal trial and the phase 2 global registration trial (ELIANA) reported high rates of initial complete response (CR) with relapse-free survival of 76% and 59%, respectively.[3,4,16] Analyses of real-world use of tisagenlecleucel by the Center for International Blood and Marrow Transplant Research and the Pediatric Real-World CAR Consortium demonstrated similar response rates.[13,17] Notably, very few patients in these studies proceeded to a consolidative HCT, and there are limited data on those who did.

Other CD19/41BB-CAR T-cell products were evaluated in pediatric patients with R/R B-ALL in early-phase clinical trials. The PLAT-02 study, a phase 1/2 trial, utilized an institutional product infused with a defined CD4/CD8 ratio.[5] Among 64 treated patients, 50 were considered eligible for potential HCT post CAR T-cell therapy. Of these, 23 proceeded to HCT (second HCT, n=10) at a median of 3 months post infusion. Rates of relapse were lower in the consolidative HCT group (5/23 patients) compared to the non-HCT group (19/27 patients). One patient died post HCT secondary to treatment complications. HCT-naive patients had a significantly improved leukemia-free survival compared to non-HCT-naive patients. Regardless of prior HCT status, patients with early loss of BCA (≤63 days post infusion) who underwent consolidative HCT had improved leukemia-free survival compared to those who did not proceed to HCT.[18] Another study with an institutional CD19/41BB-CAR T-cell product demonstrated CRs in 9 of 12 patients treated. Post CR, 5 patients (all HCT naive, including clinical case 1) went on to consolidative HCT at a median of 2.7 months post-CAR infusion. All these patients remain in remission, with 1 patient dying secondary to transplant-related complications. Conversely, the 4 who did not proceed with HCT all subsequently relapsed. Three of these patients were not considered good candidates for HCT due to prior HCT status or a history of extramedullary disease, and 1 relapsed prior to planned HCT.[8]

The benefit of consolidative transplant in pediatric patients treated with T-cell products that express CD19 CARs with a CD28.zeta signaling domain (CD19/CD28) has been demonstrated by several groups. In one study, 15 of 18 responding

Table 1. Selected studies reporting outcomes of CD19 CAR T-cell therapy +/− consolidative HCT

Center/consortium/study	Trial phase	Costim	Patients (no.)	Initial CR (%)	HCT in CR#[a]	Relapse post HCT vs no HCT
CHOP[3]	1	41BB	30	90	3	Relapse: NR vs 8/23 no HCT
ELIANA[4,16]	2		79	81	11	Relapse: 0/8[b] vs NR
Seattle[5]	1/2		64		23	Relapse: 5/23 vs 19/27 no HCT
St Jude[8]	1		12	75	5	Relapse: 0/5 vs 4/4 no HCT
PRWCC[10]	N/a		185	85	20	NR
CIBMTR[17]	N/a		255	86	34	NR
NCI[6]	1	CD28	50	62	21	Relapse: 2/21 vs 7/7 no HCT
MSKCC[15]	post HCT		15[c]	N/a	15	Relapse: 3/15
SMC[7]	2		30	86	25	Relapse: 8/25 vs 4/5 no HCT

[a]HCT while in CR.

[b]Data available for 8 out of 11 patients.

[c]One patient received a CAR/41BB T-cell product.

CHOP, Children's Hospital of Philadelphia; CIBMTR, Center for International Blood and Marrow Transplant Research; costim, costimulatory domain; hu, human; St Jude, St Jude Children's Research Hospital; MSKCC, Memorial Sloan Kettering Cancer Center; mus, murine; N/A, not applicable; NCI, National Cancer Institute; NR, not reported; Pat, patients; PRWCC, Pediatric Real World CAR Consortium; Seattle, Seattle Children's Hospital; SMC, Sheba Medical Center.

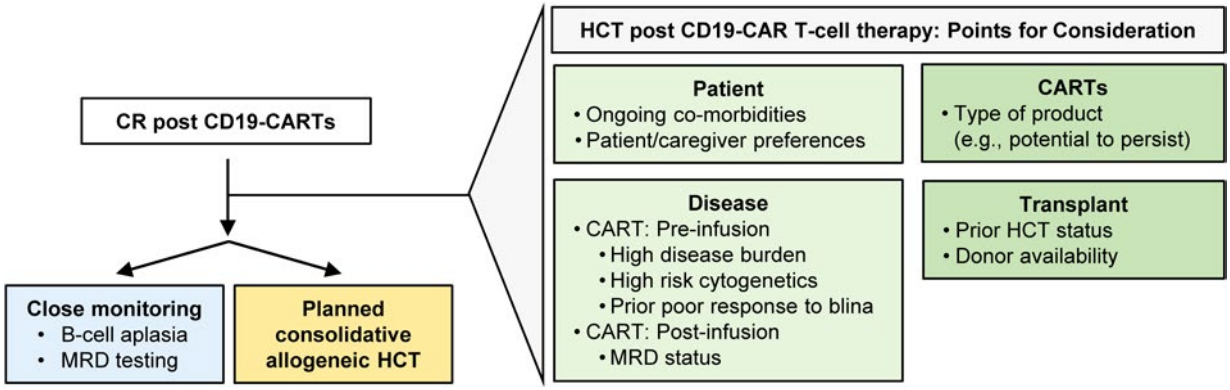

Figure 1. Management approach for patients who achieve remission after CD19 CAR T-cell therapy. The scheme highlights key factors to consider. blina, blinatumomab; CARTS, CAR T cells; MRD, minimal residual disease.

patients proceeded to consolidative HCT at a median of 57 days post infusion. Post HCT, 2 patients suffered disease relapse, and 3 died secondary to HCT complications. Investigators found that patients who received a CD34-selected, T-cell depleted graft or proceeded to HCT fewer than 80 days from CAR infusion had better post-HCT outcomes.[15] In a second phase 1 trial in which 28 patients achieved a CR after CAR T-cell therapy, 21 proceeded to a consolidative HCT, at a median of 54 days post CAR (second HCT, n=4). After transplant, 2 patients experienced subsequent disease relapse, compared to 7 of 7 patients in the nonconsolidative HCT cohort.[6] Additionally, outcomes of a third early-phase clinical study with a CD19/CD28 CAR T-cell product highlighted the benefit of consolidative HCT post CD19 CAR T-cell therapy. Among 30 patients who achieved a CR post CAR T-cell therapy, 25 (17 HCT naive) proceeded to consolidative HCT (median, 71 days). Of these, 8 patients relapsed post HCT, and 2 died secondary to treatment toxicity, with all but 1 patient relapsing in the non-HCT group.[19] In the setting of loss of BCA and rising NGS post CD19 CAR T-cell therapy, a bridging therapy prior to HCT that is readily available (eg, blinatumomab for CD19+ leukemia) might be critical prior to HCT to ensure a disease-free long-term outcome, as illustrated by clinical case 2.

These data collectively indicate that regardless of the utilized CD19 CAR T-cell product, patients who are HCT naive benefit from a consolidative HCT in the setting of limited CAR T-cell persistence. The benefit of a consolidative second HCT is less well established and, given the risk of significant toxicities, should be considered carefully and individualized based on the patient's risk of relapse and ability to tolerate this therapy. In conclusion, based on current literature, it is difficult to give clear recommendations regarding which patients should be considered for HCT post CD19 CAR T-cell therapy. Points for consideration are discussed in the next section and summarized in Figure 1.

HCT post CD19 CAR T-cell therapy: points for consideration

Prospective studies, albeit difficult to conduct, are ultimately needed to inform the critical question on the role of consolidative HCT after CD19 CAR T-cell therapy, identify high-risk pediatric patients, and support the development of evidence-based clinical-decision algorithms. In lieu of such studies, current approaches to this question weigh the risks and benefits of pursuing HCT, considering i) risk factors prior to CD19 CAR T-cell therapy and ii) monitoring for persistence and response post infusion (Figure 1).

Risk factors pre CD19 CAR T-cell therapy

Across several studies, the presence of a high disease burden prior to CD19 CAR T-cell therapy has been associated with a higher risk of relapse after treatment. While a universal cutoff of high burden has not yet been determined, some data suggest a cutoff of as little as greater than or equal to 5% blasts.[6,8,9,11,19] Prior poor response to blinatumomab has also been associated with a higher relapse risk post CAR.[8] Other traditional risk factors for treatment failure have not been recapitulated after treatment with CD19 CAR T-cell therapy, including subgroups with high-risk cytogenetics,[20] Down syndrome,[21] infants,[22,23] and extramedullary disease.[24,25] Thus, CD19 CAR T-cell therapy has the potential to redefine treatments for patients who were historically high risk.

Monitoring post CD19 CAR T-cell therapy

Longer CD19 CAR T-cell persistence is associated with improved relapse-free survival. Post CAR, close monitoring of ongoing BCA and detection of recurrent and/or persistent disease by NGS, polymerase chain reaction, and/or flow cytometry serves as a surrogate of CAR persistence.[5,8,18,21,26] Notably, relapse risk decreases with time postCAR T-cell therapy, and most relapses occur within the initial year after infusion.[6,10,17,21] Even after treatment with CD19/41-BB CAR T-cell products, the loss of BCA within 6 months or less and/or detectable disease post CAR T-cell therapy, including by NGS testing, place patients at high risk of relapse, and these patients may be considered for HCT prior to disease progression.[26] It is important to note that BCA is not a perfect surrogate for relapse risk, as patients may relapse with antigen loss variants or antigen-positive disease concurrent to findings of loss of BCA. Additional considerations include patient/caregiver preferences, provider experience, and often provider assessment of the availability of additional viable treatment options if the patient were to relapse post CAR T-cell therapy. Importantly, as CAR T-cell therapies continue to evolve and the number of patients treated with such therapies increases, continued investigation and reevaluation of such predictors will be necessary. Finally, besides patient selection the preferred HCT approach remains elusive. Ideally, the attainment of deep

remission with CD19 CAR T-cell therapy would potentially allow for conditioning regimens that do not use total-body irradiation.[27]

Enhanced CAR T cells

The limited persistence and emergence of antigen loss variants (ie, CD19− disease) have emerged as the major limitations of CD19 CAR T-cell therapies,[3-8,14] and both roadblocks will be discussed in this part of the review.

Prevention of CD19− relapse post CAR T-cell therapy

Multiple mechanisms of CD19− relapse have been described, including mutations in CD19 that lead to shedding of the extracellular domain, lineage switch with the recurring leukemia having an acute myeloid leukemia phenotype, and the emergence of a preexisting CD19− clone (Figure 2A).[28-31] Likewise, the transduction of contaminating ALL blasts in the T-cell products with the viral vector encoding the CD19 CAR can lead to masking of CD19 on the cell surface, resulting in ALL blasts that are resistant to CD19 CAR T cells.[32] The incidence of CD19− ALL relapse varies post CD19 CAR T-cell therapy and has been reported to be between 18% and 25%.[3-8]

Targeting additional antigens is actively being pursued to prevent the emergence of CD19− ALL blasts. Most efforts are focused on targeting CD22 based on the encouraging results of CD22-CAR T cells as monotherapy for pediatric ALL.[33,34] Conceptually, dual targeting of CD19 and CD22 can be achieved with 3 approaches (Figure 2B): i) sequential or coadministration of 2 CAR T-cell products, 1 expressing a CD19 and the other a CD22 CAR, ii) engineering T cells to simultaneously express a CD19 CAR and a CD22 CAR, and iii) engineering T cells to express a bispecific CAR that recognizes CD19 and CD22. All 3 approaches have been evaluated in early-phase clinical studies, with the largest cohort of patients receiving 2 CAR T-cell products.[35-37] The results of these studies indicate that all 3 approaches are safe; however, it remains to be determined which approach is best to prevent the emergence of antigen loss variants. In addition to CD22, other targets are actively being pursued to develop bispecific or trispecific CAR T-cell products for pediatric ALL.[38-40]

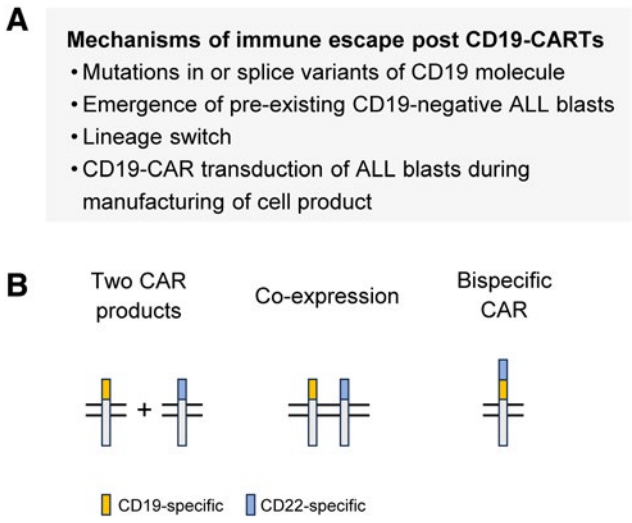

Figure 2. Mechanism and prevention of antigen loss variants post CD19 CAR T-cell therapy. (A) Mechanism of CD19-targeted immune escape. (B) CAR T-cell products to enable dual targeting of CD19 and CD22.

Enhancing persistence of functional CD19 CAR T cells

The functional persistence of CD19 CAR T cells is routinely tracked by enumerating normal CD19+ B cells in the peripheral blood post CD19 CAR T-cell infusion, with the loss of BCA either indicating the limited persistence of CD19 CAR T cells or the persistence of dysfunctional CD19 CAR T cells. Several studies have shed light on the desired characteristics of the leukapheresis product used for CD19 CAR T-cell manufacturing and the product itself associated with the functional persistence of CD19 CAR T cells.[41-44] These include infusing CD19 CAR T cells that are derived from naive T cells and are less differentiated at the end of CD19 CAR T-cell production. However, none of the identified characteristics have been validated prospectively. Likewise, 2 recent studies have highlighted that long-term persisting CD19 CAR T cells have a unique transcriptional profile,[45,46] opening up the opportunity to develop CD19 CAR T-cell products that promote the development of these gene signatures.

Combinatorial therapies

CD19 CAR T cells might routinely undergo exhaustion reprogramming as highlighted for 1 investigational CD19 CAR T-cell product, and combinatorial therapies represent 1 approach to counteract this (Figure 3A). Combining checkpoint inhibitors with CD19 CAR T cells is actively being pursued for pediatric R/R ALL, and early clinical data suggest that this approach is safe and may augment the effector function and persistence of CD19 CAR T cells.[47] In addition to checkpoint inhibitors, the provision of cytokines after initial CD19 CAR T-cell expansion and contrac-

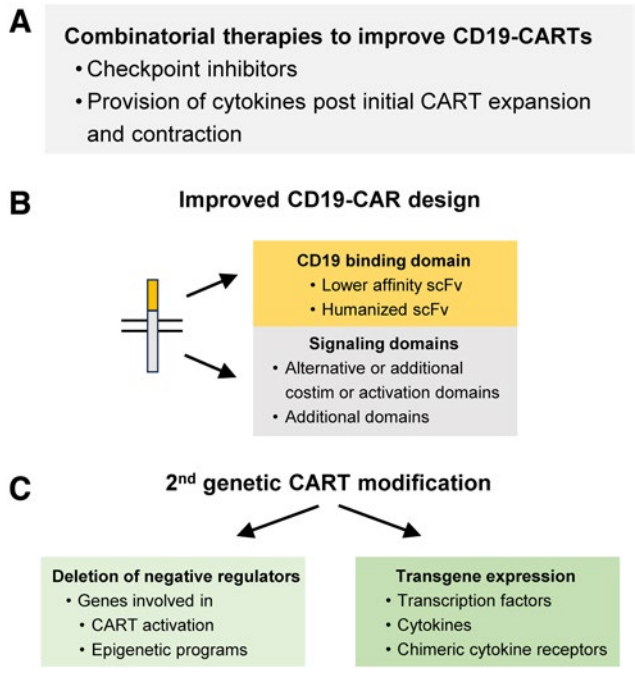

Figure 3. Strategies to enhance the effector function of CD19 CAR T cells. Examples of (A) combinatorial therapies, (B) strategies to improve CAR design, and (C) second genetic modifications of CAR T cells. costim, costimulation.

tion has demonstrated benefit in preclinical studies, and clinical studies in adults with B-cell lymphoma post CD19 CAR T-cell therapy are ongoing.

CAR design

Tisagenlecleucel as well as most investigator-initiated CD19 CAR T cells have employed a single-chain Fv that is derived from the monoclonal antibody FMC63. It has a high affinity, and studies have indicated that CAR T cells expressing a CAR that utilizes a CD19-specific scFv with a lower affinity have improved effector function.[48,49] In addition, utilizing a humanized CD19-specific scFv has the potential to reduce CAR-specific immune responses, improving persistence.[50] Currently, the choice of costimulatory domain is the most well-established factor that determines CAR T-cell persistence, with CD19/41BB CARs having longer persistence than CD19/CD28 CARs (see the section "HCT post CD19 CAR T-cell therapy: experience to date"). Finally, the incorporation of novel signaling domains holds the promise to generate CARs that endow CAR T cells with improved effector function (Figure 3B).[51-53]

Additional genetic modification to enhance the effector function of CD19 CAR T cells

Conceptually, there are 2 main approaches to enhance the effector function, including the persistence of CD19 CAR T cells (Figure 3C). One relies on deleting negative regulators and the other on transgenic expression of transcription factors, cytokines, and chimeric cytokine receptors.[54-57] Examples of deleting negative regulators include molecules that enhance CAR T-cell activation, including RASA2 and Regnase-1,[58,59] and enzymes that are critical for the epigenetic reprogramming of CAR T cells, including TET2 and DNMT3A.[60-62] These approaches are reviewed in detail in recently published articles.[55,56,63]

Conclusions

Since the infusion of the first pediatric patient with CD19 CAR T cells in 2012, CD19 CAR T-cell therapy has become an integral part of our treatment armamentarium for pediatric patients with R/R ALL. Currently, there are 2 major, complementary efforts ongoing. One focuses on increasing our understanding of how to best use tisagenlecleucel, the only US Food and Drug Administration–approved CD19 CAR T-cell product for R/R pediatric ALL, and the other focuses on developing second-generation ALL-specific CAR T-cell products with enhanced effector function. Based on our current knowledge, subsets of patients who have received tisagenlecleucel will most likely benefit from a consolidative HCT, and further studies are needed to identify these patients. Likewise, the efficacy of tisagenlecleucel might be improved with combinatorial therapies. While we know the desired characteristics of enhanced ALL-specific CAR T-cell products—namely, resistance to antigen loss variants paired with durable persistence—further preclinical and clinical studies are needed to delineate the genetic engineering approach to accomplish this.

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Conflict-of-interest disclosure

Aimee C. Talleur: no competing financial interests to declare.
Swati Naik: no competing financial interests to declare.
Stephen Gottschalk: scientific advisory board: Be Biopharma, CARGO, Immatics; honoraria: TESSA Therapeutics.

Off-label drug use

Aimee C. Talleur: none are discussed.
Swati Naik: none are discussed.
Stephen Gottschalk: none are discussed.

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Pyruvate kinase activators for treatment of pyruvate kinase deficiency

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Pyruvate kinase (PK) deficiency is a congenital hemolytic anemia with wide-ranging clinical symptoms and complications associated with significant morbidity and reduced health-related quality of life in both children and adults. The management of patients with PK deficiency has been historically challenging due to difficulties in the diagnostic evaluation, heterogeneity of clinical manifestations, and treatment options limited to supportive care with transfusions and splenectomy. An oral allosteric PK activator, mitapivat, is now a clinically available disease-modifying treatment for adults with PK deficiency. Phase 2 and 3 clinical trials of mitapivat have demonstrated sustained improvements in hemolytic anemia, hematopoiesis, and quality of life in many adults with PK deficiency and a generally reassuring safety profile with continued dosing. Additional long-term benefits include rapid and ongoing reduction in iron overload and potential stabilization of bone health. Clinical trials of treatment with mitapivat in children with PK deficiency are ongoing. In addition to disease-modifying treatment with PK activators, gene therapy is a potentially curative treatment currently under evaluation in clinical trials. With the availability of disease-targeted therapies, accurately diagnosing PK deficiency in patients with chronic hemolytic anemia is critical. PK activation and gene therapy have the potential to change the natural history of PK deficiency by improving clinical manifestations and patient quality of life and decreasing the risk of long-term complications.

LEARNING OBJECTIVES

- Evaluate the available data for pyruvate kinase (PK) activators for the treatment of PK deficiency
- Apply concepts to generate an approach to the diagnostic evaluation and treatment of PK deficiency

CLINICAL CASE

A 32-year-old man presents for a new consultation for chronic hemolytic anemia. He was evaluated for anemia in early childhood and told that he had hereditary spherocytosis. He has not seen a hematologist in many years. He received several red cell transfusions in the first few years of his life but has not since been transfused. At age 10 years, he had cholecystitis and underwent laparoscopic cholecystectomy. At the consult visit, he reports long-standing fatigue that has worsened over the prior 5 years. He works full-time as teacher but is unable to exercise, and he limits activities with his children because of fatigue and shortness of breath. He would like to know how he can improve his energy.

Introduction

Pyruvate kinase (PK) deficiency is rare congenital hemolytic anemia associated with a wide spectrum of symptoms and complications due to chronic hemolysis. PK is a tetrameric glycolytic enzyme that catalyzes the conversion of phosphoenolpyruvate to pyruvate and leads to adenosine triphosphate (ATP) production. PK deficiency is an autosomal recessive condition caused by mutations in the *PKLR* gene, which encodes the red cell and liver specific isoforms PK-R and PK-L. Given the reliance of mature red cells on glycolysis as their primary energy source, *PKLR* mutations cause an insufficiency in ATP, which leads to red cell dehydration and membrane abnormalities that cause chronic hemolysis and ineffective erythropoiesis.[1-4] PK-deficient reticulocytes are particularly susceptible to injury in the hypoxic spleen due to

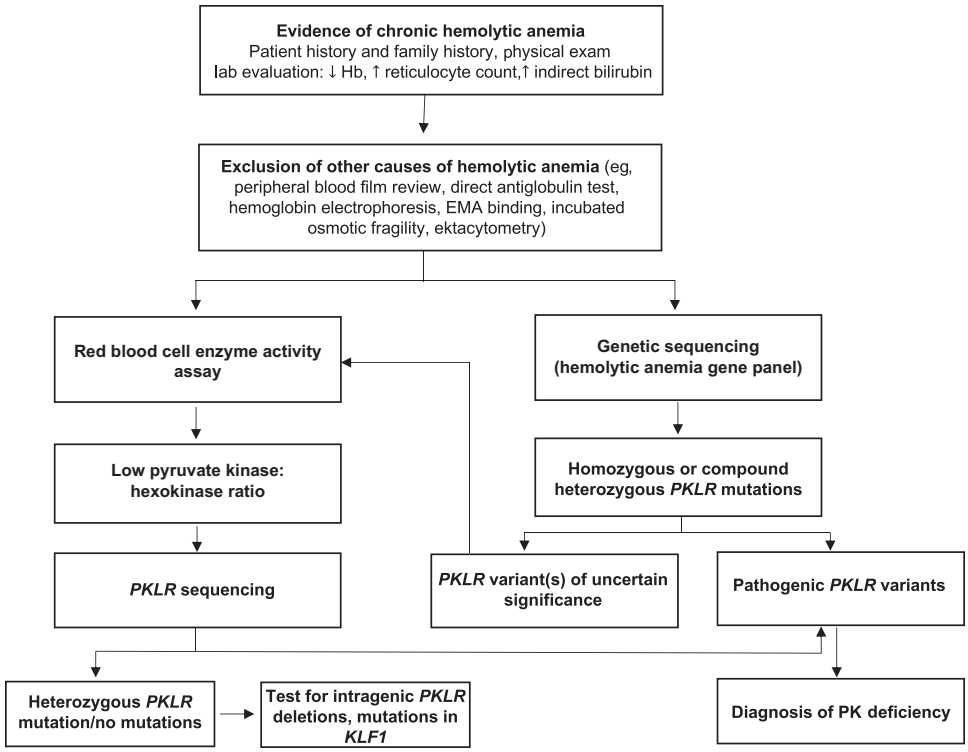

Figure 1. Algorithm for the diagnostic evaluation of PK deficiency. An accurate diagnosis of PK deficiency is key for managing patients with disease-targeted treatments. Diagnostic testing should be considered in patients of all ages with chronic hemolytic anemia. After hemolysis has been established (low hemoglobin, increased reticulocyte count and indirect bilirubin level), autoimmune hemolytic anemia, hemoglobinopathies, and membranopathies should be excluded. The peripheral blood film in PK deficiency often does not have specific red cell morphology except polychromasia. Once the more common causes of hemolysis are excluded, a red cell enzyme panel or a hemolytic anemia gene panel may be pursued. Falsely normal PK enzyme activity levels may occur with recent red cell transfusions, reticulocytosis, and/or sample contamination with other blood cells. PK enzyme activity is red cell age dependent and will be low in comparison to other red cell age dependent enzymes, such as hexokinase. In those with a low PK:hexokinase ratio on enzyme testing, *PKLR* genetic testing should be pursued given that low PK activity can also be found in carriers of PK deficiency and in those with mutations in *KLF1*.[38] Up to 20% of patients currently tested will be found to have a *PKLR* variant of uncertain significance.[12] In these patients, a low PK:hexokinase ratio can confirm the diagnosis. EMA, eosin-5′-maleimide.

high ATP needs and reliance on glycolysis rather than oxidative phosphorylation in the splenic environment.[5] The glycolytic intermediate 2,3-biphosphoglycerate (2,3-BPG) is increased in PK deficiency, causing a shift in the hemoglobin-oxygenation dissociation curve with a consequent increase in tissue oxygenation; in turn, greater tissue oxygenation can lead to improved tolerance of anemia in some patients.[6]

More than 350 pathogenic *PKLR* mutations have been reported, and most patients have compound heterozygous *PKLR* variants, leading to wide genotypic variability. The majority have at least one missense *PKLR* mutation encoding single amino acid changes that affect PK catalytic activity, stability, or expression. Prior studies have not shown a strong genotype-phenotype relationship, including among siblings, although assessment has been limited by small patient cohorts and extensive combinations of genotypes.[7]

The estimated prevalence ranges from 1 to 8 in 1 000 000.[8,9] Diagnostic testing should be considered in patients of all ages with chronic nonimmune hemolysis and requires high clinical suspicion given the wide-ranging clinical phenotype and nonspecific red cell morphology (Figures 1 and 2). When available, both evaluation of red cell enzyme activity, with a low PK:hexokinase ratio, and *PKLR* genetic testing should be pursued to allow the diagnosis to be made based on the combination of test results, since each has limitations; expert guidelines for diagnostic evaluation are available.[10,11] Although useful for diagnosis, PK enzyme activity does not correlate with clinical severity and is not predictive of clinical course.[11]

CLINICAL CASE (continued)

The patient has a hemoglobin (Hb) level of 8.2 g/dL, reticulocyte count 20%, indirect bilirubin 3 mg/dl, ferritin 1100 ng/mL, and a peripheral blood film with polychromasia but otherwise bland red cell morphology without spherocytes (Figure 2). His direct antiglobulin test is negative, and a hemoglobin electrophoresis is normal. His PK activity is 1.4 EU/g Hb (normal range: 3.2–6.5 EU/g Hb) with a hexokinase (HK) activity of 1.07 EU/g Hb (normal range: 0.14–0.37 EU/g Hb) and PK:HK ratio of 1.31 (reference normal PK:HK ratio range: 8.7–22.5). A hemolytic anemia gene

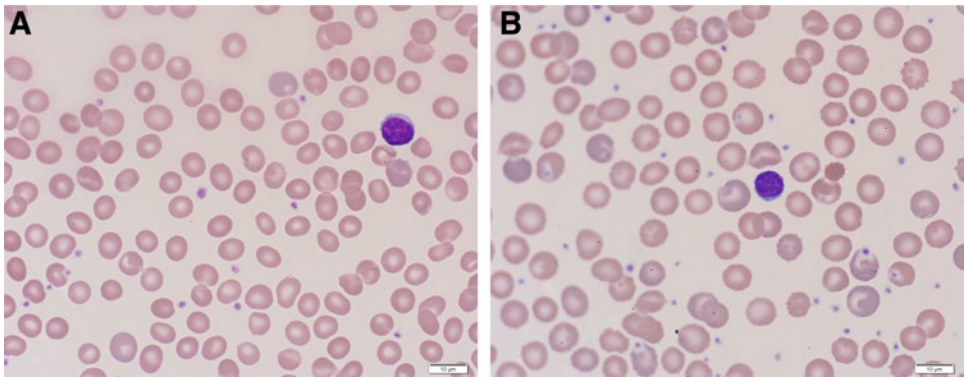

Figure 2. Peripheral blood film from a patient with PK deficiency with an intact spleen (A) and after splenectomy (B). The red cell findings in PK deficiency with an intact spleen are mild. Nonspecific or bland red cell morphology in a patient with a congenital hemolytic anemia should raise suspicion for an enzymopathy. After splenectomy, the peripheral blood film is notable for moderate polychromasia and echinocytes.

panel reveals two pathogenic missense *PKLR* variants (1529G>A, 1456C>T) consistent with a diagnosis of PK deficiency. Liver iron quantification with magnetic resonance imaging (MRI) is 7 mg/g dry weight liver. A dual energy x-ray absorptiometry (DXA) scan shows the lowest bone mineral density Z-score is −2.5 at the femoral neck, consistent with osteoporosis.

Symptoms and complications

PK deficiency has a wide clinical spectrum due to both chronic hemolysis and complications of supportive treatment (Figure 3). Severe manifestations can occur *in utero* that result in hydrops fetalis, growth restriction, and/or prematurity.[12] Newborns may have a presentation ranging widely from compensated anemia to neonatal hyperbilirubinemia to critical illness with organomegaly, hyperferritinemia, and/or liver failure.[12,13] Infants and children may have poor growth and irritability related to anemia. Affected individuals of all ages may have jaundice, scleral icterus, fatigue, cognitive effects, or limited exercise tolerance, all of which can affect quality of life.[14] Clinical findings include splenomegaly, iron overload (transfused and nontransfused), osteopenia and osteoporosis, gallbladder disease, extramedullary hematopoiesis, lower extremity ulceration, and pulmonary hypertension. Iron overload, both due to transfusions and increased iron absorption, can cause significant morbidity, including cardiac failure, liver disease, and endocrine dysfunction.[12,15,16] Regular monitoring of ferritin and MRI guides treatment with iron chelation.[17] Exacerbations of hemolytic anemia may occur with infections and pregnancy.[12] With advancing age, symptoms associated with anemia may increase in the presence of additional comorbidities.

Non–PK activator treatment strategies and associated complications

Prior to the approval of PK activators, management of PK deficiency included supportive treatments of red cell transfusions and splenectomy and potentially curative treatment with allogeneic hematopoietic stem cell transplant (HSCT). Early results from a gene therapy trial are promising. Decision-making about treatment with a PK activator should be evaluated in the context of these management options.

Red cell transfusion therapy is initiated based on growth in children, symptoms of anemia with impact on quality of life, complications, and, to some extent, baseline hemoglobin. The requirement for transfusions decreases over childhood, likely due to a decrease in the frequency of viral infections and the historic timing of splenectomy in children.[12] Erythropoiesis is supported with folic acid, particularly in the setting of dietary limitations or pregnancy. Symptoms of chronic anemia may increase during adulthood, leading to the initiation of regular transfusions.

Splenectomy is often considered in childhood for patients who undergo transfusions or have a poor quality of life due to anemia.[18] After splenectomy, many, but not all, patients have an improvement in hemoglobin (mean hemoglobin increase 1.5 g/dL) and transfusion burden.[19] Splenectomy leads to a paradoxical increase in the reticulocyte count. Those with more severe hemolysis are less likely to benefit from splenectomy.[12,14] Hemolysis continues after splenectomy with an ongoing risk of associated complications in addition to the lifelong risk of sepsis and thrombosis.[12]

Although HSCT is a curative treatment, there are no clear indications for transplant for PK deficiency. Published cohort studies reveal a higher rate of morbidity and mortality associated with transplant when compared with supportive care.[19,20] A global cohort study described 16 patients with PK deficiency who underwent transplantation in Europe and Asia (median age 6.5 years, median follow up 2.3 years).[21] Outcomes were poor, with grade 3-4 graft-versus-host disease in 7 of 16 patients and a 3-year cumulative survival of 65%.

Gene therapy also represents a potential curative option.[22-24] A phase 1 clinical trial evaluated the safety of autologous gene therapy for PK deficiency using myeloablative conditioning with lentivirus-based genetically modified hematopoietic stem cells with the corrected *PKLR* gene. Interim trial results of 2 adults have demonstrated a post–gene therapy normal hemoglobin, improvement in both hemolytic markers and quality of life, and a continuous transfusion-free period with 24 months of follow-up. To date, there have been no serious adverse events related to the product.[25] Long-term safety and efficacy data collection from the phase 1 trial is ongoing; a phase 2 trial is planned.

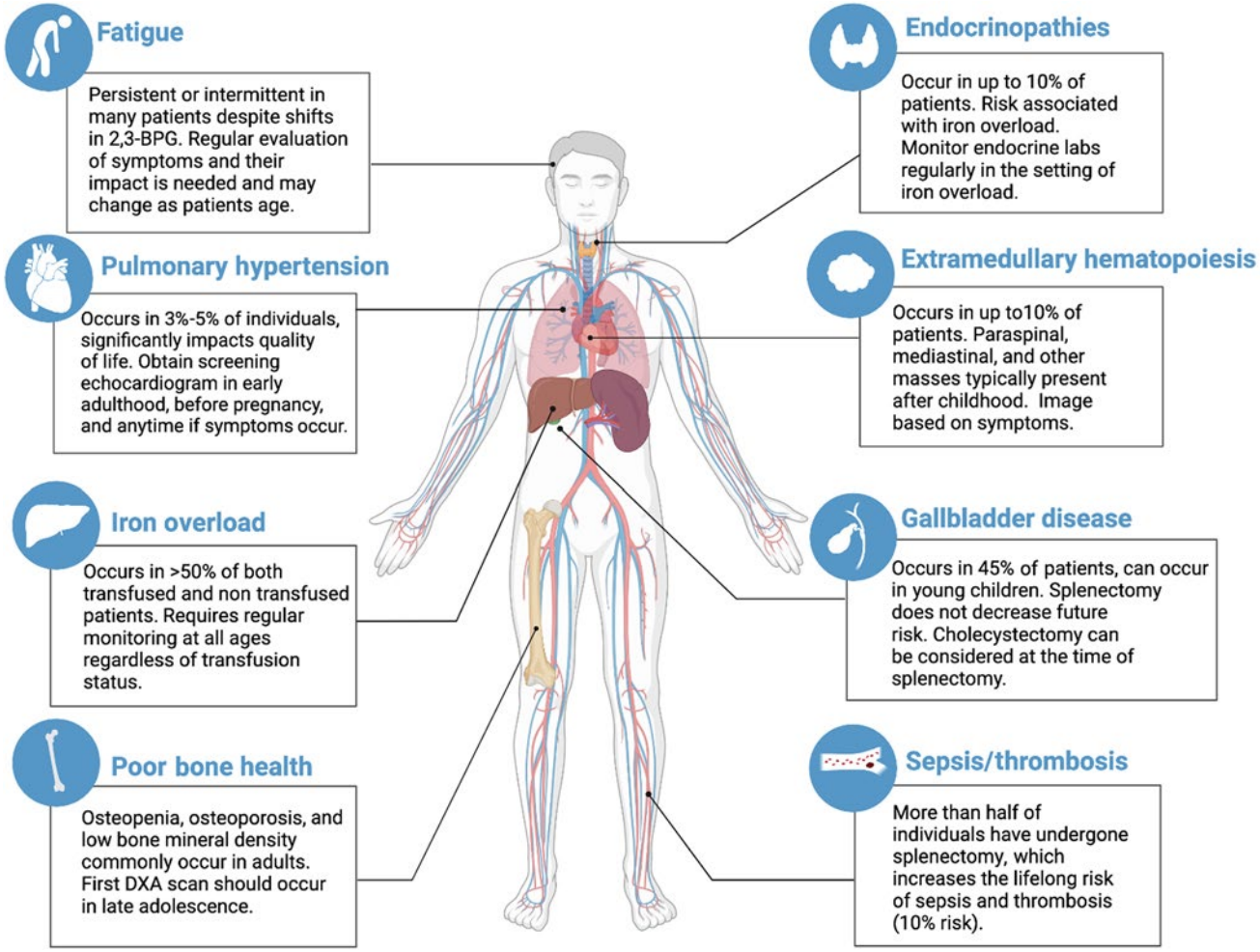

Figure 3. Signs, symptoms, and complications of PK deficiency.

PK activators for treatment of PK deficiency

Several oral PK activators are in clinical trials for treatment of red cell disorders. Mitapivat (AG-348, Agios Pharmaceuticals) is an oral allosteric erythrocyte PK activator and is the only PK activator that has been evaluated for treatment of PK deficiency (Table 1). Mitapivat binds at the dimer-dimer interface at a separate site from fructose 1,6-bisphosphate on the PK-R tetramer. The drug is orally bioavailable with or without food, with a steady state reached after 1 week with twice-daily dosing. Mitapivat is cleared through hepatic metabolism by cytochrome P450 enzymes and has an off-target effect of mild dose-dependent reversible inhibition of aromatase.[26] Ex vivo human studies demonstrate increased PK enzyme activity of both wild-type and variant PK and improvement in thermostability in variant PK with exposure to mitapivat.[27,28] In samples from patients with PK deficiency, incubation with mitapivat improved red cell deformability as measured by osmotic gradient ektacytometry, improved proliferation of erythroid progenitor cells, and led to an increase in ATP in a dose dependent manner.[28]

Two phase 1 studies assessed the pharmacodynamics, pharmacokinetics, and safety of mitapivat.[26] In the multiple ascending dose healthy volunteer study, the maximum ATP increase was 60% and the maximum decrease in 2,3-BPG was 47%, consistent with activation of PK and the glycolytic pathway. The safety data of both phase 1 studies were reassuring, with no serious treatment-emergent adverse events. The safety and pharmacodynamic data provided support for a mitapivat trial in adults with PK deficiency.

The safety and efficacy of mitapivat in adults with PK deficiency who were not receiving regular transfusions, defined as <4 transfusions in the prior 12 months, were evaluated in the open-label randomized phase 2 DRIVE-PK trial.[29] Patients were randomized to 50 mg or 300 mg of mitapivat twice daily for a 24-week period with an optional long-term extension. The primary endpoint of this study was assessment of safety. The therapy was well-tolerated; the most common reported adverse events—headache, insomnia, and nausea—tended to be transient and resolved within 1 week of drug initiation. One patient developed acute hemolysis when mitapivat was stopped, leading to a recommendation for drug taper rather than abrupt discontinuation. In this trial, 50% (26/52) of patients had a hemoglobin increase ≥1 g/dL, with a mean maximum increase of 3.4 g/dL (range: 1.1-5.8 g/dL). The hemoglobin increase occurred quickly (median

Table 1. Clinical trial data of PK activators for treatment of PK deficiency

Clinical trial	Study information	Summary of main findings	Authors, reference
Healthy Volunteer Trial of Mitapivat	Phase 1, healthy volunteers	• One grade 3 TEAE in multiple ascending dose study at a dose of 700 mg every 12 hours (abnormal liver function tests). Hormone changes consistent with reversible, dose-dependent aromatase inhibition. • Pharmacodynamic profile: maximal decrease 2,3-BPG 24 hours postdose; maximal increase in ATP between 8–14 days of dosing, durable for 48–72 h after dosing supporting twice daily dosing.	Yang H, Merica E, Chen Y, et al.[26]
Healthy Volunteer Trial of Etavopivat	Phase 1, healthy volunteers	• Treatment emergent events were mild to moderate; none led to discontinuation. No changes in hormone levels. • Pharmacodynamic profile: maximal decrease 2,3-BPG 24 hours postdose; maximal increase in ATP by day 8 of dosing, durable for 120 h after dosing supporting once daily dosing	Forsyth S, Schroeder P, Geib J, et al.[37]
DRIVE-PK trial (mitapivat)	Phase 2, adults with PK deficiency, not regularly transfused (<4 transfusions in prior 12 months)	• 26/52 (50%) of adults had an increase in Hb ≥1 g/dL; mean maximum Hb increase 3.4 g/dL • Increase in Hb occurred in median of 10 days. • Genotype-hemoglobin response relationship with all Hb responders with at least one missense *PKLR* mutation. • Well-tolerated, most common adverse events were headaches, insomnia, nausea, generally resolving within 1 week. Rebound hemolysis seen with abrupt discontinuation of drug.	Grace RF, Rose C, Layton MD, et al.[29]
ACTIVATE trial (mitapivat)	Phase 3, adults with PK deficiency, not regularly transfused (<4 transfusions in prior 12 months) and at least one missense *PKLR* mutation	• 16/40 (40%) receiving mitapivat met the primary endpoint (Hb ≥1.5 g/dL, at least 2 timepoints) compared with 0/40 (0%) receiving placebo • Hemoglobin increase correlated with improvements in markers of hemolysis and hematopoiesis and disease-specific patient reported outcome measures. • Well-tolerated; adverse events (most common: nausea, headache) were similar in the placebo and mitapivat arms.	Al-Samkari H, Galactéros F, Glenthøj A, et al.[30]
ACTIVATE-T trial (mitapivat)	Phase 3, adults with PK deficiency receiving ≥6 transfusions in prior 12 months and at least 1 missense *PKLR* mutation	• 10/27 (37%) of adults receiving mitapivat with ≥33% reduction in transfusion burden. • 6/27 (22%) transfusion free after starting mitapivat. • Adverse events included headache, nausea, increase in liver enzymes.	Glenthøj A, van Beers EJ, Al-Samkari H, et al.[31]
ACTIVATE/ACTIVATE-T open label long-term extension study (mitapivat)	Extension study of ACTIVATE/ACTIVATE-T trials	• ACTIVATE: 39.5% (15/38) randomized to placebo had a Hb response after switching to mitapivat; response by hemoglobin, hemolytic markers, and patient-reported outcomes has been sustained for more than 3 years in the majority. • ACTIVATE-T: 37% (10/27) have met criteria for transfusion response for more than 3 years; 6 patients have remained transfusion free for more than 3 years. • Well-tolerated, no new safety findings. • Decrease in iron overload by liver iron concentration and other iron markers seen in both studies with ongoing improvements over time. • Sustained stability in bone mineral density by DXA scan.	Multiple references[32–36]

DXA, dual energy x-ray absorptiometry; TEAE, treatment-emergent adverse event.

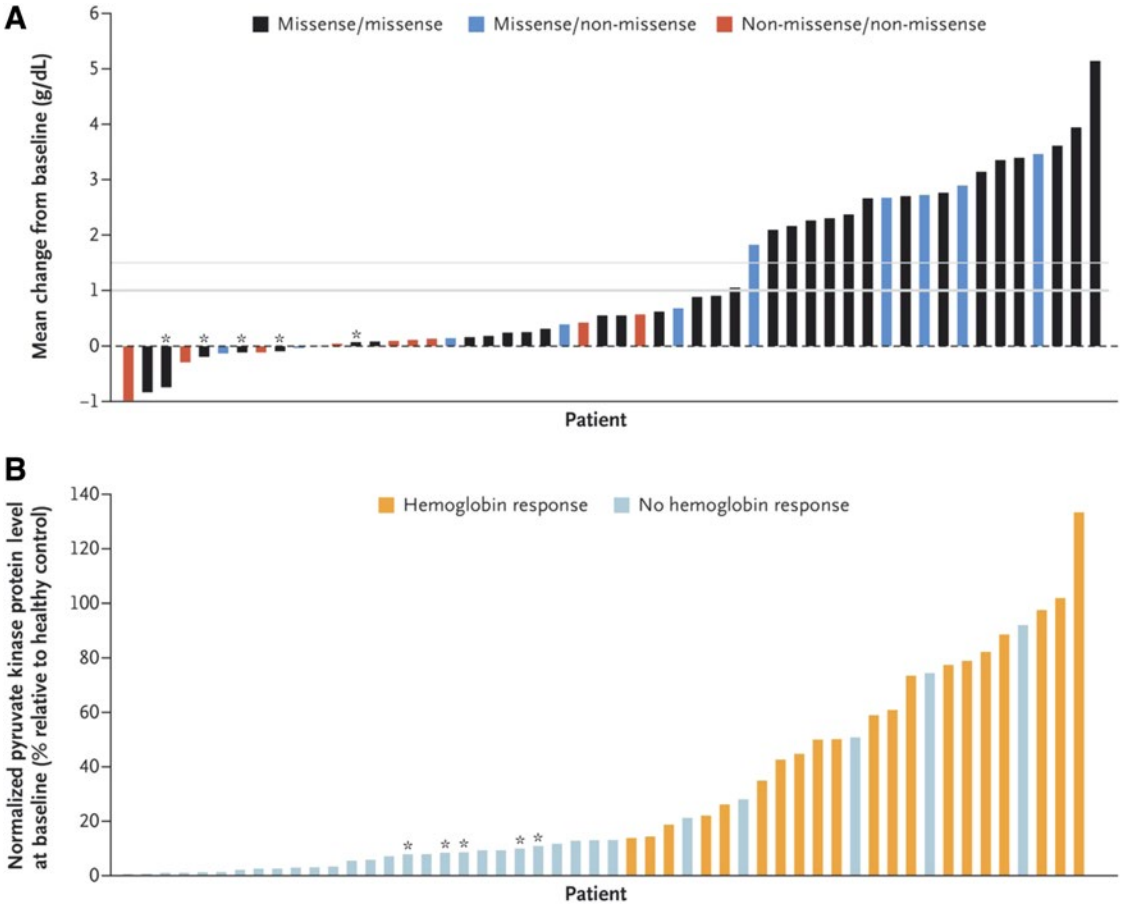

Figure 4. Change in hemoglobin according to *PKLR* genotype and hemoglobin response according to PK protein level at baseline in the DRIVEPK trial. (A) The mean change from baseline in the hemoglobin level according to the patient's *PKLR* genotype category. Of the 52 patients who received mitapivat, 20 (38%)—all of whom had at least 1 missense mutation—had a mean hemoglobin increase >1 g/dL (indicated by a thick gray line); 19 patients had a mean hemoglobin increase ≥1.5 g/dL (thin gray line). A mean hemoglobin increase >1 g/dL did not occur in any of the 5 patients who were homozygous for the R479H mutation (as indicated by an asterisk) or in any of the 10 patients who were homozygous for non-missense mutations (as indicated by a red bar). (B) Hemoglobin response according to PK protein level at baseline. Hemoglobin response is defined as >1 g/dL increase in the hemoglobin at >50% of the assessments during the core period. Baseline pyruvate kinase protein level in red cells has been normalized to the hemoglobin level (an approximation of the total red-cell protein level) and to the pyruvate kinase protein level measured in a healthy control (to allow for interassay comparisons). Patients with an increased baseline level of PK protein were more likely to have a hemoglobin response to mitapivat. The data bars in panels A and B are not aligned for each patient, so a 1:1 comparison of the individual data bars in the 2 panels is not possible. From *The New England Journal of Medicine*, RF Grace et al., Safety and Efficacy of Mitapivat in Pyruvate Kinase Deficiency, 381(1):933-944. Copyright 2019 Massachusetts Medical Society. Reprinted with permission.

response in 10 days), with a sustained hemoglobin response seen in the majority with continued dosing and with durable improvements in markers of hemolysis and hematopoiesis. There was a relationship between hemoglobin response and *PKLR* genotype: all patients with a hemoglobin response had at least 1 missense mutation, whereas the patients with 2 non-missense mutations had poor or no hemoglobin responses (Figure 4).[29] None of the 5 patients who were homozygous for the R479H mutation, which is most common in the Amish population, had a hemoglobin response. PK protein levels also correlated with hemoglobin response, consistent with the mechanism of PK activators to bind and stimulate PK.[28,29]

The safety and efficacy of mitapivat for adults with PK deficiency were demonstrated in two phase 3 clinical trials, leading to its approval by the US Food and Drug Administration (FDA).[30,31] The ACTIVATE trial, a phase 3 placebo-controlled trial, randomized 80 adults with PK deficiency who were not receiving regular transfusions to placebo or mitapivat (5 mg to 50 mg twice daily).[30] Patient eligibility required at least 1 missense *PKLR* mutation. The primary endpoint was a hemoglobin increase of ≥1.5 g/dL at 2 or more assessments, a higher threshold for response than the DRIVE-PK study. Mitapivat was well-tolerated, with similar overall adverse event rates in the drug and placebo groups. Of the 40 patients receiving mitapivat, 16 (40%)

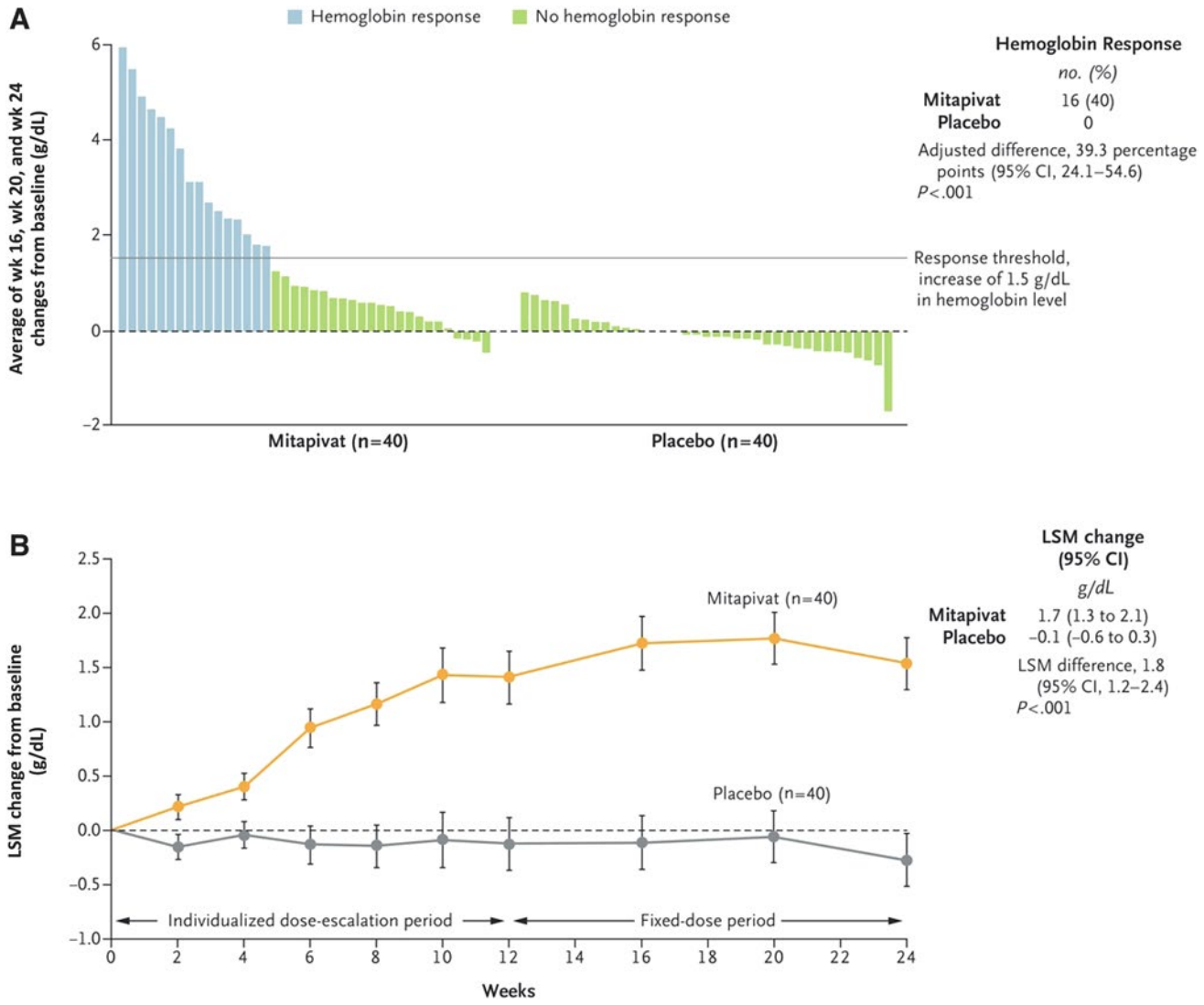

Figure 5. Change in hemoglobin in adults with PK deficiency treated with mitapivat versus placebo on the ACTIVATE T trial. (A) Hemoglobin response, defined as a hemoglobin increase from baseline of ≥1.5g/dL that was sustained at 2 or more scheduled assessments. Each bar represents an individual patient who was assigned to receive either mitapivat or placebo. (B) The least-squares mean (LSM) change from baseline in the hemoglobin level in the 2 groups during the trial period. The error bars indicate the standard error. From *The New England Journal of Medicine*, H Al-Samkari et al., Mitapivat versus Placebo for Pyruvate Kinase Deficiency, 386(15):1432-1442.[30] Copyright 2022 Massachusetts Medical Society. Reprinted with permission.

met the primary endpoint of hemoglobin response, vs 0% in the placebo arm (Figure 5). Additionally, mitapivat decreased hemolysis as measured by change from baseline in indirect bilirubin, lactate dehydrogenase, and haptoglobin and improved hematopoiesis as measured by a reduction in the reticulocyte count. Patient-reported outcomes were measured using 2 disease-specific tools, the PK Deficiency Diary and PK Deficiency Impact Assessment, which were developed to assess quality of life and evaluate the effect of treatment in PK deficiency. Significant and sustained improvements in both measures were seen during the core phase of the trial and have been sustained with continuation of the drug over several years.[32]

A phase 3 open-label trial, ACTIVATE-T, evaluated mitapivat in adults with PK deficiency who underwent transfusions regularly (≥6 transfusions in the prior 12 months).[31] Mitapivat was well-tolerated, with no major adverse events leading to treatment discontinuation. In this study, 37% (10/27) experienced a reduction in transfusion burden (33% reduction in the number of red cell units transfused during the 24-week core period compared to the historical transfusion burden), and 22% (6/27) achieved a transfusion-free response. Significant and sustained improvements in report of quality of life were also seen for this trial.

Data from the ACTIVATE and ACTIVATE-T open-label extension study have shown sustained benefits with continued administration of mitapivat, including ongoing improvement and stability in hemoglobin, hemolytic markers, markers of hematopoiesis, and patient-reported outcomes.[32–34] Several important long-term benefits have been noted, including a decrease in

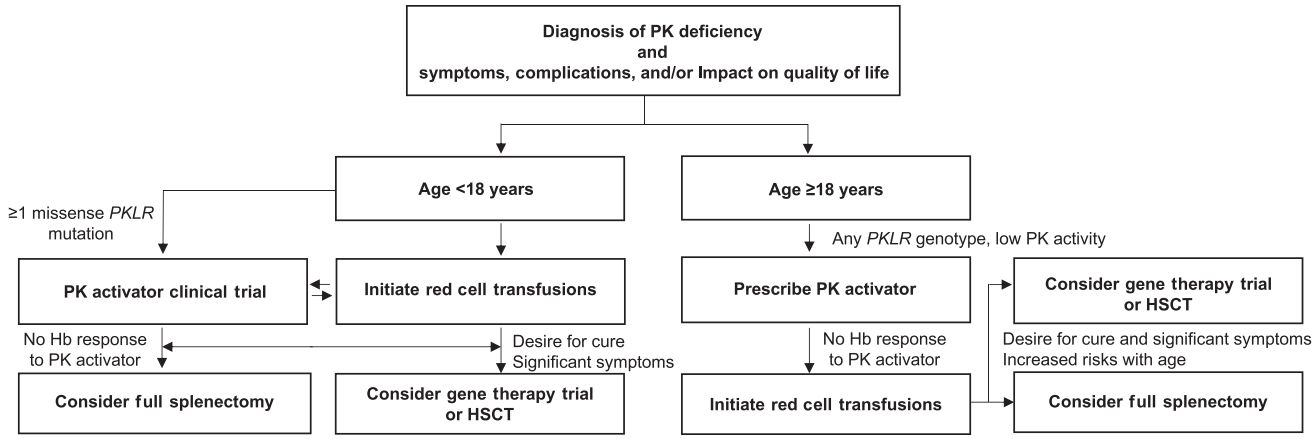

Figure 6. Considerations of treatments for PK deficiency. Supportive and/or disease-targeted treatment should be considered in patients with PK deficiency with symptoms, complications, and/or impact of the disease on quality life. For symptomatic patients ≥18 years, given the reassuring safety profile and favorable clinical efficacy in a substantial subset of adults with PK deficiency, a treatment trial with a PK activator should be initiated. If effective and well-tolerated, the PK activator should be continued with ongoing monitoring. If there is no response to a PK activator, red cell transfusions could be initiated. Hematopoietic stem cell transplant (HSCT) or enrollment in a gene therapy trial could be considered in patients with significant symptoms and/or complications of anemia who are seeking a cure. HSCT outcomes may be better at a younger age. For symptomatic patients <18 years, red cell transfusions should be initiated. Enrollment in a PK activator clinical trial should be considered in those with ≥1 missense *PKLR* variant and significant symptoms and/or complications of anemia. If there is no response to a PK activator or a patient strongly desires a cure and has significant symptoms, HSCT or enrollment in a gene therapy trial could be considered. Patients should try a PK activator, if available, before proceeding with full splenectomy. Given the complexity of management and treatment decisions in patients with PK deficiency, consideration should be made for a discussion or referral to a hematologist with expertise in PK deficiency. Hemoglobin is indicated by Hb.

erythropoiesis and iron overload with improvements in hepcidin, erythropoietin, and liver iron concentration measured by MRI, as compared with placebo in non-transfused adults in the ACTIVATE trial.[35] These changes were seen within 6 months of drug initiation and were sustained with continued improvements over time. Long-term follow-up (more than 5 years of monitoring) has also shown general stability of bone mineral density as measured by DXA.[36] Although continued follow-up is needed, this stability by DXA in patients with poor bone health prior to trial enrollment suggests that decreasing hemolysis and erythropoiesis with mitapivat may lead to an improvement in bone health.

Long-term data from the phase 2 and phase 3 trials demonstrate favorable safety profiles with more than 5 years of treatment with mitapivat. The phase 3 trials demonstrate that mitapivat has the potential for efficacy in adults with PK deficiency independent of splenectomy or transfusion status. Despite the genotype-hemoglobin response relationship observed in the phase 2 trial, adults with identical *PKLR* mutations have had different hemoglobin responses to mitapivat. In addition, although patients with 2 non-missense *PKLR* mutations were excluded from the phase 3 trials, some of these variants have a minor effect on PK protein structure or function, and patients with these variants may experience clinical efficacy with mitapivat. Therefore, a response to treatment cannot be predicted based on genotype. With the recent FDA approval of mitapivat, convincing efficacy in many patients, and a reassuring safety profile, a treatment trial of mitapivat should be considered in all symptomatic adults with PK deficiency except for those individuals who are homozygous for the R479H mutation (Figure 6). Before initiating mitapivat, testing should be performed to determine whether the PK enzyme activity level is low and whether at least 2 *PKLR* mutations of any type are present.

The approved mitapivat dose ranges from 5 mg to 50 mg twice per day; many patients may require the maximum dose. Hemoglobin response can be evaluated over the first 3-6 months of treatment and is often clear within a few weeks of titration to the maximum dose. Ongoing treatment with the drug is required for a durable effect and should be tapered rather than abruptly discontinued to avoid withdrawal hemolysis. Mitapivat should be avoided in patients who have a sulfa allergy, who have moderate to severe hepatic impairment, and who are also using strong CYP3A inhibitors or inducers. Two mitapivat trials are ongoing for children with PK deficiency. Although there have been no clear implications of the off-target effect of mild aromatase inhibition in adults, this will require careful and ongoing monitoring in children.

CLINICAL CASE (continued)

Treatment options of mitapivat, red cell transfusions, splenectomy, HSCT, and gene therapy clinical trial participation are discussed. The patient initiates mitapivat, and, 1 month after dose titration to 50 mg twice daily, his hemoglobin has increased to 11.5 g/dl. His reticulocyte count is 8%, and his indirect bilirubin is 1.5 mg/dL. He reports a substantial improvement in his energy and has increased his activity level. You start him on oral iron chelation and calcium and vitamin D supplements and refer

him to an endocrinologist. He will have regular hematology follow-up appointments to monitor and evaluate this treatment approach.

Conclusions

PK deficiency is a rare chronic hemolytic anemia with variable severity of manifestations, including fatigue, jaundice, iron overload, and decreased patient-reported quality of life. Disease-directed therapy with PK activators is now available for affected adults. Therefore, clinicians must have a high index of suspicion for this disease and send confirmatory diagnostic testing with PK enzyme activity and *PKLR* genetic sequencing. PK activators have the potential to transform the symptoms and natural history of PK deficiency in many patients by improving hemolytic anemia, hematopoiesis, and quality of life and decreasing the risk of long-term complications. A trial of this medication in symptomatic adults with PK deficiency is warranted; however, mitapivat is not effective for all individuals with PK deficiency. In a subset of patients, often with significant symptoms and complications, there may not be a clear benefit from PK activators. Gene therapy is a promising and potentially curative treatment option currently under development; although this approach may address an unmet need in this subset of patients, the long-term efficacy and safety are under evaluation. Disease-targeted treatment through PK activation and gene therapy are innovative approaches for the management of PK deficiency that may significantly improve clinical manifestations and quality of life of affected patients.

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Rachael Grace: Research funding to institution (Agios, Sobi, Novartis); consultancy (Agios, Sanofi).

Off-label drug use

Rachael Grace: No discussion of off-label drug use. Nothing to disclose.

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Pyruvate kinase activators: targeting red cell metabolism in sickle cell disease

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Hemoglobin S (HbS) polymerization, red blood cell (RBC) sickling, chronic anemia, and vaso-occlusion are core to sickle cell disease (SCD) pathophysiology. Pyruvate kinase (PK) activators are a novel class of drugs that target RBC metabolism by reducing the buildup of the glycolytic intermediate 2,3-diphosphoglycerate (2,3-DPG) and increasing production of adenosine triphosphate (ATP). Lower 2,3-DPG level is associated with an increase in oxygen affinity and reduction in HbS polymerization, while increased RBC ATP may improve RBC membrane integrity and survival. There are currently 3 PK activators in clinical development for SCD: mitapivat (AG-348), etavopivat (FT-4202), and the second-generation molecule AG-946. Preclinical and clinical data from these 3 molecules demonstrate the ability of PK activators to lower 2,3-DPG levels and increase ATP levels in animal models and patients with SCD, as well as influence a number of potential pathways in SCD, including hemoglobin oxygen affinity, RBC sickling, RBC deformability, RBC hydration, inflammation, oxidative stress, hypercoagulability, and adhesion. Furthermore, early-phase clinical trials of mitapivat and etavopivat have demonstrated the safety and tolerability of PK activators in patients with SCD, and phase 2/3 trials for both drugs are ongoing. Additional considerations for this novel therapeutic approach include the balance between increasing hemoglobin oxygen affinity and tissue oxygen delivery, the cost and accessibility of these drugs, and the potential of multimodal therapy with existing and novel therapies targeting different disease mechanisms in SCD.

LEARNING OBJECTIVES

- Learn the therapeutic mechanisms of action of pyruvate kinase activators in sickle cell disease
- Evaluate the existing evidence supporting the use of pyruvate kinase activators in the treatment of sickle cell disease

CLINICAL CASE

A 24-year-old woman with sickle cell disease (SCD; hemoglobin SS), complicated by chronic anemia with a baseline hemoglobin of 6 to 7 g/dL and significantly elevated hemolysis markers, alloimmunization, iron overload, dilated cardiomyopathy with preserved ejection fraction, cholelithiasis requiring cholecystectomy, and occasional acute pain episodes presents to an outpatient clinic for follow-up. She reports chronic fatigue and occasional dyspnea on exertion when walking up hills or flights of stairs. She has been on hydroxyurea for 2 years, with a maximum tolerated dose of 1500 mg daily due to cytopenias. She reports compliance with both hydroxyurea and deferasirox and would like to know if she has any other therapeutic options.

Chronic anemia is a hallmark complication of SCD. Anemia pathophysiology in SCD begins with hemoglobin S (HbS) polymerization and sickling of red blood cells (RBCs), leading to intravascular and extravascular hemolysis, reducing RBC life span and the oxygen delivery capacity of blood, and resulting in tissue hypoxia and chronic ischemic organ damage.[1] The focus of therapeutic development to address anemia has targeted strategies to limit HbS polymerization in deoxygenated sickle RBCs, including increasing fetal hemoglobin (HbF) levels, altering hemoglobin oxygen affinity, and RBC hydration. Remarkable clinical improvements have resulted from these efforts, particularly evident in hydroxyurea's ability to increase HbF, reducing microvascular occlusion, tissue ischemia, and pain.

However, chronic anemia remains a major contributor to morbidity and mortality in SCD, and better treatments

for anemia have the potential to further improve outcomes in SCD. Low hemoglobin concentration is associated with neurocognitive impairment in SCD, even in the absence of structural abnormalities on magnetic resonance imaging.[2] In a recent meta-analysis, a modeled increase in hemoglobin concentration of ≥1 g/dL was associated with a reduction in the risk of cerebrovascular disease, albuminuria, elevated estimated pulmonary artery systolic pressure, and mortality.[3] In addition, prominent symptoms of anemia, such as fatigue, are common in individuals living with SCD and greatly affect their quality of life and physical functioning.[4,5] However, there are no prospective data demonstrating that raising hemoglobin levels can actually improve cognitive function or clinical outcomes in SCD. At the same time, blood viscosity is abnormally increased in SCD due to decreased sickle RBC deformability, increased adhesion, and abnormal vasoreactivity, such that excessive increases in hemoglobin concentration may lead to viscosity-related complications.[1] For example, rapid and dramatic correction of anemia with simple RBC transfusions has anecdotally led to increased intracranial pressure, seizure, and stroke in patients with SCD.[6-8] A few epidemiologic studies have also found a correlation between increased hemoglobin levels and increased vaso-occlusive crisis (VOC) frequency,[9,10] and a phase 3 study of senicapoc (ICA-17043) was stopped early due to lack of efficacy in terms of reduction of VOCs despite an observed increase in hemoglobin and decrease in hemolysis.[11] This highlights a fundamental knowledge gap in the field surrounding the pathogenesis of chronic microvascular tissue ischemia in SCD: which process contributes more to tissue ischemia, the reduction in oxygen content and delivery resulting from anemia or vaso-occlusion in the microcirculation, which may be worsened by increased blood viscosity? Further research is needed to understand how therapies for anemia may influence or balance these competing mechanisms of hypoxia.

While there are limited existing treatment options for chronic anemia in SCD, the therapeutic arena for SCD is rapidly expanding. Blood transfusions are the mainstay therapy for severe anemia but carry risks of iron overload, alloimmunization, hemolytic transfusion reactions, and hyperhemolysis in SCD. Hydroxyurea, the primary drug therapy for SCD, has only a modest effect on hemoglobin level in adults, and patients who do not tolerate or remain anemic despite hydroxyurea need additional therapy. Recombinant human erythropoietin (EPO) is the standard of care for anemia of chronic kidney disease, but there are limited and conflicting data on its efficacy in SCD.[12-15] In 2019, voxelotor, a hemoglobin oxygen affinity modifier, was approved for SCD after a phase 3 study demonstrated a ~1-g/dL increase in hemoglobin in 51% of patients with SCD.[16] More recently, early-phase studies of a novel drug class in clinical development, the pyruvate kinase activators, have shown potential for raising hemoglobin level and reducing sickling and hemolysis in individuals with SCD.

RBC energetics in SCD

Erythrocytes metabolize glucose through the Embden-Myerhof pathway to produce adenosine triphosphate (ATP), the sole source of energy for the cell. Although seemingly inefficient, the pathway also produces reduced nicotinamide adenine dinucleotide to reduce methemoglobin, glucose-6-phosphate to drive the pentose phosphate shunt to make nicotinamide adenine dinucleotide phosphate to reduce glutathione and protect against oxidation of hemoglobin, cytoskeletal protein, and membrane lipids, as well as the Rapoport-Luebering shunt to generate 2,3-diphosphoglycerate (2,3-DPG; Figure 1).[17] 2,3-DPG, H^+, CO_2, CO, temperature, and HbF all affect the oxygen affinity of hemoglobin (p50, ie, the partial pressure of oxygen at which 50% of the hemoglobin is saturated with oxygen; Figure 2).[17] An increase in 2,3-DPG shifts the oxygen dissociation curve to the right and increases p50, while a decrease in 2,3-DPG decreases p50. Thus, increased levels of 2,3-DPG in SCD increase p50 and shift the oxygen dissociation curve to the right, favoring HbS deoxygenation and polymerization. In the last step of glycolysis, phosphoenolpyruvate (PEP) is catalyzed to pyruvate by pyruvate kinase (PK) and accounts for 50% of the RBC ATP production. Depletion of ATP in SCD leads to insufficient ATP for proper functioning of ATP-dependent ion pumps such as the Na^+/K^+ pump, causing defective ion transport, and Ca^{++} influx through Piezo1 activates the Gardos channel, resulting in K^+ outflux and RBC dehydration, increased intracellular HbS concentration, and promotion of HbS deoxygenation and polymerization.[18]

PK activation as a potential therapeutic approach in SCD

PK deficiency is the most common enzyme deficiency causing nonspherocytic hemolytic anemia and iron overload. PK-deficient RBCs have increased 2,3-DPG and decreased ATP, with the oxygen dissociation curve shifted to the right.[19] Similarly, increased levels of 2,3-DPG and decreased RBC ATP are seen in sickle RBCs,[20,21] causing an increase in the p50 of hemoglobin, HbS polymerization, and sickling, as well as RBC dehydration. Additionally, a recent study shows less PK stability and activity in sickle than control RBCs.[22] Furthermore, coinheritance of PK deficiency and sickle cell trait may induce sickling, causing an SCD phenotype.[23,24] There are 290 pyruvate kinase liver and red blood cell (PKLR) mutations, with 276 being pathogenic.[17] Genetic variants of PKLR have shown to be associated with acute pain in patients with SCD[25] and, together with the expected metabolic effects, provide a potential rationale for the use of PK activators as a therapeutic strategy in SCD.

The tetrameric R form of red cell PK (PKR) has low affinity for PEP and can be increased by binding of fructose-1,6-bisphosphate (FBP), the major allosteric activator of PKR. FBP binding acts to increase PEP binding affinity, promoting tetramerization and stabilizing the PKR enzyme in the tetrameric state.[26] Recently, mitapivat and etavopivat (Figure 3) were developed as oral allosteric activators to bind a pocket at the dimer-dimer interface distinct from the FBP-binding domain. This results in an increase in PKR activity in wild-type and mutant enzymes. Mitapivat (AG-348) has since been approved by the US Food and Drug Administration (FDA) and shown in PK-deficient patients to significantly increase the hemoglobin level, decrease hemolysis, and improve patient-reported outcomes.[27] Thus, targeting activation of PKR in sickle RBCs to lower 2,3-DPG and increase ATP levels seems like a reasonable strategy to increase hemoglobin and decrease sickling and hemolysis.

CLINICAL CASE (continued)

Various treatment options are reviewed with the patient, including EPO and 3 recently approved drug therapies for SCD: voxelotor (a modifier of hemoglobin oxygen affinity),

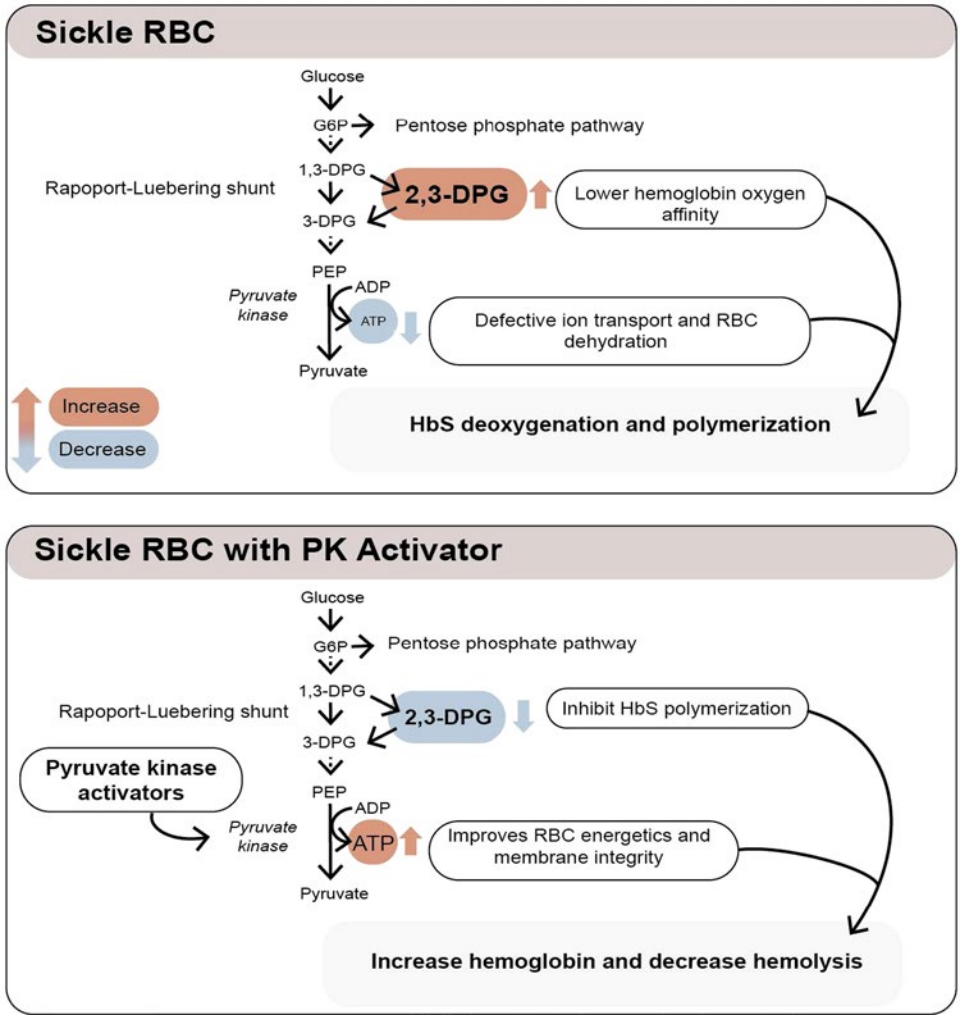


Figure 1. Mechanisms of action of pyruvate kinase activators.

L-glutamine (an antioxidant), and crizanlizumab (a P-selectin inhibitor). Due to her heavy alloimmunization and severe iron overload, chronic transfusion therapy is not recommended. Given her severe anemia and high hemolytic rate, a clinical trial of a pyruvate kinase activator is also considered.

Preclinical and clinical studies of PK activators in SCD

Mitapivat

Mitapivat activates both wild-type and mutant forms of the PK enzyme and has been evaluated for the treatment of PK deficiency, SCD, and thalassemia, as well as approved by the FDA for PK deficiency in 2022. An ex vivo study of mitapivat treatment on RBCs from patients with SCD found an increase in ATP and decreases in 2,3-DPG, p50, and point of sickling (a measure of hypoxia-induced sickling using oxygen gradient ektacytometry).[22] Findings from a preclinical study of mitapivat in the SCD (HbSS) Townes mouse model were mixed; in contrast to human data, the HbSS mice had higher PKR protein and ATP and lower 2,3-DPG compared to the control (HbAA) mice.[28] Mitapivat did not significantly impact 2,3-DPG or hemoglobin levels but did further increase ATP levels and decrease spleen size, leukocytosis, RBC reactive oxygen species levels, and RBC mitochondrial retention in HbSS mice, suggesting potential beneficial mechanisms apart from inhibition of HbS polymerization by reducing 2,3-DPG.

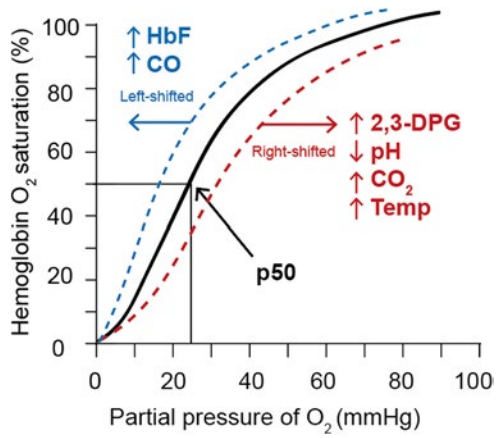
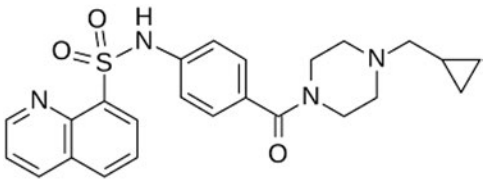
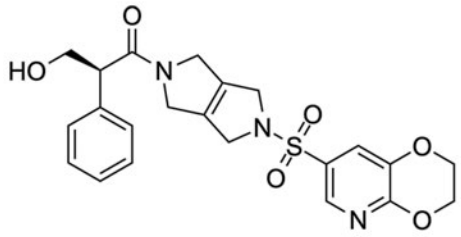


Figure 2. Factors influencing the hemoglobin oxygen dissociation curve.



Mitapivat



Etavopivat

Figure 3. Biochemical structures of first-generation pyruvate kinase activators.

Completed phase 1 and phase 2 single-center multiple ascending dose studies (NCT04000165, EudraCT 2019-003438-18; Table 1) demonstrated promising clinical effects of mitapivat in patients with SCD.[29,30] Pharmacokinetic and pharmacodynamic effects of mitapivat were demonstrated to be similar to data from healthy volunteers, with a dose-dependent increase in serum drug and ATP levels and a dose-dependent decrease in 2,3-DPG levels. In the phase 1 study,[29] participants with SCD treated with mitapivat had a significant increase in mean hemoglobin of 1.2 g/dL, and 56.3% of participants achieved a ≥1-g/dL increase in hemoglobin from baseline. Mean reductions in hemolytic markers were observed as well. Furthermore, there was a trend toward decreased p50 and increased time to sickling, t50; while not significant, these findings support the mechanism of action of decreasing 2,3-DPG to increase hemoglobin oxygen affinity and delay sickling. The phase 2 study (ESTIMATE) showed similar results, with a mean increase in hemoglobin level of 1.3 g/dL and 1.1 g/dL during the 8-week dose finding and the 1-year fixed-dose extension period, respectively,[30,31] as well as a significant increase in ATP and reduction in 2,3-DPG, hemolytic markers, p50, and point of sickling during the extension period, presented as a conference abstract.[31]

Most treatment-related adverse events were nonserious in both studies. The phase 1 study reported 2 VOCs as related to drug, 2 VOCs not related to drug, and a serious adverse event (SAE) of pulmonary embolism not related to drug.[29] The first VOC that was possibly related to drug occurred during the drug taper period, leading to a protocol amendment to extend the drug taper length. The second possibly drug-related VOC also occurred during drug taper, although in the setting of self-reported VOC triggers. The ESTIMATE study reported a massive pulmonary embolism due to COVID-19 resulting in death and a grade 4 SAE of urinary tract infection with hypotension that were both unrelated to drug.[30,31] During the extension period, 4 VOCs occurred, with 3 in the setting of documented noncompliance the week before. The mean annualized VOC rate and SCD-related hospital admission days were decreased compared to baseline, although these differences were not significant.[30,31] Overall, the 2 studies concluded that mitapivat was safe and tolerable in participants with SCD, and a phase 2/3 multicenter study, RISE UP

Table 1. Summary of ongoing and completed clinical trials of pyruvate kinase activators in sickle cell disease

	Trial	Subjects	Study design	Status
Mitapivat (AG-348)	NCT04000165[29]	N=17, age ≥18, HbSS	Phase 1, open-label, multiple ascending dose study	Completed
	ESTIMATE (EudraCT 2019–003438–18)[30,31]	N=9, age ≥16, HbSS, HbS/β^0- or HbS/β^+-thalassemia	Phase 2, open-label, multiple ascending dose phase, followed by fixed-dose extension study	Ongoing
	NCT04610866	N=15, age 18–70, HbSS	Phase 1/2, open-label extension study	Ongoing
	RISE UP (NCT05031780)	N=267, age ≥16, any SCD genotype	Phase 2/3, randomized, placebo-controlled, double-blind, followed by open-label extension study	Ongoing
Etavopivat (FT-4202)	NCT03815695[34–36]	N=130, healthy volunteers and patients with SCD (any genotype) age 12–65	Phase 1, randomized, placebo-controlled, double-blind, single ascending and multiple ascending dose study	Completed
	NCT04987489	N=60, age 12–65, patients with thalassemia and any SCD genotype	Phase 2, open-label study	Ongoing
	NCT05725902	N=12, age 12–21, HbSS or HbS/β^0	Phase 2, open-label study	Ongoing
	HIBISCUS (NCT04624659)	N=344, age 12–65, any SCD genotype	Phase 2/3, randomized, placebo-controlled, double-blind, followed by open-label extension study	Ongoing
	HIBISCUS-KIDS (PACTR202209604592389)	N=50, age 12 to <18, any SCD genotype	Phase 1/2, open-label, single-arm, followed by extension study	Ongoing
AG-946	NCT04536792	N=64, age 18–70, healthy volunteers and patients with SCD (any genotype)	Phase 1, open-label, single ascending and multiple ascending dose study	Ongoing

(Table 1), is in progress, with primary end points of hemoglobin response, treatment-emergent adverse events (phase 2), and annualized rate of VOC (phase 3).

Etavopivat

Etavopivat (FT-4202) is another small-molecule PK activator in clinical development for SCD and thalassemia. Preclinical studies of etavopivat in the Berkeley sickle cell anemia (BERK SCA) mouse model showed significantly decreased 2,3-DPG and increased ATP levels, which were associated with decreases in p50, point of sickling, number of irreversibly sickled cells on blood smear, and increased RBC deformability.[32] In addition, treatment with etavopivat led to a mean hemoglobin increase of 1.7 g/dL, a significant reduction in hemolytic markers, and a 28.5% increase in RBC half-life compared to untreated BERK SCA mice, demonstrating clear mechanistic benefits of decreasing 2,3-DPG and increasing ATP in sickle RBCs. Ex vivo studies similarly showed a significant decrease in p50 and point of sickling with etavopivat treatment of RBCs from patients with HbSS and HbSC disease.[33]

While the full results of a completed phase 1/2 study of etavopivat in SCD (NCT03815695; Table 1) have not yet been published, results to date have been presented as conference abstracts.[34-36] In the 12-week open-label extension phase, the mean maximal hemoglobin increase from baseline was 1.5 g/dL, with 73.3% achieving a hemoglobin increase of >1 g/dL.[34] Increases in hemoglobin and decreases in hemolytic markers were significant at all time points between 2 and 12 weeks of treatment.

In line with the etavopivat preclinical and mitapivat clinical data, 2,3-DPG decreased and ATP increased from baseline throughout the 12 weeks of treatment, followed by a return to baseline after 4 weeks of washout.[35] A significant decrease in mean p50, point of sickling, and dense RBCs and an increase in RBC deformability were observed at 12 weeks of treatment.[34] Additionally, data from the 2-week and 12-week treatment phases of the study demonstrated an increased mean corpuscular volume, decreased mean corpuscular hemoglobin concentration, increased antioxidant capacity, and decreased markers of inflammation, hypercoagulability, adhesion, and serum erythropoietin level, suggesting potential pleiotropic salutary effects of PK activation in SCD.[34,35]

Most safety events were grades 1 to 2, most commonly sickle cell pain events and headache.[34,36] On study, there were 2 SAEs of VOC unrelated to study drug and an SAE of left femoral vein deep vein thrombosis possibly related to study drug. Compared to historical data, the annualized rate of VOC requiring hospitalization during the 12-week treatment period was lower, although this difference was not significant. A phase 2/3 randomized, placebo-controlled study (HIBISCUS; Table 1) in adults and adolescents with SCD and a phase 1/2 open-label study (HIBISCUS-KIDS) in children with SCD are ongoing, with primary end points of hemoglobin response and annualized VOC rate (HIBISCUS) and pharmacokinetic assessments plus safety and tolerability (HIBISCUS-KIDS), respectively.

AG-946

AG-946 is a second-generation PK activator in clinical development for the treatment of SCD and low-risk myelodysplastic syndrome. AG-946 differs pharmacokinetically and pharmacodynamically from first-generation mitapivat in that AG-946 has greater potency, a longer half-life (~80-110 hours vs 3-6 hours), and more prolonged effects on 2,3-DPG and ATP levels (observed at least 14 days after last dose in healthy controls),[37] which provides a potential self-tapering effect. Preclinical data presented in a conference abstract demonstrated AG-946's ability to normalize levels of glycolytic intermediates, decrease 2,3-DPG levels, and increase hemoglobin levels in a Townes HbSS mouse model, although it did not significantly affect ATP levels or degree of RBC sickling.[38] Ex vivo treatment of RBCs from patients with SCD with AG-946 also decreased p50 and point of sickling, similar to first-generation PK activators.[39] In an ongoing phase 1 clinical trial, enrollment in single and multiple ascending dose cohorts for healthy volunteers has been completed and was deemed to be safe; this study is now enrolling multiple sequential dosing cohorts in participants with SCD (NCT04536792).

CLINICAL CASE (continued)

The patient elects to participate in a phase 2/3 clinical trial of a pyruvate kinase activator. Her hemoglobin level, hemolytic markers, and rate of VOC will be monitored closely.

Concerns about increasing hemoglobin oxygen affinity

Recent concerns were raised about the importance of oxygen delivery by RBCs in anemia and drugs that affect the oxygen affinity of hemoglobin.[40] Individuals with a normal hemoglobin level have baseline 20% hemoglobin oxygen unloading, while in anemia, 30% is unloaded due to increased levels of 2,3-DPG. This compensatory effect can be observed in PK deficiency, which markedly shifts the oxygen dissociation curve to the right through increased 2,3-DPG, allowing for more oxygen unloading; exercise tolerance on a bicycle ergometer was greater in a PK-deficient individual than an individual with hexokinase deficiency, with a similar hemoglobin but lower 2,3-DPG level. The article reminds hematologists of this basic physiologic principle when considering PK activators or voxelotor, drugs that alter oxygen affinity but increase hemoglobin by a modest 1 to 2g/dL. It is important to keep in mind that compared with other types of anemias, the lowering of 2,3-DPG through PK activation may increase the delay time in HbS polymerization and prevent sickling, providing a uniquely beneficial effect in SCD. Further concerns have been raised about cerebral oxygen delivery with drugs that alter oxygen affinity in SCD,[41] with conflicting evidence in the literature; 1 recent conference abstract showed that voxelotor treatment in children with SCD reduces cerebral metabolic stress by improving cerebral oxygen delivery,[42] while another abstract showed no changes in cerebral blood flow and oxygen metabolic rate ($CMRO_2$), despite increased cerebral oxygen delivery due to improvement of hemoglobin levels.[43] These data reflect the complex physiology underlying cerebral hemodynamics and metabolism in SCD, which requires further investigation.

Additional considerations

While the preclinical and clinical data to date for PK activators are promising, additional placebo-controlled studies and demonstration of effects on clinical outcomes such as VOC frequency are needed in the SCD population. There has also been some concern over withdrawal of therapeutic effect with abrupt cessation of

therapy. In patients with PK deficiency, treatment cessation at higher doses of mitapivat led to episodes of withdrawal hemolysis and anemia, prompting the addition of a drug taper, with no further episodes of withdrawal hemolysis on study.[44] While there was no clear evidence of withdrawal hemolysis associated with the VOCs occurring during the clinical trials in SCD, a sudden stop in drug dosing due to noncompliance or side effects could theoretically translate into an abrupt increase in 2,3-DPG levels and a consequent shift of the oxygen dissociation curve to the right, promoting an acute increase in deoxy-HbS polymerization, sickling, and vaso-occlusion. On the other hand, increased RBC ATP may improve RBC survival, which may provide a longer-term protective effect. The results of the ongoing phase II/III studies of mitapivat and etavopivat should shed additional light on this question.

Another consideration is the cost of novel therapies and global access to these therapies. Insurance approval has proven a barrier to access for recently approved drug therapies for SCD in the United States, and the out-of-pocket cost has been prohibitive for the use of these new drugs in low- and middle-income countries (LMICs). As drug development advances in the SCD arena, more effort needs to be focused on equitable distribution of drug access to LMICs, where most of the SCD disease burden is concentrated.

Finally, with multiple drug therapies now available for SCD and numerous novel therapies coming down the pipeline, further research into how to compare, combine, or sequence drug therapies is needed to establish data-driven guidelines for the treatment of complications in SCD. With existing and potential drugs targeting HbF induction, hemoglobin oxygen affinity, RBC metabolism, hemolysis, adhesion, oxidative stress, and complement activation, we are approaching a reality in which multimodal drug therapy will hopefully usher in an era of precision medicine in SCD,[45] providing targeted treatment of different disease phenotypes.

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Conflict-of-interest disclosure

Julia Z. Xu is the US national principal investigator for the phase 1 clinical trial of AG-946 in patients with sickle cell disease. Julia Z. Xu receives research funding and serves on an advisory committee for GlaxoKlineSmith.

Gregory M. Vercellotti receives research funding from CLS Behring, Omeros, and Novartis and serves on the DSMB of Novo Nordisk/Forma, Alexion, Hillhurst, and Sangamo.

Off-label drug use

Julia Z. Xu: There is nothing to disclose.

Gregory M. Vercellotti: There is nothing to disclose.

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Pyruvate kinase activators: targeting red cell metabolism in thalassemia

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Thalassemia is an inherited red blood cell disorder whereby the qualitative and/or quantitative imbalance in α- to β-globin ratio results in hemolysis and ineffective erythropoiesis. Oxidative stress, from the precipitated excess globin and free iron, is a major factor that drives hemolysis and ineffective erythropoiesis. Pyruvate kinase activity and adenosine triphosphate availability are reduced due to the overwhelmed cellular antioxidant system from the excessive oxidative stress. Mitapivat, a pyruvate kinase activator in development as a treatment for thalassemia, was shown to increase hemoglobin and reduce hemolysis in a small phase 2 single-arm trial of patients with α- and β-thalassemia. The ongoing phase 3 studies with mitapivat and the phase 2 study with etavopivat will examine the role of pyruvate kinase activators as disease modifying agents in thalassemia.

LEARNING OBJECTIVES

- Understand thalassemia as an inherited red blood cell disorder where both oxidative stress and hemolysis play a major role in the pathogenesis
- Describe the results of the phase 2 single-arm trial of pyruvate kinase activator mitapivat in patients with α- and β-thalassemia
- Appreciate that pyruvate kinase activation is the first mechanism targeted in clinical trials for patients with α-thalassemia

CLINICAL CASE

A 34-year-old female with non-deletional Hemoglobin H disease ($\alpha^{-3.7}$/Hemoglobin Constant Spring compound heterozygote) is referred by another hematologist for a second opinion regarding splenectomy. Patient receives on average 2–3 units of packed red blood cells per year for episodes of symptomatic anemia and has a liver iron concentration of 3.0 mg/g dw. She was advised to undergo splenectomy by the referring hematologist in hopes of improving her hemoglobin (Hb) and her symptoms, but the patient is concerned about the risk of infection and thrombosis associated with asplenia and wishes to explore alternatives to splenectomy. Patient on review has fatigue, exertional dyspnea, and exercise limitations. She comments on being frustrated by her profound fatigue and the need to take a nap almost daily. Other secondary causes of fatigue including depression have been ruled out. On examination, the patient has scleral icterus and palpable splenomegaly at 5cm below the costal margin. Laboratory findings included a hemoglobin of 7.0 g/dL, MCV 65 fL, mean corpuscular hemoglobin 21 pg/cell, absolute reticulocyte count 336×10⁹/L, lactate dehydrogenase level 462 U/L, total bilirubin 3 mg/dL (indirect bilirubin 2.8 mg/dL), haptoglobin undetectable. Abdominal ultrasound demonstrated a spleen size of 18 cm in craniocaudal length.

Introduction

Thalassemia is an inherited red blood cell (RBC) disorder whereby the qualitative and/or quantitative imbalance in α- to β-globin ratio results in ineffective erythropoiesis and hemolysis.[1] A paucity or qualitative deficiency in β-globin results in β-thalassemia and vice versa. Presentation is highly variable, ranging from those who do not require regular transfusions to survive (non-transfusion-dependent thalassemia [NTDT]) to severely anemic patients who are transfusion-dependent (TDT). NTDT is a highly prevalent condition in many areas around the globe and is becoming

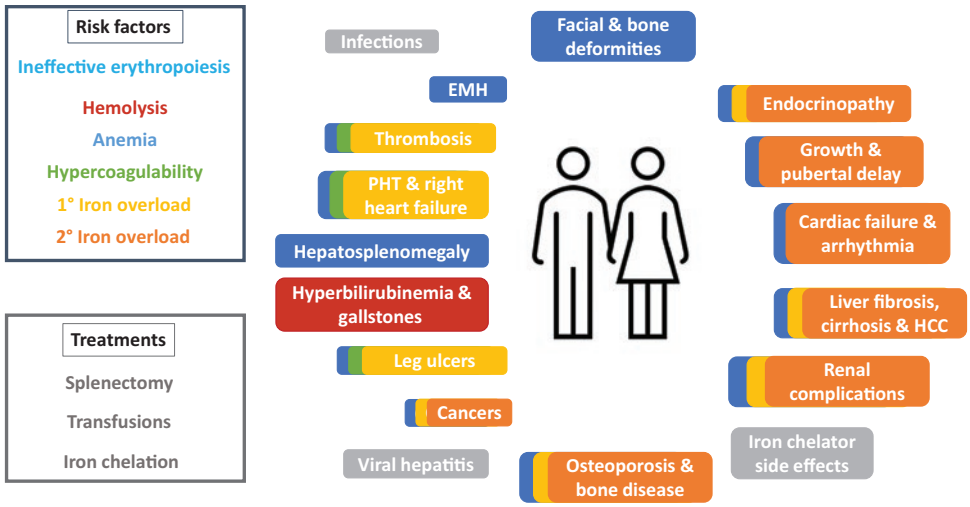

Figure 1. Multisystem complications from risk factors and treatments of thalassemia. Risk factors and their corresponding complications are color-coded. Complications with overlapping colors denote those stemming from and exacerbated by the multiple risk factors involved. Modified from Taher AT, Musallam KM, Cappellini MD. β-Thalassemias. *N Engl J Med.* 2021;384(8):727–743. 1°, primary; 2°, secondary; EMH, extramedullary hematopoiesis; HCC, hepatocellular carcinoma; PHT, pulmonary hypertension.

more prevalent in others due to migration. There is growing evidence that patients with NTDT face a high burden of morbidity and mortality despite not being transfusion dependent.[1,2] Ineffective erythropoiesis and hemolysis drives anemia, hypercoagulability, and primary iron overload, resulting in a myriad of complications including gallstones, hepatosplenomegaly, bone deformities, osteoporosis, extramedullary hematopoiesis (EMH), leg ulcers, thrombosis, pulmonary hypertension, endocrinopathies, growth delay, liver fibrosis and cirrhosis, heart failure, and arrhythmia, among others (Figure 1).[1] Treatment for NTDT is currently confined to treatment of these complications (e.g., hypertransfusion for EMH) since there is no approved disease-modifying therapy, and in particular, for patients with α-thalassaemia. Likewise, treatment for TDT is also largely confined to regular transfusions and supportive care. The ensuing iron overload from regular transfusions further exacerbates the liver, cardiac, endocrine, and renal complications (Figure 1).[1] Use of splenectomy for both NTDT and TDT are in decline because of the risk of infection and thrombosis.[1] While luspatercept is currently approved for β-TDT, it reduces the transfusion burden by one-third or more (and at least 2 units over 12 weeks) from baseline in only 21.4% of patients and must be administered as subcutaneous injections every 3 weeks.[3] Gene therapy is approved for use in a small segment of patients with β-TDT thalassaemia. Since hemolysis plays a crucial role in the pathogenesis of thalassemia, agents that can combat the hemolysis would be desirable. To this end, two compounds, mitapivat and etavopivat, have been developed and are currently undergoing clinical trials in patients with thalassemia. Mitapivat and etavopivat are two oral small molecular allosteric activators of the RBC pyruvate kinase (PKR), the last enzyme in glycolysis that converts phosphoenolpyruvate to pyruvate. Mitapivat is approved by the FDA for treatment of pyruvate kinase deficiency, and both mitapivat and etavopivat are currently evaluated for the treatment of thalassemia and sickle cell disease (SCD). RBCs are entirely dependent on glycolysis (due to the lack of mitochondria) to produce energy (e.g., adenosine triphosphate [ATP]), needed for the maintenance of cellular metabolism, hydration, membrane integrity, and deformability, and to provide antioxidant capacity to protect hemoglobin molecules and other proteins from continuous oxidative stress.[4] Any mismatch in energy (ATP) supply and demand leads to reduced RBC health and lifespan. How this mismatch occurs in thalassemia and can be attenuated by pyruvate kinase (PK) activation will be detailed below.

Ineffective erythropoiesis and hemolysis in β-thalassemia

In β-thalassemia, excess α-globin overwhelms the α hemoglobin stabilizing protein, which normally stabilizes the free α-globin chains. The free α-globin aggregates and precipitates, forming inclusion bodies called Fessas bodies.[5] In normal erythropoiesis, GATA1 promotes terminal erythroid differentiation, and GATA1 is protected by Heat Shock Protein 70 (HSP70) in the nucleus. In β-thalassemia, the free α-globin sequesters HSP70 in the cytoplasm, aided by exportin-1 (Figure 2).[6] HSP70, having been lured away, is unable to protect GATA1 from degradation by Caspase 3, leading to accumulation and apoptosis of the erythroid precursors in the bone marrow, termed ineffective erythropoiesis.[7] Moreover, free α-globin molecules auto-oxidize and form hemichromes (α-globin with oxidized ferric iron) and reactive oxygen species (ROS). The hemichromes precipitate and intercalate into the RBC plasma membrane and oxidize the membrane lipid and proteins.[7] The resultant destabilization of the RBC membrane cannot be readily detoxified by the cellular antioxidant system, and the excess ROS leads to intramedullary cell death. If by chance the RBCs survive the maturation journey, these cells have diminished lifespan due to the oxidative stress, and hemolysis ensues in the peripheral circulation (Figure 2). Oxidative injury causes Band 3 to cluster, producing a neoantigen that binds to the immunoglobulin G and complement, resulting in removal of the damaged RBC by macrophages in the circulation. In addition, the free iron liberated from the hemolysis creates highly reactive hydroxyl and hydroperoxyl radicals, which exerts further oxidative stress and tissue damage.[7]

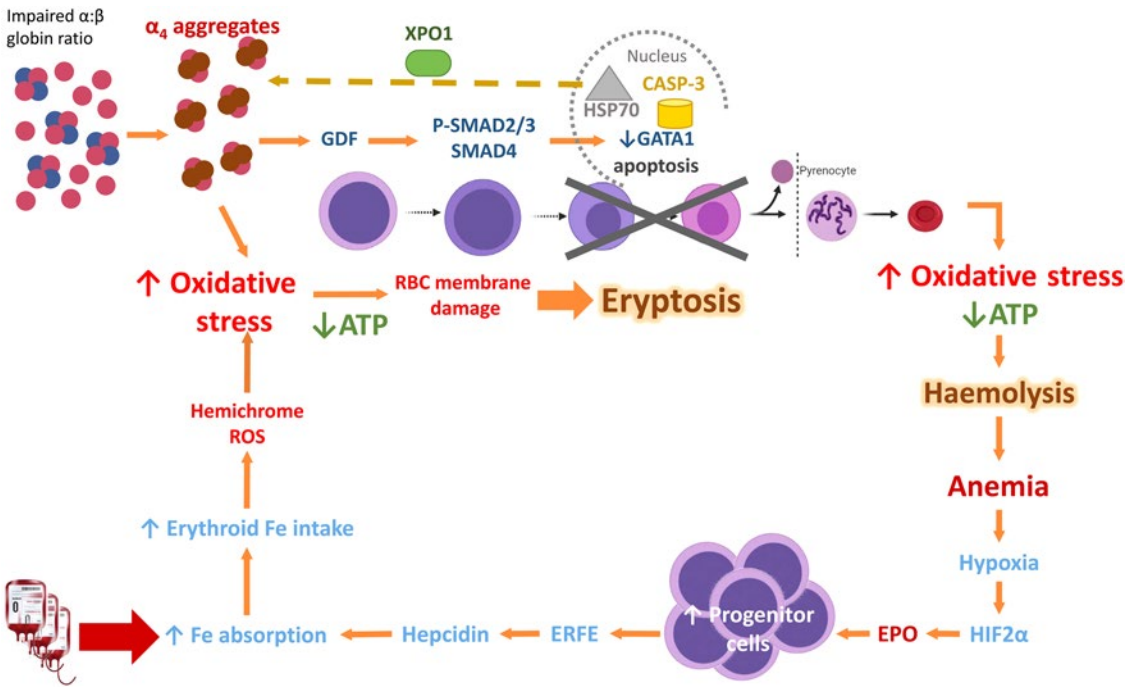

Figure 2. Mechanism of oxidative stress, ineffective erythropoiesis and hemolysis in β-thalassemia. Impaired α- to β-globin ratio results in aggregation and precipitation of the excess α-globin, exerting oxidative stress on the developing RBC. Concurrently, α-globin aggregates induce the sequestration of HSP70 in the cytoplasm, GDF activation and phosphorylation of SMAD2/3, and activation of SMAD4. Both processes lead to reduced GATA1 resulting in eryptosis. RBCs that exit the marrow soon undergo hemolysis because of shortened RBC lifespan. Modified from Longo F, Piolatto A, Ferrero GB, Piga A. *Int. J. Mol. Sci.* 2021, 22, 7229, Taher A and Saliba AN. *Hematology Am Soc Hematol Educ Program.* 2017 Dec 8;2017(1):265–271; Musallam KM, et al. *Haematologica.* 2011;96(11):1605–1612; Rivella S. *Haematologica.* 2015;100(4):418. ATP, adenosine triphosphate; EPO, erythropoietin; ERFE, erythroferrone; GDF, growth differentiation factor; HIF2α, hypoxic inducible factor 2α; HSP70, Heat Shock Protein 70; RBC, red blood cell; ROS, reactive oxygen species; XPO-1, exportin-1.

Oxidative stress and hemolysis in α-thalassemia

Conversely, in α-thalassemia, excess β-globin and γ-globin form tetramers called hemoglobin H and Barts, respectively. Although hemoglobin H (HbH) and Barts are more stable than α globin tetramers, they are still liable to induce the ROS formation and cause damage to the developing RBC. The phenotype of HbH disease, a form of α-thalassemia where three of the four α-globin genes are mutated, is characterized by chronic hemolytic anemia.[8] The exact mechanism by which α-thalassemia leads to hemolysis and ineffective erythropoiesis is less clear, but abundant evidence suggests that the process may be analogous to β-thalassemia in that oxidative stress plays an important component in the pathophysiology. This includes findings of low glutathione levels,[9] hepcidin, total antioxidant capacity levels,[10] and elevated malondialdehyde levels in patients with α-thalassemia.[11]

Impairment of glycolysis in thalassemia

There is also evidence to show that in thalassemia, the glycolytic pathway is affected by the oxidative stress. Ting et al determined, via ^{13}C and ^{31}P magnetic resonance spectroscopy, that glucose metabolism in the RBCs of patients with β-thalassemia intermedia were significantly (approximately three times) higher than in healthy patients or patients with β-thalassemia trait, even when contribution by reticulocytes were excluded.[12] However, ATP concentrations in the RBCs of patients with β-thalassemia major or Hemoglobin E/β-thalassemia were significantly lower than healthy controls.[13] These two studies together show that glucose metabolism is shifted away from the glycolytic pathway and toward the pentose phosphate pathway. PK enzyme activity and stability were found to be reduced in patients with TDT with no pathogenic PKLR mutation, even when adjusted for RBC age and the presence of reticulocytosis.[14] Matte et al have shown that ROS levels were increased in the RBCs of $Hbb^{th3/+}$ mice compared to wild-type mice, and that PKR and PKM2 expression was higher in both the reticulocyte and older RBC fractions.[15] PKM2 was also upregulated in the RBCs of $Hbb^{th3/+}$ mice and may act as a compensatory response to the increased oxidative stress.[15] These discoveries led to the hypothesis that pyruvate kinase activation may reduce oxidative damage, thus reducing hemolysis, improving ineffective erythropoiesis and RBC lifespan, and resulting in improvement of anemia. Also, improvement in ineffective erythropoiesis may alleviate the dysregulated iron metabolism, leading to reduction in iron overload (Figure 3).

Pyruvate kinase activation in thalassemia mouse model

To examine the effect of pyruvate kinase activation in thalassemia, Matte et al administered mitapivat to $Hbb^{th3/+}$ mice at 50 mg/kg twice daily for 21 days (by gavage) and 56 days (by oral diet), respectively. After 21 days, improvements were observed in Hb, mean corpuscular volume (MCV), mean corpuscular Hb, RBC morphology, and survival (14 vs 9.6 days in treated and untreated $Hbb^{th3/+}$ mice compared to 18.9 days in wild-type mice). Concomitant reduction in markers of hemolysis (lactate dehydrogenase [LDH], total and indirect bilirubin), erythropoietin (EPO) level,

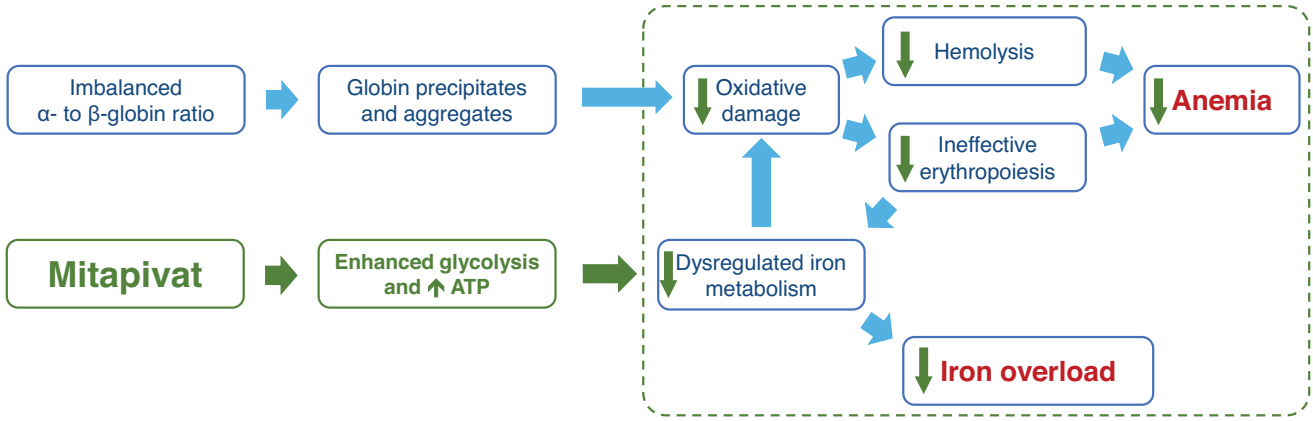

Figure 3. Hypothesis on how pyruvate kinase activation, through enhanced glycolysis and increased in ATP availability, reduces oxidative damage and hemolysis and improves ineffective erythropoiesis and dysregulated iron metabolism, thereby improving anemia and iron overload.

reticulocyte count, ROS, hemichromes, and α-globin membrane precipitates was observed, accompanied by an increased glutathione to glutathione disulfide ratio. An increase in ATP was also observed. In addition to the amelioration of hemolysis, mitapivat improved ineffective erythropoiesis. Splenic extramedullary hematopoiesis was reduced, and PK activity was increased in RBC polychromatic and orthochromatic erythroblasts of mitapivat-treated $Hbb^{th3/+}$ mice, accompanied by reduced apoptotic (annexin-V$^+$) erythroblasts and ROS in maturing erythroblasts.[15] This also resulted in a reduction in the erythroferrone (ERFE) level, followed by upregulation of liver hepcidin expression and a reduction in hepatic iron overload. There is evidence that mitapivat may also reduce duodenal iron absorption via the PKM2-HIF2α axis by downregulation of HIF2α, NF-κb p65 active form, FPN1, and Dmt1 in the enterocytes of the mitapivat-treated $Hbb^{th3/+}$ mice.[15]

Recently, Mattè et al demonstrated that in chronically transfused $Hbb^{th3/+}$ mice treated with mitapivat, the results were a longer interval between transfusions (13.8 vs 10.5 days in mitapivat and vehicle-treated mice, respectively) and reduced splenic iron accumulation.[16] Similar to the $Hbb^{th3/+}$ mice not on transfusion treated with mitapivat, α-globin membrane precipitates, EPO level and liver iron accumulation were reduced, hepcidin increased, and splenic macrophage function was shifted from a pro-inflammatory to a pro-resolving state.[16] Concomitant administration of mitapivat with deferiprone had a similar effect.[16] These findings provided the preclinical evidence for subsequent trials of pyruvate kinase activation in both TDT and NTDT.

CLINICAL CASE (continued)

The patient was once again counseled on the benefits and risks of splenectomy, including the potential benefit of increasing total hemoglobin by 1–2 g/dL, but she declined this approach based on the four- to five-fold risk of venous thromboembolism and pulmonary hypertension compared to non-spelenectomized patients.[17] Regular transfusion was also offered as an option, but the patient was concerned about the need for chronic iron chelation. Given the patient's hemolytic profile, she was offered the opportunity to participate in a clinical trial of a pyruvate kinase activator.

Pyruvate kinase activation in thalassemia patients

Mitapivat

The phase 2, open-label, multicenter study investigated the efficacy and safety of mitapivat in adult patients with α- and β-NTDT.[18] The primary objective of the study was to evaluate the efficacy of mitapivat in NTDT. Twenty patients with NTDT from four sites across the US, UK, and Canada were enrolled. The genotype inclusion criterion was broad and included β-thalassemia with or without α-globin gene mutations, HbE/β-thalassemia, or α-thalassemia. Patients had to have a hemoglobin less than 10 g/dL, and non-transfusion dependency was defined as less than 6 RBC units transfused in the preceding 24 weeks and none in the 8 weeks prior to study drug-dosing. Following screening, patients entered a 24-week core period where they received an initial dose of mitapivat 50 mg twice a day orally. At week 6, the dose was increased to 100 mg twice a day based on safety and tolerability. After the 24-week core period, patients may enter an optional 10-year extension period. The primary endpoint was defined as an increase of hemoglobin by at least 1 g/dL from baseline between weeks 4 and 12. Secondary and exploratory endpoints included sustained or delayed Hb response, markers of hemolysis, hematopoietic activity, and safety. The secondary endpoint was defined as meeting the primary response and at least a 1 g/dL increase in hemoglobin in at least two readings between weeks 12 and 24. The median age in this cohort was 44 years, 50% of the population was Asian, and the median Hb at baseline was 8.4 g/dL.[18] Overall, 5 patients had α-thalassemia (HbH), and 15 patients had β-thalassemia. The primary endpoint was met in 80% (16/20) of the participants; all 5 patients with α-thalassemia and 11/15 patients with β-thalassemia met the primary endpoint. The secondary endpoint of sustained hemoglobin response was met in 65% of the 20 patients, with all 5 patients with α-thalassemia meeting this endpoint. The mean change in Hb from baseline over a 12-week interval between weeks 12 and 24 was similar between α- and β-thalassemia (1.2 and 1.3 g/dL, respectively), and the mean time to ≥1 g/dL increase in Hb among the responders was

4.5 weeks.[18] Qualitatively, markers of hemolysis (LDH and bilirubin) and erythropoiesis were reduced or remained stable in most patients. Consistent with the mechanism of action of mitapivat, the mean ATP change in blood ranged from 62-87%, similar to what was previously observed in a multiple-ascending dose study in healthy volunteers (60% maximum increase in ATP).[18] Note that all participants in this cohort had nonpathogenic PKLR genotypes.

Safety of mitapivat in thalassemia

In terms of safety, 17 (85%) patients experienced a treatment-emergent adverse event during the 24-week core period, with most being grade 1 or 2; the majority were self-limiting. The most commonly reported adverse events were initial insomnia ($n = 10$ [50%]), dizziness ($n = 6$ [30%]), and headache ($n = 5$ [25%]).[18] The safety profile was similar to prior studies of mitapivat in healthy volunteers and patients with PK deficiency. In the 17 participants who enrolled in the 10-year long-term extension (LTE), Hb increase was sustained with a mean increase in Hb of 1.7 g/dL. Qualitatively, continued reduction in LDH, indirect bilirubin, and EPO was observed in the LTE up to week 72, as well as a reduction (expressed as median change from baseline) in ERFE (−5430 ng/L), reticulocytes/erythrocyte ratio (−0.007), and soluble transferrin receptor level (−1.82 mg/L), while hepcidin remained stable over time (+650 ng/L).[19] The type and frequency of adverse events in the extension period were consistent with those observed in the core period. Initial insomnia was confined to the core period and was not observed in the LTE. Adverse events occurring in ≥15% of patients during the extension period were headache (5/17) and back pain (3/17), none of which were grade ≥3. In patients who have had repeat bone mineral density in the extension period, no trends for decreases were observed.

Phase 3 trial of mitapivat in thalassemia

The encouraging results from the phase 2 study have led to the development of two phase 3, multicenter, randomized, double-blind, placebo-controlled studies of mitapivat, in patients with non-transfusion dependent (ENERGIZE; NCT04770753) and transfusion-dependent α- or β-thalassemia (ENERGIZE-T; NCT04770779) (Table 1).[20] In ENERGIZE, 171 adults with either α- or β-thalassemia (based on Hb electrophoresis, Hb HPLC, and/or DNA analysis), a Hb ≤10 g/dL, and non-transfusion dependency (defined as ≤5 RBC units during the 24-weeks before randomization and no RBC transfusions within the 8 weeks prior) are enrolled in the 24-week double-blind core period and randomized in a 2:1 fashion to receive either mitapivat 100mg or placebo twice daily. The primary endpoint is Hb response, its definition being similar to the primary endpoint in the phase 2 study. However, the secondary endpoints include a patient-reported outcome and functional assessment (change in Functional Assessment of Chronic Illness Therapy [FACIT]-Fatigue subscale score, patient-reported global measures of fatigue, 6-minute walk test distance) in addition to change in average Hb concentration, markers of hemolysis, erythropoiesis, and iron metabolism. The design of ENERGIZE-T is similar, with key differences being a 48-week double-blind core period enrolling 240 adult α- or β-TDT (e.g., patients who are transfusion-dependent with HbH disease or α-thalassemia major/Hb Barts hydrops fetalis would qualify), the primary endpoint being transfusion reduction response (defined as a 50% or greater reduction in transfused RBC units with a reduction of two or more units of transfused RBCs in any consecutive 12-week period through week 48 compared with baseline), and the secondary endpoints examining transfusion reduction at different degrees and intervals within the 48-week core period. Transfusion-dependency is defined as 6 to 20 RBC units transfused and no transfusion-free period for more than 6 weeks during the 24-weeks before randomization. In both studies, eligible patients have the option of entering the 5-year open-label extension. Both studies also permit the enrollment of patients on stable hydroxyurea dose for at least 16 weeks before randomization, recognizing that it is used by some centers in the treatment of patients with β-thalassemia.[17]

Etavopivat

Etavopivat is also being evaluated in NTDT and TDT following the conclusion of the single-ascending dose and multiple-ascending dose phase 1 trial (NCT03815695) in 90 healthy adults.[21] The ongoing phase 2 open-label study (NCT04987489) aims to evaluate the safety and efficacy of etavopivat in patients 12 to 65 years old with SCD on chronic transfusions (cohort A), TDT (cohort B), and NTDT (cohort C) (Table 1).[22] Patients will receive etavopivat 400mg once daily for 48 weeks for all cohorts. The primary endpoint for the TDT cohort is erythroid response, defined as the proportion of patients with 20% or greater reduction in transfusion over a continuous 12-week treatment period compared to baseline. The primary endpoint for the NTDT cohort is defined as a 1.0g/dL or greater increase from baseline at week 12 in Hb.[22] Both the TDT and NTDT cohorts plan to enroll 12 to 20 patients and are powered to detect a response rate of 60% and 50% to the primary endpoint in the TDT and NTDT cohorts, respectively. Secondary and exploratory endpoints include other transfusion

Table 1. Clinical trials of pyruvate kinase activators in thalassemia

Category	Phase	Drug	N	Duration	Identifier	Status
NTDT	2 (single-arm, open-label)	Mitapivat	20	24 weeks	NCT03692052	Completed
		Etavopivat	20	48 weeks	NCT04987489 (cohort C)	Ongoing
	3 (double-blind RCT)	Mitapivat: placebo (2:1)	171	24 weeks	NCT04770753	Ongoing
TDT	2 (single-arm, open-label)	Etavopivat	20	48 weeks	NCT04987489 (cohort B)	Ongoing
	3 (double-blind RCT)	Mitapivat: placebo (2:1)	240	48 weeks	NCT04770779	Ongoing

RCT, randomized controlled trial.

reduction and hemoglobin response parameters, changes in HRQoL (SF-36 and PROMIS), serum ferritin levels, liver iron, 2,3-BPG, ATP, PK, and safety parameters.[22]

CLINICAL CASE (continued)

The patient was enrolled in the phase 2 study of mitapivat in NTDT. Her hemoglobin increased by 1.0 g/dL from baseline within 4 weeks of starting treatment and reached 1.4 g/dL at 24 weeks. She had initial insomnia, which abated by day 5 on treatment without intervention. Her markers of hemolysis improved and EPO level reduced. Subjectively, the patient noted that her exercise capacity increased, and most important to her, she no longer needed to take a nap in the afternoon.

Considerations on the role of pyruvate kinase activation in the treatment of thalassemia

The preclinical and clinical evidence for pyruvate kinase activation in thalassemia and other hemolytic disorders like pyruvate kinase deficiency and SCD thus far points to its effectiveness at ameliorating hemolysis brought on by oxidative stress. As such, one may surmise that it may be more effective in thalassemia genotypes that result in a predominantly hemolytic phenotype (e.g., non-β^0/β^0 thalassemia or HbH disease). However, no distinct relationship between genotype and hemoglobin response could be gleaned from the phase 2 data of mitapivat in thalassemia because 80% of patients met the primary endpoint, despite their diverse thalassemia genotype, as noted by Kuo et al.[18] The fact that mitapivat also improved ineffective erythropoiesis in $Hbb^{th3/+}$ mice suggests that mitapivat may have comparable efficacy in patients whose pathophysiology is dominated by ineffective erythropoiesis. The ongoing phase 2 and 3 trials may provide further insight into how pyruvate kinase activation may be effectively used in the treatment of patients with thalassemia.

Conclusion

Despite advances in the understanding in the pathophysiology of thalassemia, treatment of patients with NTDT, and in particular α-thalassemia, remains supportive. Luspatercept is the only approved treatment for patients with TDT aside from transfusion. Pyruvate kinase activation was able to increase hemoglobin concentration and reduce hemolysis in the majority of the patients with thalassemia in the phase 2 study, despite their diverse genotypic heterogeneity, which provides the proof of concept that suppression of hemolysis may improve the pathology of both α- and β-thalassemia. The ongoing phase 3 trials of mitapivat and the phase 2 trials of etavopivat in α- and β-thalassemia will determine whether pyruvate kinase activation will improve patient-important outcomes by assessing their functional and quality-of-life impact beyond change in hemoglobin and markers of hemolysis and erythropoiesis.

Conflict-of-interest disclosure

Kevin H.M. Kuo: consultancy and research funding: Agios Pharmaceuticals, Pfizer; consultancy: Alexion Pharmaceuticals, NovoNordisk, Vertex Pharmaceuticals; consultancy and honoraria: Bristol Myers Squibb; data safety monitoring board: Bioverativ/Sanofi/Sangamo.

Off-label drug use

Kevin H.M. Kuo: nothing to disclose.

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EVIDENCE-BASED MINIREVIEW

When should gene therapy be considered for transfusion-dependent β-thalassemia patients?

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LEARNING OBJECTIVES

- Understand the void filled by gene therapy in the treatment paradigm for transfusion-dependent β-thalassemia
- Evaluate the appropriateness of gene therapy for individual patients with transfusion-dependent β-thalassemia

When should gene therapy be considered for transfusion-dependent β-thalassemia patients?

Case: The parent of a 12-year-old boy of Greek ancestry who has transfusion-dependent β-thalassemia are asking about curative options. He does not have any matched related donors, and a search through the international bone marrow donor registry has not identified a match either. He is transfused every 3 weeks to maintain a pretransfusion hemoglobin level of 9.5 to 10.5 g/dL and is well chelated, with a most recent liver magnetic resonance imaging showing a liver iron concentration of 3.4 mg/g dry weight and a cardiac T2* of 42 ms. The parents are concerned about school absences and their son's desire to participate in competitive sports as he gets older.

Transfusion-dependent β-thalassemia is a lifelong condition with a high physical and emotional burden of disease with tethering to the medical establishment, a propensity for many systemic complications, and impairment of quality of life. The recent approval of lentiviral gene addition therapy and the likely approval of CRISPR/CAS9-based gene-editing therapy dramatically increase "curative" options for this disease.[1] However, as a treatment with significant potential for toxicity and side effects, patient selection will be key to ensuring excellent long-term outcomes. Using a population, intervention, comparison, outcome (PICO)-based analysis, we suggest an algorithm to assist in this. We have deliberately not included the economics of gene therapy to be able to focus on the clinical decision-making only.

Patient population: Individuals with transfusion-dependent (conventionally accepted as ≥8/y) β-thalassemia.

Intervention: Autologous hematopoietic stem cell transplant with lentiviral gene addition (the only currently approach approved by the US Food and Drug Administration uses beti-cel [a product of $CD34^+$ cells transduced with the BB305 lentiviral vector encoding the β-globin (βA-T87Q_) gene])[2]

Comparison: Continued standard-of-care management with regular transfusions and chelation or allogeneic hematopoietic stem cell transplantation (HSCT).

Outcome: Achievement of transfusion independence with improvement in quality of life.

Intervention: Gene therapy

Gene therapy for β-thalassemia involves harvesting stem cells by pheresis after plerixafor/granulocyte colony stimulating factor stimulation, modification of these cells (transduction of a lentiviral vector containing a copy of the β-globin gene, or genome editing to knock down *BCL11A* to allow for reactivated γ-globin expression so as to restore fetal hemoglobin production), and reinfusion after myeloablative conditioning.[2,3] Once engraftment has occurred (taking somewhat longer than an allogeneic transplant), endogenous production of red cells containing HbA or HbF ameliorates the anemia, leading to transfusion independence. Data from clinical trials have confirmed durable engraftment with stable hemoglobin levels in both adults and children with transfusion-dependent thalassemia (91% of patients achieving transfusion independence in the Northstar trials using the lentiviral vector and 95% in the CLIMB trials using CRISPR/CAS9 editing).[1,4] Early gene addition trials had better outcomes in those with non-β^0/β^0 genotypes, but more recent data suggest that age, genotype, and splenectomy status do not play a role. Complications are mostly related to the transplant procedure itself, including infections during the engraftment period, some delayed platelet recovery, and veno-occlusive disease (now not seen following introduction

Table 1. Gene therapy trials for β-thalassemia

Trial name	Study design	Inclusion criteria	Exclusion criteria	Intervention	Comparison	Outcome
NorthStar Hgb 204[2,6]	Phase 1/2 N=19 Multisite	TDT age 12–35 years Any genotype	Severe iron overload Prior transplant	Beti-cel	Standard of care: Transfusion dependence and chelation	Transfusion independence for 24 months in 61%
Hgb205[2,6]	Phase 1/2 Single site (Paris) N=4	TDT Age 5–35 years Any genotype	Malignancy Organ damage Infection Neutropenia	Beti-cel	Standard of care: Transfusion dependence and chelation	Transfusion independence for 24 months in 75%
Northstar 2 Hgb 207[2,3]	Phase 3 N=23 Multisite	TDT Age <50 years Non-β0/β0 genotype	β0/β0 genotype Known HLA match	Beti-cel	Standard of care: Transfusion dependence and chelation	Transfusion independence in 91%
Northstar 3 Hgb 212[2,3]	Phase 2 Multisite N=23	TDT β0/β0, β0/β+ IVS-I-110, and β+ IVS-I-110/β+ IVS-I-110 genotypes Age <50 years	Presence of a mutation characterized as other than β0 (eg, β+, βE, βC) on at least 1 β-globin gene (HBB) allele	Beti-cel	Standard of care: Transfusion dependence and chelation	Transfusion independence in 86%
LTF-303[2,8]	Multiphase Multisite N=41	TDT Previously treated with beti-cel	None	Follow-up after beti-cel 13 years	Standard of care: Transfusion dependence and chelation	Transfusion and chelation independence in 68% in phase 1/2 and 89% in phase 3
β-Thalassemia Major With Autologous CD34+ Hematopoietic Progenitor Cells Transduced With TNS9.3.55 a Lentiviral Vector Encoding the Normal Human β-Globin Gene[1,9]	Phase 1 Multisite N=4	TDT of any genotype	Infection Diabetes Myelodysplasia	Autologous CD34+ HSPCs transduced with TNS9.3.55, a lentiviral vector encoding the normal human β-globin gene Reduced-intensity busulfan	Standard of care: Transfusion dependence and chelation	Transfusion requirements reduced by 50%
GLOBE[1,10]	Phase 1/2 N=7	TDT of any genotype Age >3 years	If age <18 years with an HLA match	GLOBE lentiviral vector–transduced CD34+ cells	Standard of care: Transfusion dependence and chelation	Three adults with reduced transfusion requirements Four pediatric patients with transfusion independence
CLIMB-THAL 111[1,4]	Phase 1/2	TDT of any genotype Age 3–64 years	Infection Malignancy	Autologous CRISPR-Cas9–edited CD34+ HSPCs	Standard of care: Transfusion dependence and chelation	42/44=95% transfusion independence 2/44=5% reduced transfusion requirements

HLA, human leukocyte antigen; HSPC, hematopoietic stem cells; TDT, transfusion-dependent thalassemia.

of standard prophylaxis with defibrotide), with no deaths, minimal graft-vs-host-disease, and no major concerns for marrow dysplasia.[5] (Caveat: the bone marrow in individuals who have successfully undergone gene therapy still shows evidence of ineffective erythropoiesis.[6]) Infertility and risk for clonal disease may be comparable to allogeneic HSCT. Successful treatment resulted in improvements in health-related quality of life, and normalization of day-to-day activity.[2] Table 1 provides a summary of trials and the key outcomes described in each.

Comparison: Standard transfusion and chelation

Management of transfusion-dependent thalassemia is extremely patient time-intensive and burdensome, necessitating frequent all-day visits to the hospital for transfusions and the potential for long-term complications, including transfusion-associated infections, alloimmunization, with systemic iron overload and organ dysfunction despite chelation therapy. Chelation therapy requires excellent compliance to prevent iron-related organ damage and has its own toxicities. This has a tremendous physical, mental, emotional, and economic burden on patients and families, with symptoms from chronic anemia, organomegaly (extramedullary hematopoiesis), endocrinopathy, bone disease and vasculopathy (pulmonary hypertension, silent cerebral infarction), anxiety and depression, and loss of school or work days. Median age at death in the United States is ~37 years, half that of the average American, mostly related to iron overload–induced heart failure.[2] Until recently, allogeneic HSCT from a matched related donor (MRD) (more recently matched unrelated donor as well) has been the best "curative" therapy, achieving transfusion independence in over 90% of individuals when performed at a younger age.[7] However, the nonavailability of a matched donor limits this as a viable option in most patients.

Patients should have a detailed discussion on the process and timeline, efficacy and safety profile, short- and long-term complications, and alternatives. It would be appropriate to offer gene therapy to motivated patients after further discussion of the following:

1. Metanalysis of registry data showed the best results in children under age 7 years (with limited fertility preservation options) who had MRD, with less than optimal results in patients over the age of 15 years, especially without matched donors.[7] No such age-related trend was seen in the gene therapy clinical trials. While most trials limited participation to patients between 4 and 35 years, age has not been defined in the recent Food and Drug Administration approval. It would be appropriate to offer gene therapy to younger patients, since they would be most likely to have good outcomes, and derive the longest benefit. It may be reasonable to offer this to even older individuals, particularly those who have been well transfused and chelated and do not have the preexisting morbidities described, and would therefore be less likely to have adverse outcomes.[2]
2. Individuals with available MRDs were excluded from the trials.[1,2] However, with demonstrated equivalent efficacy in achieving transfusion independence and the low risk of graft-vs-host-disease, gene therapy may now be a consideration even in when a MRD is available so long as there is a continued demonstrable durable response without increased risk of development of clonal disease (which may be related to an inherent risk in thalassemic stem cells).
3. Individuals with iron overload–induced hepatic fibrosis or cirrhosis, as well as those with renal dysfunction, would be high-risk candidates to undergo HSCT and likely should be excluded.
4. Fertility preservation is an important consideration and should feature in all discussions. Being able to receive gene therapy with equivalent success at an older age has the benefit of being able to wait until postpubertal fertility preservation is possible or family planning is completed.
5. Continuing transfusion and chelation therapy may be considered by patients/families who feel the risks of stem cell transplantation are too great, and the discussion should include (1) recent data[7] showing improving outcomes with allogeneic HSCT and comparable results with gene therapy, (2) the potential for development complications related to iron overload or vascular disease, (3) potential reduced life expectancy, and (4) the ongoing burden of being tightly tethered to the medical system.

Outside of the clinical trial setting, the challenge is to offer the option of gene therapy to patients who will not only be most likely to benefit but also be least likely to have adverse events. Given that there may still be some uncertainty around durability and potentially some unknown risk for clonal disease, we believe the certainty of benefit in the form of transfusion independence and reduced risk of future complications is high, compared with continuing transfusions and chelation with the attendant risks of iron overload, endocrinopathies, bone disease, and vasculopathy.[2] Gene therapy has benefits over allogeneic HSCT, but uncertainties around long-term safety and durability limit this comparison.

Most pressing need: Transfusion-dependent thalassemia has a high burden of disease, potential for many systemic life-limiting morbidities, and limited conventional "curative" options. A durable, well-tolerated treatment approach with acceptable toxicity which achieves transfusion independence would provide a viable "curative" option for more patients.

Conflict-of-interest disclosure

Cheryl Mensah reports consultancy fees from Vertex.
Sujit Sheth reports consultancy fees from Agios Pharmaceuticals, bluebird bio, Bristol Myers Squibb, Forma, and Chiesi and serves on a clinical trial steering committee for CRISPR/Vertex CTX001 for thalassemia.

Off-label drug use

Cheryl Mensah: There is no off-label drug use mentioned.
Sujit Sheth: There is no off-label drug use mentioned.

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Clonal evolution in inherited marrow failure syndromes predicts disease progression

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Progression to myelodysplastic syndromes (MDS) and acute myeloid leukemia is one of the most serious complications of the inherited bone marrow failure and MDS-predisposition syndromes. Given the lack of predictive markers, this risk can also be a source of great uncertainty and anxiety to patients and their providers alike. Recent data show that some acquired mutations may provide a window into this risk. While maladaptive mechanisms, such as monosomy 7, are associated with a high risk of leukemogenesis, mutations that offset the inherited defect (known as somatic genetic rescue) may attenuate this risk. Somatic mutations that are shared with age-acquired clonal hematopoiesis mutations also show syndrome-specific patterns that may provide additional data as to disease risk. This review focuses on recent progress in this area with an emphasis on the biological underpinnings and interpretation of these patterns for patient care decisions.

LEARNING OBJECTIVES

- Understand the types of somatic clonal mutations in inherited bone marrow failure/MDS syndromes
- Identify somatic genetic rescue mutations in specific bone marrow failure/MDS syndromes
- Understand how to diagnosis rescue mutations in clinical practice

Introduction

The stressed hematopoietic system in individuals with inherited bone marrow failure and myelodysplastic syndrome (MDS) predisposition syndromes is remarkably adaptable. Due to the high turnover nature of hematopoiesis, *de novo* adaptations that provide a fitness advantage, either in terms of survival or proliferation, compete with the germline failing hematopoiesis and persist over time.[1,2] These adaptive mechanisms are remarkably varied, with some exquisitely specific for a single inherited disorder while others are shared across different syndromes broadly. Here, I will discuss these mechanisms of adaptation and examine how these findings may impact risk stratification and inform patient care. I will review general progress in this area, but for illustrative purposes and because of the predominance of the recent evidence, I will focus on three disorders: (1) SAMD9/9L syndromes, a common cause of monosomy 7 MDS in early childhood; (2) Shwachman-Diamond syndrome, a ribosomopathy associated with MDS and acute myeloid leukemia (AML) in later childhood and young adults; and (3) the short telomere syndromes, a common adult-onset inherited syndrome.

Diagnosis of genetic predisposition to MDS/AML informs patient care

Syndromes associated with genetic predisposition to MDS and/or AML include both the inherited bone marrow failure (BMF) syndromes and MDS predisposition syndromes; these will collectively be referred to as BMF/MDS syndromes in this review.[3] These genetic disorders are diverse in their etiology but share a risk of myeloid neoplasm evolution as a hallmark with variable risk of antecedent marrow failure, extra-hematopoietic manifestations, and non-myeloid cancers. At least 14 common syndromes and roughly 100 genes have been linked to BMF/MDS syndromes due to defects in in many mechanisms, including DNA repair, ribosome function, telomere maintenance, or hematopoietic transcription factor signaling, among others.[4,5] The mechanism for more recently identified syndromes, such as the RNA helicase *DDX41* and *SAMD9/9L*, whose gene products suppress hematopoiesis, remains poorly understood.[6-8] Some syndromes present with severe cytopenia or immunodeficiency in early childhood, and others can remain cryptic until adulthood.[9] Even within a single genetic disorder, there can be variable severity and

heterogeneity in presentation as well as variable risk of leukemogenesis. Distinguishing these presentations from *de novo* disease is clinically relevant because of implications for treatment, long-term surveillance, and family counseling.[10,11] While some BMF/MDS syndromes present with progressive stem cell failure, in a number of cases, the first presentation may be as an MDS or overt AML.[12] Timely diagnosis of an underlying genetic predisposition has critical implications for timing of hematopoietic stem cell transplant, related donor selection, preparative regimens, and posttransplant care.[13,14]

Age-dependent presentation of MDS/AML in inherited BMF/MDS syndromes

The onset of MDS/AML in these syndromes has some age-dependent patterns with SAMD9/9L syndromes and GATA2 deficiency presenting more often in childhood.[15,16] In older adults, mutations that cause telomere shortening (collectively known as the short telomere syndromes[17]) and germline *DDX41* mutations account for the majority of known inherited predisposition.[6,18] RUNX1-familial platelet disorder with associated myeloid malignancies (FPDMM) is more rare and can present at nearly any age with myeloid or lymphoid malignancy.[19,20] Other more rare autosomal recessive inherited causes of MDS/AML that are seen in both children and younger adults include Shwachman-Diamond syndrome and Fanconi anemia.[21,22] Figure 1 summarizes the age-dependent patterns of MDS/AML diagnosis in these frequently diagnosed syndromes and reflects the peaks and ranges of MDS/AML diagnosis obtained from numerous published family and cohort studies.

Common inherited causes of childhood-onset MDS

Among young children with MDS, germline mutations in *SAMD9* or *SAMD9L* are the most common inherited cause. Mutations in these genes account for 8%-17% of pediatric MDS and up to 40% in children with monosomy 7 MDS who are younger than 5 years.[16,23] *SAMD9* and *SAMD9L* are homologous genes located on chromosome 7q21; they have proposed roles in antiviral immunity and protein translation and also function to negatively regulate cellular proliferation.[8,24] While their precise function is not known, in experiments of hematopoietic cells in culture, expression of pathogenic germline *SAMD9* or *SAMD9L* mutations enhances the growth suppressing effect of the wild-type protein, resulting in a profound inhibition of cell growth. As such, mutations in these genes mediate their effect through gain-of-function.[8,25]

In older children and adolescents, mutations in *GATA2* predominate, accounting for approximately 10% of cases of pediatric MDS and up to 50% of monosomy 7 MDS in children who are older than 12 years.[23,26] GATA2 is a zinc finger transcription factor that is required for maintenance and proliferation of hematopoietic and immune cells.[27] Germline coding and noncoding mutations in *GATA2* cause loss of function of the mutated allele and exert their effect through haploinsufficiency.[28,29]

Germline predisposition to MDS/AML in older adults

At the other end of the age spectrum, among adults over age 50 with MDS/AML, the short telomere syndromes are among the most common inherited causes.[18,30-32] Mutations in genes encoding telomerase components and the other telomere

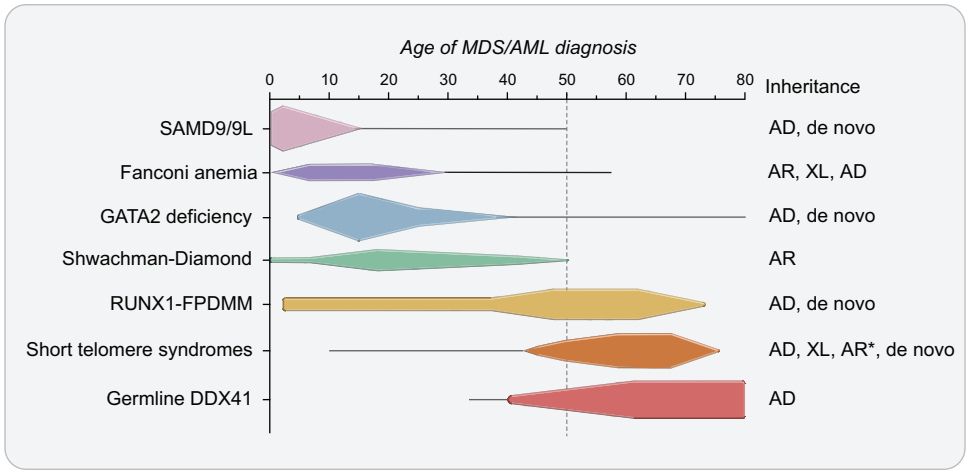

Figure 1. Age-dependent presentation of myeloid malignancy in inherited bone marrow failure and MDS predisposition syndromes. A schematic demonstrating the typical and peak ages of myeloid malignancy diagnosis in individuals with inherited bone marrow failure and MDS predisposition syndromes. There is an age-dependent onset depending on the underlying genetic cause that informs approaches to surveillance and patient counseling. Schema is based on a comprehensive review of myelodysplastic syndrome (MDS) and/or acute myeloid leukemia (AML) cases reported in the literature in individuals with confirmed germline predisposition. The reported incidence of MDS/AML in these disorders is impacted by rates of hematopoietic stem cell transplant for bone marrow failure and other competing risks, such as pulmonary fibrosis in the short telomere syndromes which accounts for the drop off of MDS/AML diagnosed in older ages. Horizontal lines demonstrate the published age range where there are isolated cases at those age extremes. Queried publications are referenced in reference 13 in addition to references 23, 51, and 72 and PubMed IDs 32088370, 30578959, and 34469508. *Autosomal recessive inheritance is rarely found in the short telomere syndromes (<5%) and more often manifests as aplastic anemia with few reports of MDS/AML in these individuals. AD, autosomal dominant; AR, autosomal recessive; XL, X-linked.

maintenance genes, which are associated with telomere shortening, explain 5%-10% of suspected, but clinically unresolved, BMF/MDS syndromes in children and young adults.[33,34] Among unselected MDS in adults, mutations in the telomerase reverse transcriptase (*TERT*) gene alone account for 3% of cases.[32] This supports an even higher prevalence of the short telomere syndromes in adult-onset MDS, as germline loss-of-function mutations in *TERT*, resulting in TERT haploinsufficiency, account for the genetic cause in only half the cases.[30] The genetic basis and mechanisms of telomere shortening for the other 15 germline mutant genes have been recently reviewed.[9] In the bone marrow, short telomeres result in slowly progressive hematopoietic stem cell loss that leads to both BMF and primary immunodeficiency.[35,36] Telomere length is the primary determinant of disease severity in the short telomere syndromes. Among children with more severe forms of telomere shortening, stem cell depletion usually manifests as aplastic anemia. In contrast, among older adults with relatively milder telomere shortening deficits, the risk of MDS/AML is higher and up to 15% for those over age 50.[18,37] However, even among those over age 50 with MDS/AML, two-thirds of the patients suffer from extrahematopoietic disease, and the primary cause of death is pulmonary disease.[18,38]

MDS/AML comprise nearly 70% of all short telomere syndrome malignancies, with the remaining cancers being mostly squamous cancers that arise in the setting of a T-cell immunodeficiency.[30,36] Although genome instability has been the primary hypothesized mechanism of carcinogenesis, short telomere syndromes have a lower than expected incidence of most cancers, and somatic alterations that arise in the setting of BMF, rather than genome instability, are the primary drivers of MDS/AML progression. The somatic landscape of the rare solid tumors seen in these patients is also quiescent and lacks the hallmarks of genome instability consistent with T-cell surveillance defects being their primary driver. Of note, a recently recognized entity associated with abnormally long telomere length has also been linked to the risk of myeloid as well as lymphoid neoplasms and clonal hematopoiesis. The risk of malignancy in this long telomere syndrome is associated with extended replicative potential of hematopoietic stem cells, and these disorders, in contrast to the short telomere syndromes, lead to hyperproliferative disease.[9,39]

In contrast to the short telomere syndromes, germline *DDX41* mutations do not have obvious extrahematopoietic phenotypes and are more frequently are diagnosed in the setting of seemingly *de novo* MDS and AML.[40] Germline mutation in *DDX41* accounts for 2%-4% of unselected MDS/AML cases and represents a late-onset genetic predisposition, with a median age of MDS/AML diagnosis in the 7th decade.[41,42] While initial reports were not associated with preceding bone marrow failure, recent data demonstrate antecedent cytopenia in nearly half of patients presenting with myeloid neoplasms due to germline *DDX41* mutations.[41] *DDX41*, located on chromosome 5q35, encodes an RNA helicase, and germline mutations have been proposed to alter pre-mRNA splicing and RNA processing.[6] *DDX41* is thought to have a tumor suppressor function whereby second-hit loss-of-function mutation or acquired del(5q) involving the wild-type *DDX41* allele is seen at time of MDS/AML diagnosis in up to 60% of germline *DDX41* cases.[6]

Distinct types of clonal hematopoiesis in inherited bone marrow failure/MDS predisposition syndromes

Clonal hematopoiesis (CH) is an overrepresentation of cells with an acquired somatic mutation, and this phenomenon is common in patients with inherited BMF/MDS syndromes. Hematopoietic stem cells harboring a mutation that results in a fitness advantage are selected and can rise to detectable levels. In some BMF/MDS syndromes, there are more selective pressures due to the underlying hypofunctioning bone marrow. In individuals with BMF/MDS syndromes, these acquired changes generally fall into two categories: (1) mutations that are commonly shared across many disorders and also seen with age-related clonal hematopoiesis (or clonal hematopoiesis of indeterminate potential, CHIP),[43] and (2) those that are *specific* to one particular disorder.

CHIP in BMF/MDS syndromes

CH mutations that are age-related fall in a relatively small subset of genes, with approximately one dozen genes responsible for the vast majority of clonal hematopoiesis seen in unselected populations.[43-45] High-risk somatic mutations in leukemia driver genes are the largest identifiable risk factor for hematologic malignancy in the general population.[46] Several BMF/MDS syndromes demonstrate premature onset of age-related CH several decades earlier than the general population, suggesting antecedent CH may contribute to MDS/AML risk (Table 1).[18,19,47,48] For example, *TP53* mutations are common among some BMF/MDS syndromes. In the short telomere syndromes, somatic *TP53* mutations allow the cell to bypass the short telomere checkpoint; these mutations are seen in approximately 15% of adults with short telomere syndromes but are distributed evenly between those with and those without MDS/AML.[18,49] Among the *TP53*-mutant short telomere patients without MDS/AML, pulmonary fibrosis was the leading cause of mortality, not their hematologic disease. In children with Shwachman-Diamond syndrome, ultradeep sequencing identified somatic *TP53* mutations in nearly half of those studied, although there was no association with hematologic phenotype.[50] Consistent with sporadic MDS, acquisition of biallelic *TP53* alterations through copy-neutral loss of heterozygosity (CN-LOH) at 17p, *TP53* gene deletion, or second-hit mutation was associated with MDS/AML in both the short telomere syndromes and Shwachman-Diamond syndrome.[47,49] In single-cell analysis of one individual with Shwachman-Diamond syndrome and p53-mutant AML, CN-LOH was documented subclinically (variant allele fraction [VAF] <0.1%) 4 years prior to AML diagnosis, suggesting that more advanced clinical diagnostic modalities may be able to identify the patients with exceptionally high risk of transformation to MDS/AML and allow early intervention.[47] However, not all the inherited syndromes cause antecedent CHIP and, in individuals with germline mutations in *DDX41*, for example, progression to MDS/AML is instead associated with a second hit in the wild-type *DDX41* allele (Table 2).[51] Other recurrent CHIP mutations documented in inherited BMF/MDS syndromes have been recently reviewed.[52]

Somatic genetic rescue in BMF/MDS syndromes

Somatic genetic rescue (SGR) is a term that encompasses disease-specific genetic changes found in individuals with Mendelian hematopoietic and immunodeficiency disorders.[53]

Table 1. Risk of myeloid malignancy and age-related clonal hematopoiesis in inherited BMF/MDS syndromes

	Incidence of MDS/AML	Peak age of MDS/AML diagnosis	Prevalence of CHIP in carriers without hematologic malignancy
SAMD9/9L syndromes	Moderate*	Early childhood	Absent
Fanconi anemia	30%-40%	Childhood	**
GATA2 deficiency	75%	Late childhood through young adult	20%-50%
Shwachman-Diamond syndrome	10%-30%	Late childhood through young adult	60% *TP53* Other CHIP <10%
RUNX1-FPDMM	20%-40%	Any age	25%-60%
Short telomere syndromes	15%	Adults >50 years	30%
Germline DDX41	40%-50%	Adults >60 years	Rare

*Lack of cohort studies limits definition of MDS/AML prevalence in SAMD9/9L syndromes.

**Large-scale NGS studies in individuals with Fanconi anemia without MDS/AML have not been published.

RUNX1-FPDMM, RUNX1 familial platelet disorder with associated myeloid malignancies.

Initially thought to be rare events, the availability of deep sequencing underscores that SGR is common and explains some of the varied penetrance of bone marrow failure and myeloid malignancy in BMF/MDS syndromes.[25,49,54,55] Mechanisms of SGR can directly correct the mutant genotype (a process termed reversion) or can function indirectly to offset the effect. These mechanisms improve fitness on the cellular level, but, for the bone marrow as a whole, some mechanisms are beneficial (adaptive SGR) while others are pro-leukemogenic (maladaptive SGR). Understanding SGR mechanisms is important for patient care because adaptive mutations that theoretically offset the inherited defect and associated pressures toward leukemogenesis may identify a low-risk subset of patients.

The presence of beneficial versus leukemogenic adaptation influences disease course in SAMD9/9L syndromes

SGR has been documented in over half of individuals with SAMD9/9L syndromes and this occurs by varied mechanisms.[23–25] One is the acquisition of a second *SAMD9* or *SAMD9L* mutation in cis that offsets the effect of the germline mutation (termed second-site mutation). Another is the removal of the mutant allele through one of several processes, including (1) mitotic recombination, resulting in uniparental isodisomy of chromosome 7q (UPD7q), (2) aneuploidy, resulting in monosomy 7 or, more rarely, (3) focal gene deletion encompassing the mutant gene (Figure 2).[23,25,55] In *in vitro* studies, coexpression of the second-site somatic loss-of-function mutation with the germline

Table 2. Common adaptive somatic genetic rescue mechanisms and maladaptive responses in inherited bone marrow failure and MDS predisposition syndromes and their association with risk of progression to MDS/AML

	Lower risk (adaptive rescue)	Higher risk (maladaptive response)
SAMD9/9L syndromes	Second-site loss-of-function mutation (cis) UPD 7q	-7 / del7q
Shwachman-Diamond syndrome	*EIF6* inactivating mutation Deletion 20q Isochromosome 7q	*TP53* mutation
GATA2 deficiency	Direct reversion*	-7 / del7q / der(1;7)
Fanconi anemia	Direct reversion	
RUNX1-FPDMM	UPD 21q**	Somatic 2nd-hit *RUNX1* mutation (trans)
Short telomere syndromes	Direct reversion *TERT* promoter mutation *POT1* loss-of-function mutation RNA exosome mutation	-7 / del7q / der(1;7) *TP53* mutation
DDX41	—	Somatic 2nd-hit *DDX41* mutation (trans)

Direct reversion in Fanconi anemia and the short telomere syndromes encompasses multiple mechanisms, including uniparental isodisomy of the wild-type allele, somatic second-site loss-of-function mutation, and back mutation, that individually are seen more rarely.

*Single case report by Catto et al found somatic reversion of a germline nonsense mutation in *GATA2* to a synonymous mutation in an asymptomatic 61-year-old adult.[54]

**Single case report by Glembotsky et al of somatic reversion via uniparental isodisomy of chromosome 21 in an individual with germline *RUNX1* mutation and gradual improvement platelet number and function.[75]

RUNX1-FPDMM, *RUNX1* familial platelet disorder with associated myeloid malignancies; UPD, uniparental isodisomy.

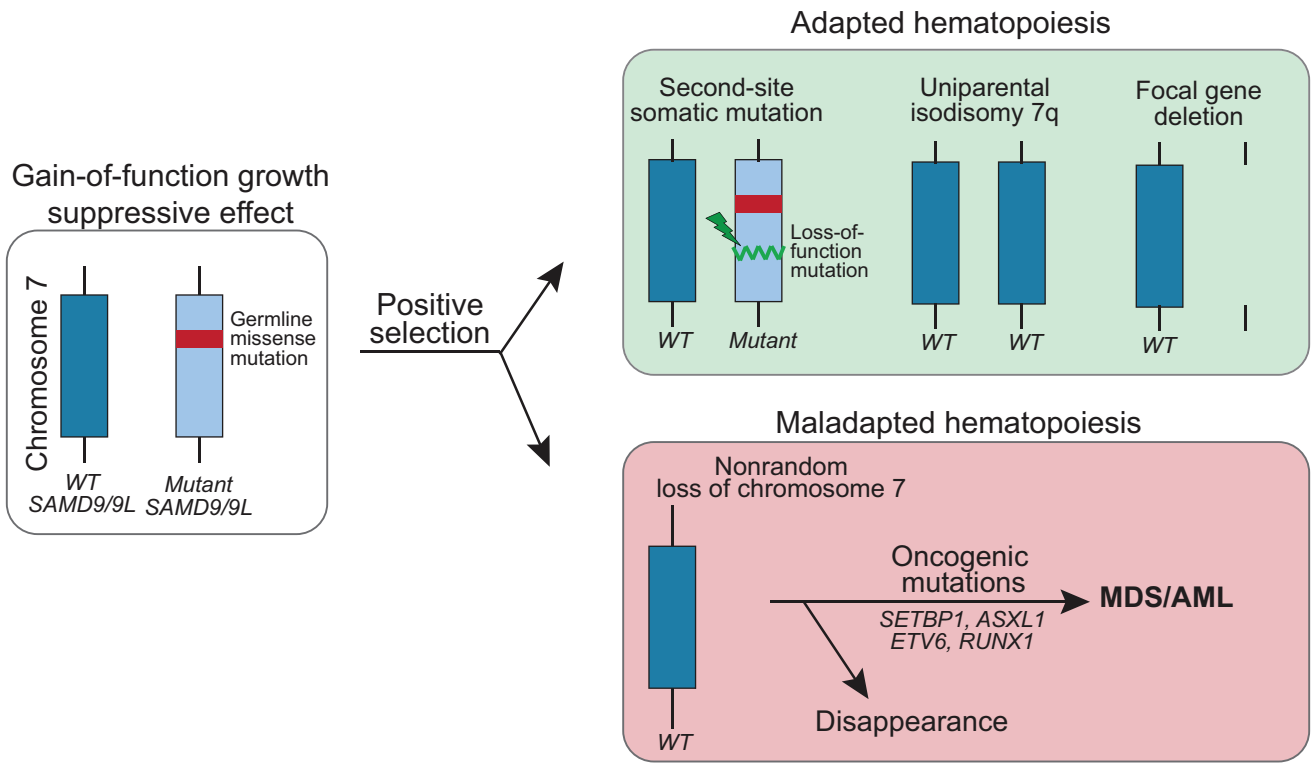

Figure 2. Mechanisms of somatic genetic rescue in the SAMD9/9L syndromes. Pathogenic germline mutations in *SAMD9* and *SAMD9L* are gain-of-function and have a growth suppressive effect on hematopoiesis. Cells that acquire adaptations that improve growth or survival are selected in the high turnover environment of the bone marrow. Somatic second-site loss-of-function mutations in cis or removal of the mutant allele via uniparental isodisomy of chromosome 7q or focal gene deletion are not associated with progression to MDS/AML. In contrast, the monosomy 7 clone often acquires additional leukemia driver mutations with high risk of disease progression. WT, wild type.

gain-of-function mutation ameliorated the growth suppressive defect.[23,24] Interestingly, second-site somatic mutations can be either truncating or missense mutations, and both types show comparable restoration of growth *in vitro*.[23] UPD7q, detected as CN-LOH at 7q by microarray, reverts cells harboring it to the wild-type genotype and is hypothesized to be a more complete rescue effect.[23] In contrast, preferential loss of chromosome 7 or 7q containing the germline mutation, resulting in monosomy 7 or del(7q), is a maladaptive, pro-leukemogenic event that, as in other diseases, is associated with development of MDS/AML, most likely due to haploinsufficiency of additional genes on 7q.[56] Unique to SAMD9/9L syndromes, there are reports of monosomy 7 clones disappearing and the patient obtaining hematologic and morphologic remission.[23,25] Alternatively, and perhaps more often, the monosomy 7 clone acquires additional leukemia driver mutations in genes such as *SETBP1*, *ASXL1*, *RUNX1*, *EZH2*, and Ras pathway genes with high risk of disease progression.[23]

Multiple distinct SGR events can converge in one individual, and many different rescue mechanisms can be found in the same family (Figure 3A). Single-cell data recently confirmed these SGR events are mutually exclusive on the cellular level.[23] In a cohort of children *with* MDS and SAMD9/9L syndromes, monosomy 7 predominated (55% with monosomy 7 at the time of MDS diagnosis).[23] Of note, nearly half (18 of 37) of the children with monosomy 7 MDS also had concurrent somatic second-site mutations or UPD7q supporting monosomy 7 as a stronger driver of the hematologic phenotype toward MDS/AML than the adaptative mechanisms. However, family-based studies of children and adults with SAMD9/9L syndromes without MDS suggests adaptive UPD7q and somatic second-site mutations with remission potential may be more common than monosomy 7 overall, and there are many examples of carriers with sizable or multiple adaptive SGR clones experiencing a benign hematologic course into adulthood.[25,55] Assessing for somatic second-site *SAMD9/9L* mutations, UPD7q, and monosomy 7 at initial SAMD9/9L syndrome diagnosis can be done through clinically available testing, and their detection, in conjunction with peripheral blood count monitoring, can improve risk stratification and tailor surveillance interventions.

Somatic *TERT* promoter and *POT1* mutations protect against MDS/AML in the short telomere syndromes

Some of the earliest identified rescue mechanisms were in the short telomere syndromes. As with the adaptive genetic mechanisms seen in SAMD9/9L syndromes, the initial reports identified mechanisms that directly repaired the germline mutant allele itself via mitotic recombination with the wild-type allele, somatic second-site mutations, or back mutations.[57-59] In adults with heterozygous germline deletions in the telomerase RNA (*TR*) gene (located on chromosome 3q), mitotic recombination with the

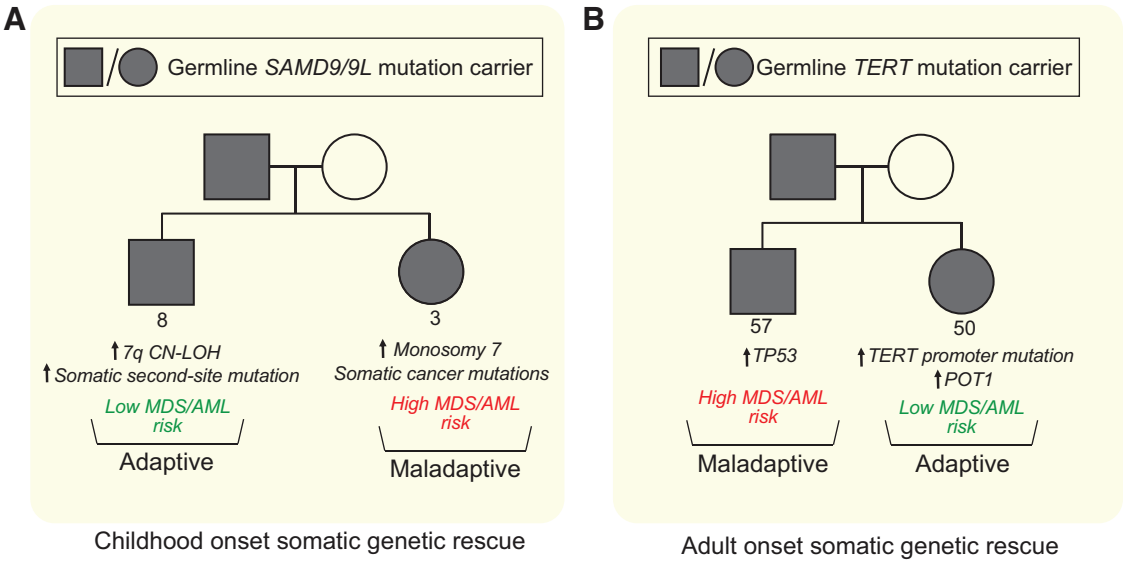

Figure 3. Distinct somatic genetic rescue mechanisms can be present within the same family and impact clinical course. Representative pedigrees of SAMD9/9L syndrome (A) and short telomere syndromes (B) showing that within 1 family carrying the same germline mutation, different somatic adaptations can arise. The consequence of these mutations influences subsequent risk of progression to MDS/AML, and the stochastic nature of these acquired changes on the cellular level may explain some of the variability in penetrance of MDS/AML in families with inherited bone marrow failure and MDS predisposition syndromes.

wild-type allele, which lead to uniparental isodisomy of 3q, resulted in reversion to the wild-type genotype in the affected blood cells.[58] This was documented in 4 of 12 individuals with small (1-4 nucleotide) deletions in *TR* but has not been replicated in other cohorts.[58] In an adult with a germline gain-of-function mutation in *TINF2*, a somatic second-site frameshift deletion in cis provided an advantage and abolished the BMF phenotype.[57] In a child with dyskeratosis congenita due to germline mutation in the 5′ UTR of *DKC1*, acquired back mutation led to correction of the germline mutation and normalization of dyskerin in the cells bearing the reversion.[59]

More recently, deep sequencing has uncovered multiple mechanisms of SGR that may be seen in up to 30% of older adults with short telomere syndromes.[49] The most common are somatic mutations in canonical sites in the promoter region of *TERT*.[49,60,61] These *TERT* promoter mutations are a common telomere maintenance mechanism in many malignancies and create a *de novo* ETS transcription factor binding site, which upregulates transcription of the *TERT* allele.[62] In patients with germline loss-of-function *TERT* mutations, these somatic *TERT* promoter mutations occur on the wild-type allele (i.e; in trans with the germline mutation) and thus preferentially upregulate expression of wild-type *TERT* (Figure 4).[60,61]

A diverse number of additional SGR mechanisms appear to converge on enhancing telomerase levels and function.[49] For example, loss-of-function mutations in *POT1* appear to offset germline defects in *TERT* as well as multiple other genes. In patients with inherited mutations in *TR* or related pathways, somatic mutations that minimize telomerase RNA degradation or restore telomerase RNA levels appear to be restricted to that group of patients. Some of these mutations are also identical to those that are seen in cancer, but in the short telomere syndromes, they appear to avert the telomere crisis and to be protective against MDS/AML. The evidence that these somatic rescue mutations are beneficial when at high allele frequency (VAF >10%) is that they were not seen in patients with short telomere MDS/AML and are generally mutually exclusive with cytogenetic abnormalities, such as monosomy 7.[9,49] Multiple SGR mechanisms can coexist in separate clonal populations in an individual (Figure 3B).[49] *TERT* promoter and *POT1* mutations are commonly included on clinical next-generation sequencing (NGS) panels and may provide clinically relevant information in assessing the course of telomere-mediated BMF in older adults.

Somatic genetic rescue in Shwachman-Diamond syndrome

Shwachman-Diamond syndrome is an autosomal recessive disorder that, in 90% of cases, is caused by homozygous or compound heterozygous mutations in the *SBDS* gene, located on chromosome 7q11.[63] Biallelic loss-of-function mutations in *SBDS* lead to low levels of SDS protein, which is required along with EFL1 to remove eIF6 from the 60S ribosome subunit prior to ribosome maturation; hence, these mutations result in defective ribosome assembly.[64,65] Interstitial deletion of chromosome 20q (del20q), which encompasses the *EIF6* gene, and isochromosome i(7)(q10) (i7q) were observed over a decade ago in the blood and bone marrow in approximately 20% of patients with biallelic SBDS mutations.[66,67] Studies of cases with the common, hypomorphic *SBDS* germline mutation, c.258+2 T>C, in a compound heterozygous state, showed that i7q nonrandomly occurs on the same allele containing c.258+2 T>C. The c.258+2 T>C mutation results in expression of an alternatively-splice truncated protein in addition to a scant amount of normal protein.[68,69] Thus, when this particular mutant gene is duplicated, relatively more protein is produced. Large del20q clones (>10% of bone marrow cells)

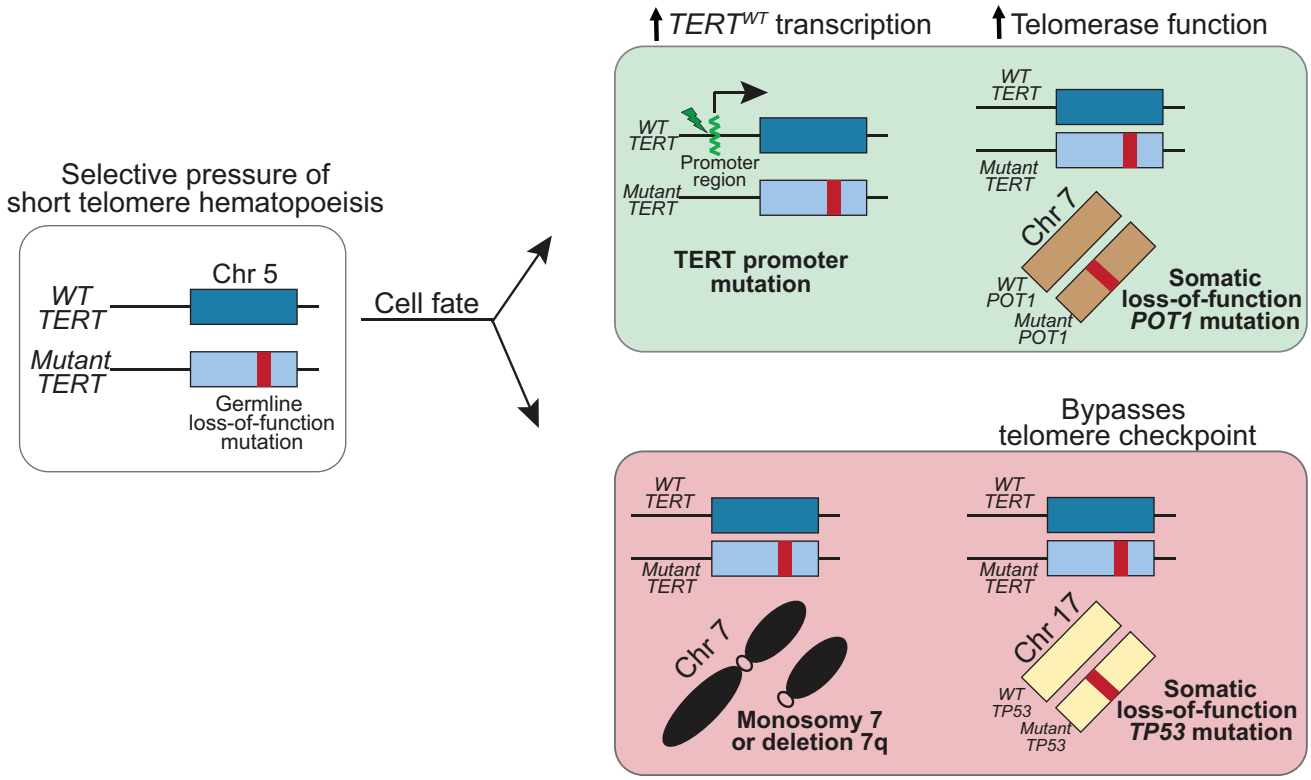

Figure 4. Somatic genetic rescue mutations that promote telomere elongation are protective of developing MDS/AML in the short telomere syndromes. *TERT* promoter mutations arise in trans with the germline *TERT* loss-of-function mutation and upregulate transcription of the wild-type *TERT*. *POT1* mutations are a more universal rescue mechanism with no preference for germline mutant gene, and *POT1* loss-of-function mutation improves telomerase access to the telomere and/or increases telomerase processivity. Monosomy 7 and biallelic *TP53* mutations, also shared risk factors in other BMF/MDS syndromes, are associated with progression to MDS/AML. Chr, chromosome; WT, wild type.

had been associated with a benign course and low risk of progression to MDS.[70,71] However, a recent registry-based cohort study found del20q and i7q were transient findings in up to one-quarter of patients.[72] Further, there are reports of MDS/AML in some individuals with these cytogenetic alterations, although the del20q and i7q were often absent from the malignant clone. Thus, the clinical significance of these as low risk or protective findings remains less clear.

Using ultradeep sequencing of two independent cohorts, somatic mutations in *EIF6* itself are found in up to 60% of individuals with Shwachman-Diamond syndrome due to biallelic *SBDS* mutations.[47,73] Expression of the *EIF6* mutations in leukemia cell lines resulted in either decrease in eIF6 protein amount or disruption in eIF6 and 60S binding. When expressed in SBDS-deficient human CD34+ cells, inactivating *EIF6* mutations resulted in increased colony formation and improvement in the ribosome assembly defect supporting a functional compensation for *SBDS* deficiency.[47] The presence of somatic *EIF6* mutations was also *not* associated with severe BMF, leukemic transformation or, by single cell sequencing, *TP53* co-mutation.[47] Additional longitudinal follow-up is needed to further assess the impact of *EIF6* mutations on MDS/AML risk, particularly since most mutations were present at low abundance (<1% VAF, below the limit of clinical detection).

Diagnostic evaluation for SGR events in clinical practice

The identification of these mechanisms has revealed the importance of incorporation of somatic genomic testing into routine hematologic surveillance for individuals with BMF/MDS syndromes. Many SGR mechanisms described here can be assessed on commonly-utilized clinical molecular tests, while others require dedicated testing (Table 3). Hematologic malignancy somatic NGS panels can detect second-site reversion mutations, indirect SGR mutations, and second-hit mutations, as long as the genes or regions of interest are captured by the particular panel. Genomic microarray is needed to detect copy number alterations, such as CN-LOH indicative of uniparental isodisomy, which is particularly important in SAMD9/9L syndromes among others (Table 2).

Concordance of mutation detection between peripheral blood and bone marrow, particularly for larger clonal changes (VAF >5%), may allow screening and monitoring of SGR mechanisms from peripheral blood in some settings.[74] For example, clinically relevant *TERT* promoter and *POT1* mutations can be reliably detected from the peripheral blood in individuals with short telomere syndromes.[49] Our practice is to use peripheral blood NGS to screen older adults with short telomere syndromes (at least over age 40) for protective SGR mutations. Presence of a *TERT* promoter mutation with VAF >10% identifies an individual

Table 3. Clinically available molecular tests to detect common adaptive mutations in patients with inherited bone marrow failure and MDS predisposition syndromes

Somatic genetic rescue mechanism	Molecular test
Somatic 2nd site mutations **Other direct reversion mutations**	Somatic NGS of gene of interest*
Indirect rescue mutations such as: *TERT* promoter** *POT1*	Somatic NGS of gene of interest*
Clonal cytogenetic abnormalities such as: Interstitial deletion 20q Isochromosome 7q Monosomy 7	Conventional karyotype FISH
Copy neutral-LOH to detect UPD	Chromosomal microarray

*Whether the gene of interest is included in clinically available somatic NGS panels is gene dependent. Clinical assays to date also have a higher threshold for detection of somatic mutations than the more sensitive deep sequencing approaches utilized in the research setting.

**Somatic genetic rescue mutations at three canonical sites in the *TERT* promoter are found in patients with short telomere syndromes: c.-124, c.-146 and c.-57. That latter site, c.-57 C>T, is less commonly included in clinical somatic mutation panels.

FISH, fluorescence *in situ* hybridization.

at lower risk of progression to MDS/AML for whom monitoring via serial blood counts and peripheral blood NGS is appropriate. Additional studies and longer follow-up are needed to more definitively test the predictive utility of SGR testing in clinical settings and to refine actionable thresholds for each specific BMF/MDS syndrome.

Summary and future directions

The availability of higher resolution sequencing has uncovered a pattern of rescue hematopoiesis in individuals with inherited BMF/MDS syndromes. The adaptative rescue mechanisms identified to date are exquisitely disease-specific and driven by the underlying biology; their detection in seemingly sporadic cases should prompt consideration of an underlying germline BMF/MDS predisposition, if not already identified. The recent evidence demonstrates that adaptive SGR mechanisms are often more common than the overall prevalence of MDS/AML. These emerging observations identify a leukemogenesis paradigm that is shared across multiple BMF/MDS syndromes whereby, in the highly replicative environment of hematopoiesis, disease-specific adaptive modifications arise that overcome the inherited defect. More rarely, maladaptive responses emerge with increased leukemogenic potential. Future studies aimed at enhanced detection of leukemogenic alterations have the potential to improve clinical outcomes by enabling preemptive intervention in the highest risk patients.

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Conflict-of-interest disclosure

Kristen Schratz: no competing financial interests to declare.

Off-label drug use

Kristen Schratz: Nothing to disclose.

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Minimal intensity conditioning strategies for bone marrow failure: is it time for "preventative" transplants?

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Hematopoietic cell transplantation (HCT) can cure blood dyscrasias and reduce the risk of hematologic cancers in patients with inherited bone marrow failure syndromes (IBMFS). However, because of its high mortality rate, HCT is generally reserved until patients with IBMFS manifest life-threatening cytopenias or myeloid malignancy, at which point outcomes are poor. Screening tests that accurately predict transformation and enable timely intervention are lacking. These unknowns and risks limit the use of HCT in patients with IBMFS, sometimes until significant disease-related sequelae have occurred. A major goal for IBMFS is to reduce cellular therapy–related complications to the point that earlier intervention can be considered before significant transfusion exposure, occurrence of comorbidities, or malignant transformation. In recent decades, disease-specific allogeneic HCT trials have yielded significant improvements in outcomes in IBMFS conditions, including Fanconi anemia and dyskeratosis congenita. This is in large part due to marked reductions in conditioning intensity to address the increased sensitivity of these patients to cytotoxic chemotherapy and radiation. The success of these approaches may also indicate an ability to leverage intrinsic fitness defects of hematopoietic stem and progenitor cells across IBMFS disorders. Now with advances in tracking somatic genetic evolution in hematopoiesis and tailored minimal intensity conditioning regimens, this question arises: is it time for preventative HCT for IBMFS?

LEARNING OBJECTIVES

- Describe determinants of HCT timing for IBMFS
- Compare disease-specific factors and approaches for HCT among IBMFS

CLINICAL CASE

A boy born at 32 weeks' gestation with a history of intrauterine growth retardation and esophageal strictures requiring dilation was found to have moderate pancytopenia at age 8 years. Bone marrow evaluation showed hypocellularity but no evidence of myelodysplastic syndrome/acute myeloid leukemia (MDS/AML). Referral and evaluation for inherited bone marrow failure syndromes (IBMFS) were notable for skin pigmentation abnormalities, oral leukoplakia, and dystrophic nails, leading to a diagnosis of dyskeratosis congenita (DC). The diagnosis was confirmed using a newly available flow cytometry–based fluorescence in situ hybridization telomere length test, showing mean lymphocyte telomere length less than the first percentile for age. A genetic workup for known telomere biology disorder–associated genes was negative. In further evaluation, he was found to have cerebellar hypoplasia, dysarthria, lacrimal duct obstruction, and a markedly abnormal DL_{CO} of 50% on pulmonary function testing. His sibling was a full human leukocyte antigen match but showed mean lymphocyte telomere length at the first percentile. He had multiple 10/10 HLA-matched unrelated donors (MUD), but reported outcomes for hematopoietic cell transplantation (HCT) for DC were poor, with a 100-day mortality ~25%. Androgen therapy was thus initiated when the boy was 9 years old, with a substantial response in his hemoglobin and platelets, obviating the need for transfusions for the next 6 years. This was, however, complicated by hyperlipidemia and suppression of puberty. During this time, he was found to have compound heterozygous mutations in the *RTEL1* gene, consistent with his diagnosis. His parents and sibling were carriers.

Diagnosis and HCT decision-making in IBMFS

Genetic discoveries in recent decades have transformed our understanding of IBMFS. Unlike the case described, some patients do not follow the textbook presentation. The use of gene panels and functional tests has greatly increased diagnostic capability and enabled grouping based on the underlying biology rather than clinical phenotype alone. For patients presenting with bone marrow failure (BMF), accurate and timely diagnosis of an acquired vs inherited disorder is critical for decision-making,[1,2] with different therapeutic interventions for their cytopenias depending on this categorization. These include immunosuppressive therapy (cyclosporine A/antithymocyte globulin), eltrombopag, androgens, corticosteroids, or HCT. The choice of frontline approach(es) depends on factors beyond diagnosis, however, and in many cases the evidence base underlying these choices is still not well established. Although successful HCT would cure the blood dyscrasias in most IBMFS, treatment-related mortality across the board can be estimated to be at least 10%, and often higher. Therefore, many factors need to be taken in account to determine the feasibility and timing of HCT for IBMFS, including patient status, donor suitability, and prior experience in the treating center and field (Figure 1).

With respect to patient hematologic status, cytopenias including severe neutropenia (defined as an absolute neutrophil count <500/μL) and transfusion-dependent thrombocytopenia or anemia are considered indications to move to HCT because of accumulating infection risk, iron overload, and allosensitization. Patients with IBMFS and evidence of MDS or AML based on adverse cytogenetics and blast count also require HCT for cure, with or without prior cancer-directed therapy. More moderate cytopenias and low-grade myelodysplastic syndrome constitute "gray zones" requiring careful consideration of HCT vs observation or other treatments, depending on estimates of disease evolution, treatment response, and expected toxicities. For example, androgens have been shown to increase blood counts in approximately two-thirds of patients with Fanconi anemia (FA) or DC within 3 months of treatment.[3,4] However, the degree and durability of response are unpredictable, and side effects that vary based on the age and gender of the patient limit adherence. Finally, patient-specific features, including age, disease-related comorbidities, and infection status, are carefully weighed to determine HCT candidacy and timing.

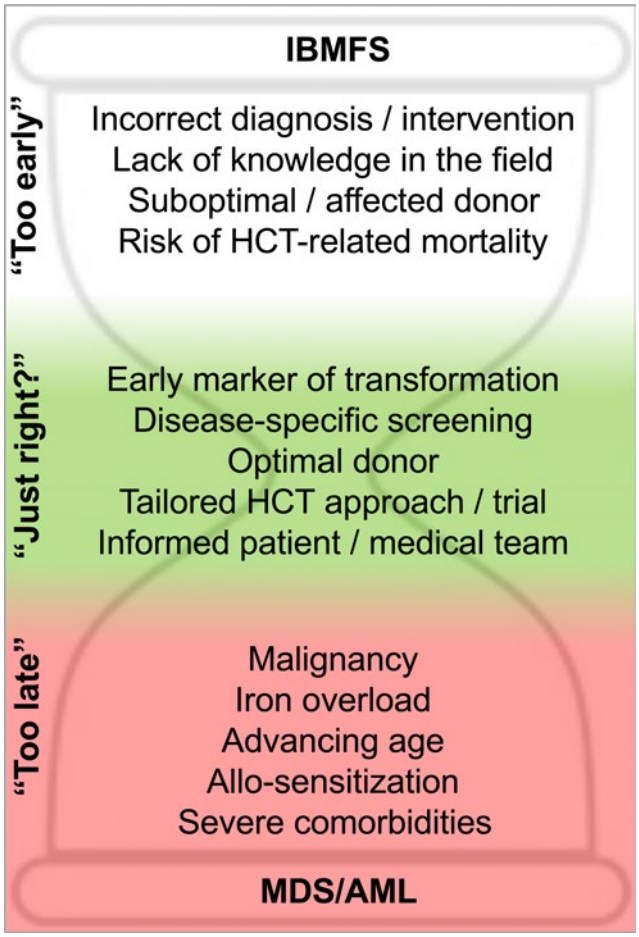

Figure 1. Determinants of optimal HCT timing in IBMFS.

Regarding donor factors, as for HCT in general, the use of HLA-matched family donors is preferred but complicated in IBMFS by the need to evaluate and triage family members for the possibility of subclinical disease.[5] The complexity increases when a genetic etiology has not been firmly established, or when the clinical significance of carrier status is unclear, as seen in the sibling in our case who had borderline telomere length and a heterozygous *RTEL1* mutation. Thus, in equivocal cases, well-matched unrelated bone marrow donors might be prioritized instead of related donors. Except for FA, data are relatively sparse on haploidentical transplants and alternative graft sources in IBMFS disorders, limiting their appeal.

Beyond patient and donor factors, HCT candidacy and counseling for IBMFS patients have relied heavily on anecdotal experience and local practice. For most IBMFS other than FA, the literature is composed primarily of retrospective studies with small patient numbers, often reflective of different HCT "eras" and disease categorizations (eg, based on clinical phenotypes), and with variable regimens and supportive care that may not accurately reflect current practices. It is not uncommon for centers to have limited experience in HCT for particular IBMFS disorders, with the outcomes of 1 or a few prior patients influencing local decision-making.

With these considerations in mind, there is a clear need for prospective trials in IBMFS to guide HCT and other therapeutic interventions. Ideally, the eligibility criteria of a disease-specific protocol could thus be used to help determine HCT timing. In the case described, lacking an open prospective trial, androgen therapy was initially chosen by the team based on bleak retrospective HCT data in DC. This approach temporized cytopenias and allowed progress in the field that impacted later decision-making.

CLINICAL CASE (continued)

The patient underwent yearly surveillance for DC, including bone marrow exams, which consistently showed severe hypocellularity but no morphologic or cytogenetic evidence of transformation. Somatic mutation screening by next-generation sequencing became available during this time and was performed but did not show any abnormalities in bone marrow DNA. At 15 years of age, the patient's platelet count and hemoglobin

waned, and the oxymetholone dose was increased with partial effect, but hyperlipidemia worsened, requiring statin therapy. He was reevaluated for allogeneic MUD HCT and deemed eligible for a new minimal intensity prospective trial tailored for DC, but the family deferred due to the relatively small number of patients treated on the protocol to date.

By 18 years of age, the patient's platelets had decreased to ~20 K/μL and hemoglobin to ~8 g/dL on high doses of oxymetholone, and he required platelet transfusions for procedures. He was found to have a positive contrast echocardiogram suggestive of pulmonary arteriovenous shunting. His bone marrow exams continued to show hypocellularity ~5%-10% without morphologic, cytogenetic, or somatic mutation abnormalities indicative of MDS/AML. He was again evaluated for HCT on the minimal intensity disease-specific protocol, which by this time had treated several additional patients with up to 5 years of follow-up. The HCT team, patient, and family contemplated the waning androgen response, ongoing MDS/AML risk, progressive systemic disease, and quality of life. He remained eligible for the HCT protocol and participation was offered. After careful consideration, the now-adult patient decided to proceed with MUD HCT in the year between high school and college.

Surveillance and earlier HCT intervention in IBMFS

Surveillance and screening tests in general are only useful if (1) there is a reliable early marker; (2) there is a predictable, adverse disease outcome; and (3) there is an effective intervention to prevent or treat the outcome, relative to the side effects of the intervention itself.

Surveillance for cancer risk and disease-specific comorbidities

IBMFS are highly heterogenous in their hematologic presentation and progression not only between but also within biologically linked disease categories. After diagnosis, many patients with IBMFS find themselves in one of the "gray zones" of HCT timing, with only mild-moderate cytopenias and uncertain trajectories. Several factors might push the equation toward earlier intervention, including MDS/AML risk, progression of nonhematologic disease, and individual decision-making (Table 1).

Several forms of IBMFS, including FA, DC, Shwachman-Diamond syndrome (SDS), and *GATA2* deficiency, carry high risks of transformation to MDS/AML,[6-8] up to thousands of times higher than the general population.[9] The goal of early detection of MDS/AML underlies the recommendations for bone marrow screening in these patients.[10,11] While bone marrow morphology, blast count, cytogenetics, and fluorescence in situ hybridization have been used to evaluate for transformation to MDS/AML, it is unclear whether these measures or the frequency at which they are assessed has a meaningful impact on improving outcomes. From anecdotal experience, MDS/AML certainly can occur within a year-long interval without abnormalities detected in the peripheral blood or on the preceding bone marrow exam.

One promising approach to address this gap is next-generation sequencing for somatic mutations in the blood and bone marrow of patients with IBMFS. On a clinical basis, somatic mutations are identified by targeted capture and sequencing of MDS/AML-associated genes to detect clonal changes with high sensitivity, with implications for disease stratification and therapy.[12] Similar analyses are now being conducted in IBMFS patients on a research basis, with early results indicating that somatic genetic changes may be adaptive to the underlying germline lesion, or alternatively may serve as early markers of malignant transformation.[13] However, the analyses are still in the early stages, and the utility of somatic genetic analysis in clinical decision-making for IBMFS remains to be tested, ideally in prospective trials. In the case presented, somatic mutation testing was arguably introduced prematurely, prior to establishing its performance characteristics for MDS/AML screening in DC. Importantly, the prospective trial protocol remained silent on its use for determining eligibility.

Beyond MDS/AML, IBMFS carry risks of nonhematologic sequelae that may complicate or exclude eligibility for HCT. Notable examples include pulmonary and liver disease in DC, solid cancers in DC and FA, and cardiac and liver iron overload from chronic red cell transfusions in Diamond Blackfan anemia (DBA). Several reports suggest inferior HCT outcomes with increasing age, possibly due to such comorbidities.[14-16] Thus, patients with IBMFS require comprehensive screening beyond the hematopoietic system in order to anticipate and identify a window of opportunity for interventions. For example, patients with telomere biology disorders may present with a simultaneous need for lung or liver transplant and HCT and may be excluded from either intervention due to increased risks. Optimizing outcomes in these cases requires rethinking the typical parameters of transplant timing, via close collaboration, alignment, and often advocacy by organ transplant and HCT teams. As children with IBMFS transition to adulthood, their own wishes and priorities may become clearer, sometimes differing from those of their parents. Ideally, they have been engaged in their care and thus better able to make

Table 1. IBMFS and risks/surveillance impacting HCT timing

Disorder	MDS/AML risk	Solid tumor risk	Other surveillance
Fanconi anemia	+	+	
Dyskeratosis congenita/ telomere diseases	+	+	Immune deficiency Liver disease Lung disease
Shwachman-Diamond syndrome	+	+/–	Immune deficiency
Diamond Blackfan anemia	+/–	+/–	Iron overload
GATA2 deficiency	+		Immune deficiency
SAMD9/SAMD9L syndromes	+		Immune deficiency
RUNX1	+		
ANKRD26	+		
ERCC6L2	+		
MECOM	+		
DDX41	+		

informed choices,[17] as in the case at hand, in which the patient decided his HCT timing upon reaching adulthood.

Earlier intervention with minimal intensity HCT

HCT is the only known intervention expected to prevent MDS/AML in IBMFS. To realize the benefit of improved surveillance, the outcomes of HCT in the "gray zone" or BMF stage of hematologic disease must be improved. Features of an ideal HCT regimen for IBMFS include (1) ensuring full engraftment and eliminating host HSPCs with their cell-intrinsic risk of MDS/AML; (2) minimizing acute toxicity and treatment-related mortality (TRM); (3) avoiding acceleration of other disease-specific organ pathology and cancer risk. Conditioning intensity is driven by doses of alkylating agents and/or radiation and correlates with higher engraftment, but also with organ toxicity, TRM, and accelerated carcinogenesis. While the relative risks of these trade-offs of conditioning intensity are difficult to quantify, Khan et al applied mathematical modeling to estimate event-free survival (EFS) of patients with FA after "pre-emptive" HCT (ie, before the onset of severe BMF or MDS).[18] Indeed, significant improvements in EFS were predicted if HCT was performed in early childhood, and if TRM and acceleration of solid tumors were minimized.[18]

Therefore, based on these parameters, successfully reducing conditioning intensity while maintaining engraftment would be expected to improve long-term IBMFS outcomes and enable preemptive or "preventative" HCT. Fortunately, a track record of substantially reduced-intensity conditioning in IBMFS has been established via disease-specific studies in the past 4 decades. The 3 examples provided here are illustrative and not exhaustive and primarily aim to encourage additional prospective studies in IBMFS based on their success (Table 2).

Fanconi anemia

Tailoring HCT intensity in IBMFS has its origins in attempts to mitigate the heightened toxicity to alkylator and radiation exposure manifested by patients with FA.[19] Remarkably, cyclophosphamide was able to be reduced by up to ten times that of conventional doses and radiation exposure also substantially reduced, while engraftment rates were preserved and survival was improved.[20] The evolution of reduced-toxicity regimens in FA has been reviewed, with highlights including the introduction of fludarabine to enable successful MUD HCT without radiation, and T-cell–depleted grafts to minimize graft-versus-host disease (GVHD).[15] Notably, in a multi-center prospective trial, reduced-dose busulfan was evaluated in radiation-free unrelated donor HCT using $CD34^+$-selected grafts and yielded 1-year overall survival in the BMF cohort of 85% (Table 2).[21] Taken together, the international experience in FA over the past 4 decades improved 5-year overall survival to >90% for young patients with BMF and reduced graft rejection and GVHD to <10%, setting the stage for preventive transplants in select patients and clinical settings.[15]

Dyskeratosis congenita

The success of tailored approaches in FA stimulated disease-specific HCT trials in DC, as it was apparent early on that these patients also suffered high TRM ~25% within 4 months post-transplant.[22,23] Regimens incorporating fludarabine to reduce alkylator and radiation exposure, and alemtuzumab for in vivo T-cell depletion to prevent GVHD were described in single-center studies, improving alternative donor HCT outcomes but with ~70% overall survival.[24,25] A prospective HCT trial for BMF in DC patients initiated in 2012 (NCT01659606) asked whether myeloid engraftment could be achieved without using radiation or DNA alkylating agents. The multicenter study utilized fludarabine and alemtuzumab conditioning alone, based on the theory that the immune suppression may be sufficient in a BMF disorder in which host hematopoietic stem and progenitor cells with impaired replicative capacity due to short telomeres might be outcompeted by healthy donor cells. An interim report of 20 patients treated between 2012 and 2018 demonstrated primary engraftment in 95% of patients and overall survival of 90%.[26] The study reached its accrual goal of 40 patients over the course of 10 years and will be reported soon. Long-term follow-up is planned to define the natural history in DC after HCT without exposure to agents that would accelerate organ toxicity and carcinogenesis.

Treosulfan for IBMFS

Alongside attempts to reduce toxicity by minimizing or eliminating alkylators, alternative agents may hold promise in IBMFS. Treosulfan is a pro-drug of alkylating agents that are effective in myeloablation and immunosuppression, but with more predictable pharmacokinetics and potentially decreased organ toxicity.

Table 2. Prospective, multicenter HCT trials for IBMFS

Disorder(s)	Trial	Regimen	Donors	Graft/GVHD ppx	N (IBMFS)	1° graft	OS	Ref
Fanconi anemia	NCT00987480	PK-targeted busulfan; FLU 140 mg/m^2; CY 40 mg/kg; rATG 10 mg/kg	HLA 7/8 or better URD; HLA 4/8 to 7/8 RD	CD34-selected PBSC CSA	34 (non-MDS)	100%	85%	21
SDS, *GATA2*, DBA, other IBMFS	NCT00919503 (completed) NCT04965597 (ongoing)	Treosulfan 42 g/m^2 FLU 150 mg/m^2	MRD or MUD	Unmanipulated BM or PBSC TAC/MTX	10 (non-PNH)	100%	100%	27
Dyskeratosis congenita/telomere biology disorders	NCT01659606	FLU 180 mg/m^2 C1H 1 mg/kg	HLA 7/8 or better URD or RD	Unmanipulated BM CSA or TAC/MMF	20	95%	90%	26

1° graft, primary engraftment rate; BM, bone marrow; BU, busulfan; C1H, alemtuzumab; CSA, cyclosporine A; CY, cyclophosphamide; FLU, fludarabine; HLA, human leukocyte antigen; MMF, mycophenolate mofetil; MRD, matched related donor; MTX, methotrexate; N, number treated; OS, overall survival; PBSC, peripheral blood stem cell; PK, pharmacokinetic; PNH, paroxysmal nocturnal hemoglobinuria; ppx, prophylaxis; rATG, rabbit antithymocyte globulin; RD, related donor; TAC, tacrolimus; URD, unrelated donor.

Table 3. Considerations for optimal HCT trial design for IBMFS

• Prioritize prospective, multicenter trials
• Articulate diagnostic categorization and hematologic disease status based on molecular features, in eligibility/stratification criteria
• Assess adherence to eligibility criteria across centers and ensure timely revision thereof to reflect real-world experience (ie, minimize "picking and choosing")
• Include broad age ranges
• Account for disease-specific comorbidities
• Develop strata encompassing MDS/AML, including de novo presentations
• Consider accessibility of diagnostics (eg, genetic and functional testing) and interventions (eg, graft type/manipulation) at different centers/different parts of the world
• Develop trials in collaboration with patient advocacy groups
• Include quality-of-life and long-term follow-up measures

In a prospective, multicenter trial, Burroughs et al reported 100% engraftment and 100% survival in 10 patients with IBMFS (3 SDS, 4 DBA, 2 *GATA2*, and 1 undefined) who underwent MSD or MUD HCT after conditioning with treosulfan, fludarabine±rabbit anti-thymocyte globulin.[27] All patients had improvements in cytopenia(s) and achieved transfusion independence. Notably, 2 patients had cytogenetic abnormalities (17p deletion in SDS and trisomy 8 in *GATA2*), which resolved on bone marrow examination post-HCT. Treatment-related toxicities were minimal, including no liver toxicity despite pre-HCT iron overload in all the patients with DBA. The ability to minimize nonhematopoietic toxicity while providing some degree of myeloablation with treosulfan extends the possibility of earlier HCT to patients with higher cellularity or evidence of clonal changes without overt MDS/AML. This encouraging study has been expanded in a multicenter blood & marrow transplant clinical trials network prospective trial (NCT04965597) to evaluate treosulfan-based conditioning in 40 patients with a range of IBMFS.

CLINICAL CASE (continued)

The patient underwent MUD HCT on the radiation- and alkylator-free protocol for DC. He developed hemoptysis during conditioning with alemtuzumab and fludarabine and required oxygen but recovered with supportive care. He proceeded to have an uneventful HCT course with full engraftment of donor cells and normalized peripheral blood counts. He developed mild steroid-responsive gut GVHD but no other complications. He began college the following winter. In the years that followed, his engraftment and hematologic parameters remained intact, but he developed avascular necrosis requiring multiple joint replacements and radiographic evidence of pulmonary fibrosis without hypoxia.

Conclusions and future directions

This case demonstrates the changing equation around HCT for a patient with DC, BMF, and risk for MDS/AML over 10 years of his life. Noncurative therapy with androgens bought time, during which a disease-specific minimal intensity prospective HCT trial was developed and successfully undertaken. His acute pulmonary complications may indicate a sensitivity that would have complicated higher-intensity conditioning. The patient's MDS/AML risk post-HCT is now considered negligible, but he continues to develop non-hematologic manifestations of DC.

As exemplified, significant progress in minimal intensity HCT for IBMFS has been achieved through disease-specific and prospective studies over 4 decades, with important lessons and considerations for the future (Table 3):

1. The implementation of screening tests for MDS/AML and non-hematopoietic disease progression in IBMFS should ideally be coupled to prospective trials with carefully determined eligibility criteria. In this manner, prospective trials in IBMFS will inform the efficacy of HCT approaches in specific clinical contexts and help guide HCT timing.
2. Because of the slow accrual expected and the long-term follow-up needed for meaningful interpretation of HCT for IBMFS, it is expected that such trials will take years to complete. Thus, multi-institutional studies should be promoted. As needed, eligibility criteria should be amended early on to align as closely as possible with real-world experience to be most useful in practice once the trial is complete.
3. Increased awareness and testing are identifying more patients with de novo presentations of MDS/AML, particularly adults, who have an underlying IBMFS.[28,29] Therefore, while preventative HCT for IBMFS is a goal, there remains a need to improve HCT strategies for hematologic malignancy in IBMFS.
4. As shown in our case, it is difficult to ascertain definitively how minimal-intensity HCT impacts nonhematologic sequelae in IBMFS, highlighting the importance of integrating long-term follow-up into prospective trials.[30,31]

Conflict-of-interest disclosure

Suneet Agarwal has disclosed the following financial relationship: owned stock in Rejuveron Telomere Therapeutics.

Off-label drug use

Suneet Agarwal: All drug use mentioned in this article should be considered off-label except eltrombopag for severe aplastic anemia.

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Posttransplant complications in patients with marrow failure syndromes: are we improving long-term outcomes?

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Inherited bone marrow failure syndromes (IBMFS) encompass a group of rare genetic disorders characterized by bone marrow failure, non-hematologic multisystemic comorbidities, disease defining congenital anomalies, and a susceptibility to myelodysplastic syndrome, acute myeloid leukemia,and in some instances solid tumors. The most common IBMFS include Fanconi anemia, Shwachman-Diamond syndrome, Diamond-Blackfan anemia, and telomere biology disorders/dyskeratosis congenita. Allogeneic hematopoietic stem cell transplant (HCT) is a well-established curative treatment to correct the hematological manifestations but does not halt or reverse the nonhematological complications and may hasten them. With advances in HCT and in our ability to care for patients with IBMFS, an increasing number of survivors are making it imperative to not only diagnose but also treat late effects from the pre-, peri-, and post-HCT course and complications relating to the natural history of the syndrome. As the field of HCT evolves to allow for the incorporation of alternate graft sources, for expansion of donor options to include unrelated and mismatched donors, and for use of reduced-intensity conditioning or reduced toxicity myeloablative regimens, we have yet to determine if these advances modify the disease-specific course. While long-term outcomes of these patients are often included under one umbrella, this article seeks to address disease-specific post-HCT outcomes within IBMFS.

LEARNING OBJECTIVES

- Examine the disease-specific post-HCT outcomes of patients with IBFMS to determine the impact of HCT on long-term disease outcomes
- Propose screening recommendations for individual IBMFS post-HCT

Introduction

Inherited bone marrow failure syndromes (IBMFS) are a heterogeneous group of genetic disorders clinically characterized by congenital anomalies, bone marrow failure, and a predisposition myelodysplastic syndrome (MDS) with evolution to acute myeloid leukemia (AML) and other solid tumors. Fanconi anemia (FA), Shwachman-Diamond syndrome (SDS), Diamond-Blackfan anemia (DBA), and telomere biology disorders/dyskeratosis congenita (TBD/DC) are the most common IBMFS with defined clinical characteristics. Allogeneic hematopoietic stem cell transplant (HCT) is the only curative option for the hematological impairment and for halting the progression to myeloid malignancies. While it can affect the hematological course, HCT does not improve and may hasten progression to long-term complications of the underlying disorder, including solid tumors. Although historical HCT outcomes for IBMFS have been challenging, the overall practice of HCT has improved owing to detailed multidisciplinary approaches, advancements in disease-defining screening recommendations, and refinement of HCT approaches using alternative donor and graft manipulation. However, the lack of long-term follow-up with these newer approaches makes predicting the impact on the late-effect course challenging, for which there are already minimal data. Individuals who receive an HCT may have significantly inherently worse biological disease, confounding our understanding of these outcomes. Here we offer insight into post-HCT complications and offer supplemental screening tools for disease-specific toxicity (Tables 1–5).

Fanconi anemia (FA)

FA is an inherited chromosomal breakage disorder characterized by bone marrow failure, congenital anomalies,

Table 1. General IBMFS post-HCT screening recommendations

Late effect/organ system	Disease-specific abnormality/concern	Recommendations
Audiology	• Risk for hearing loss	• Audiology screening if ear anomalies identified or if high risk of impairment from treatment course
Ophthalmology	• Vision changes, cataracts, lacrimal duct stenosis	• History and physical for changes in vision, lacrimal duct stenosis, cataracts, etc
Neurocognitive	• Difficulties in school/employment/home settings	• If neurological dysfunction is identified on screening history, perform diagnostic neuropsychological testing and consider referral for school support/employment guidance
Endocrinology	• Thyroid impairment • Growth and puberty • Fertility	• Annual TSH, T_4 screening • Close height/weight monitoring, evaluation of bone health per bone mineral density recommendations • Tanner staging, physical exam, and long-term screening for premature ovarian/testicular insufficiency: FSH, LH, AMH, inhibin B
Bone mineral density	• Impaired bone mineral density/osteopenia with risk for fragility fractures	• Vitamin D monitoring and repletion • DXA scan posttransplant[i] and close monitoring in those who develop GVHD requiring systemic treatment
Pulmonary	• Impairment of lung function	• Annual history and physical screening • PFT every 6–12 months with referral if decline in lung function
Renal	• Chronic kidney disease with and without congenital anomalies • Hypertension	• Hypertensive screening at each visit or annually • Urine protein: creatinine, cystatin C urinalysis annually
Iron overload	• Iron overload impacting cardiac, liver, and endocrine organs	• Serum ferritin screening until resolution of overload • Consider T2-weighted MRI if screen positive or significant transfusion history • Phlebotomize posttransplant to target normal LIC if possible
Psychosocial/lifestyle	• Family planning/genetic counseling • Access to support networks, mental health professionals, and overall screening	• Support for patients living with chronic conditions • Family counseling with referral to genetics for those wishing to conceive • Lifestyle counseling with avoidance of smoking, limiting sun exposure/application of SPF, healthy diet • Revaccination with a focus on HPV with high malignancy risk • Referral to mental health specialist as needed

These recommendations have been adapted from published guidelines.[1–4]

AMH, anti-Mullerian hormone; DXA, dual energy X-ray absorptiometry analysis; FSH, follicle-stimulating hormone; HPV, human papillomavirus; LH, luteinizing hormone; LI, liver iron content; MRI, magnetic resonance imaging; PFT, pulmonary function testing; SPF, sun protection factor; T_4, thyroxine; TSH, thyroid stimulating hormone.

[i]Swauger S, Sabulski A, Hornung L, Wasserman H, Myers KC, Howell JC. Bone health outcomes at 1 year after hematopoietic stem cell transplantation in a heterogeneous pediatric population. *Transplant Cell Ther.* 2022;28(1):44.e1–44.e6. doi:10.1016/j.jtct.2021.08.019.

susceptibility to clonal evolution to MDS/AML, and squamous cell carcinoma (SCC). The Fanconi core complex along with its associated protein complexes are required to ensure timely DNA repair.[5] Mutations in this complex lead to excessive DNA damage and a high risk for transformation to malignancies. The National Cancer Institute IBMFS cohort studies[6,7] reported a cumulative incidence of severe BMF of 70% by age 50 years and 20% overall solid tumor risk by 65 years. The observed to expected rate (O/E) for AML was >200-fold, surpassing other IBMFS. Elevated O/E ratios unique to FA included vulvar cancer and brain tumors, with 2098-fold and 64-fold, respectively, making long-term outcomes more challenging given the limited tolerance for chemotherapy and radiation. Comparing cancer risk for transplanted vs untransplanted patients, the O/E ratio was 55 to 19 and with an earlier median age at presentation, making posttransplant cancer surveillance high priority.

HCT for FA was initiated in the late 1970s to 1980s, with excess regimen-related toxicity and mortality secondary to high-dose alkylating agents like cyclophosphamide and the use of total-body irradiation given the underlying hypersensitive to DNA cross-linking agents.[8,9] The following 30 years of advancement have been summarized,[10,11] highlighting the introduction of fludarabine, a powerful adjunct to dose de-escalation of total-body irradiation/cyclophosphamide, reducing graft failure and improving overall survival.[12] Recently, there has been an effort to eliminate use of radiation therapy.[13] In vivo T-cell depletion has been routinely used, but other graft manipulation strategies using CD34+ enrichment techniques with and without T-cell add-back or T-cell receptor alpha/beta depletion[14] have

Table 2. FA post-HCT screening recommendations

Late effect/organ system	Disease-specific abnormality	Recommendations
Cancer screening	• SCC: head, neck, anogenital region • Nonmelanoma skin cancer: basal cell carcinoma and SCC • Breast cancer • CNS tumors • Wilms tumor	• ENT evaluation of the head and neck region every 6–12 months starting at age 10 years with possibility of nasolaryngoscopy • Dermatological evaluation yearly • Gynecological exam annually starting at puberty • Pap smear starting at 18 years or sexual activity • Encourage HPV vaccination • Additional surveillance and genetic counseling for those with *FANCD1* (*BRCA2*)—leukemia, brain tumors, Wilms tumor, breast cancer; *FANCJ* (*BACH1*), *FANCN* (*PALB2*), *FANCO* (*RED51C*)—familial breast cancer gene[i,ii] • Patients with total-body irradiation or chest RT require screening mammography starting at age 25 or 8 years after radiation exposure (no later than 40 years)[1] • Chronic GVHD screening
Endocrinology	• Hypothyroidism • Glucose dysregulation: insulin resistance/diabetes • Growth hormone deficiency/bone health • Metabolic syndrome	• Annual TSH, free T_4 • Oral glucose tolerance testing, insulin levels annually • Growth evaluation, BMI, and bone health, 25-hydroxy vitamin D levels, DXA scan annually • Dyslipidemia screening
Renal	• Renal anomalies	• Close follow-up on renal-specific anomalies
Reproductive-gonadal	• Genitourinary malformations • Premature ovarian and testicular insufficiency	• Follow-up with urology on genitourinary-specific anomalies • Tanner staging, physical exam, and long-term screening for premature ovarian/testicular insufficiency: FSH, LH, AMH, inhibin B • Fertility counseling services
Audiology	• Conductive hearing loss	• Audiology screening and referral for hearing-assistive devices with abnormalities

These recommendations have been adapted from published guidelines.[1–4]

AMH, anti-Mullerian hormone; BMI, body mass index; DXA, dual energy X-ray absorptiometry analysis; ENT, otolaryngology; FANC, Fanconi anemia complementation; FSH, follicle-stimulating hormone; HPV, human papillomavirus; LH, luteinizing hormone; RT, radiation therapy; T_4, thyroxine; TSH, thyroid stimulating hormone.

[i]Woodward ER, Meyer S. Fanconi anaemia, childhood cancer and the BRCA genes. *Genes.* 2021;12(10):1520. doi:10.3390/genes12101520.

[ii]Zierhut HA, Bartels DM. Waiting for the next shoe to drop: the experience of parents of children with Fanconi anemia. *J Genet Couns.* 2012;21(1):45–58.

Table 3. SDS post-transplant screening recommendations

Late effect/organ system	Disease-specific abnormality	Recommendations
Cancer screening	• Potential risk for future solid tumor	• Close monitoring with lifestyle modifications, eg, avoidance of smoking and excessive alcohol intake • Encourage SPF usage • Encourage regular self-exams and general population cancer screening recommendations • Follow general population screening recommendations • Patients with total-body irradiation or chest RT require screening mammography starting at age 25 or 8 years after radiation exposure (no later than 40 years)[1]
Skeletal	• Rib cage deformities, scoliosis, short stature	• Bone mineral density evaluation • Growth hormone evaluation • Consultation with orthopedics for bracing/other interventions
GI/pancreatic	• Nutritional deficiencies secondary to pancreatic insufficiency	• Pancreatic dysfunction may become subclinical over time but those who are symptomatic may require long-term pancreatic enzyme replacement and close monitoring of stool output, nutrition, and growth
Cardiac	• Baseline cardiac dysfunction compounded by regimen-related toxicity	• Baseline echocardiogram and exposure-modified frequency post-HCT; referral to cardiology if abnormalities identified
Hepatic	• Potential for hepatic dysfunction	• Close monitoring of liver enzymes

These recommendations have been adapted from published guidelines.[1–4]

SPF, sun protection factor.

Table 4. DBA post-HCT screening recommendations

Late effect/organ system	Disease specific abnormality	Recommendations
Cancer screening	• Colon cancer • Osteogenic sarcoma	• Colonoscopies starting at 1 year after HCT and every 5 years if normal[i] • Awareness and education of risk of osteogenic sarcoma • Patients with total-body irradiation or chest RT require screening mammography starting at age 25 or 8 years after radiation exposure (no later than 40 years)[1]
Ophthalmological	• Cataracts	• Evaluation for vision changes/cataracts in patients pretreated with steroids
Endocrinology	• Iron overload depositing in endocrine organs	• Evaluation of all endocrine organs: growth hormone, TSH, free T_4, diabetes screening, parathyroid, gonadal axis screening on an annual basis
Neurocognitive	• Impact on neurodevelopment and educational performance	• Screening for neurodevelopmental issues particularly in patients with craniofacial abnormalities
Iron overload	• Iron overload burden increased in DBA compared with rest of IBMFS	• T2-weighted MRI of the heart and liver • Monitor liver function testing and consider referral to hepatology if abnormal • Phlebotomizing per Table 1
Bone mineral density	• Fractures/osteopenia secondary to steroid use	• Screening with DXA scan prior to HCT and every 2–3 years if abnormal post-HCT
Obstetrics	• Risk for premature delivery	• Discussion with obstetrician for pregnancy planning

These recommendations have been adapted from published guidelines.[1–4]

DXA, dual energy X-ray absorptiometry analysis; MRI, magnetic resonance imaging; T_4, thyroxine; TSH, thyroid stimulating hormone.

[i]Lipton JM, Molmenti CL, Hussain M, et al. Colorectal cancer screening and surveillance strategy for patients with Diamond Blackfan anemia: preliminary recommendations from the Diamond Blackfan Anemia Registry. *Pediatr Blood Cancer*. 2021;68:e28984. doi:10.1002/pbc.28984.

Table 5. DC post-HCT screening recommendations

Late effect/organ system	Disease-specific abnormality	Recommendations
Cancer screening	• HNSCC • Gynecological tumors/anal cancer	• Encourage HPV vaccination • Yearly gynecological evaluation for Pap smear and HPV screening • Oral and skin exams for cancer screening every 6–12 months • Patients with total-body irradiation or chest RT require screening mammography starting at age 25 or 8 years after radiation exposure (no later than 40 years)[1]
Liver	• Liver cirrhosis/fibrosis	• Baseline liver labs yearly • Early assessment of iron overload and aggressive phlebotomy/chelation as needed after HCT • Elastography-based ultrasound with concerns • Early referral for liver consult with concerns
Pulmonary	• Pulmonary fibrosis • Pulmonary AVMs	• Close monitoring with screening history and physical • PFTs yearly with early referral to pulmonology with decline in pulmonary function • Imaging studies as required with a focus on avoiding unnecessary radiation exposure • Consider xenon MRI if available at local center[i] • Bubble echocardiogram for AVM surveillance
Esophageal/GI	• Esophageal stenosis • Risk of GI bleed due to telangiectasias/varices	• Screening for stenosis with dilatation as needed • Endoscopies with GI bleed concerns
Reproductive	• Urethral stenosis	• Follow-up with urology for dilations
Bone health	• Osteopenia • Avascular necrosis	• Screening with DXA scan prior to HCT and every 2–3 years if abnormal post-HCT

These recommendations have been adapted from published guidelines.[1–3]

AVM, arteriovenous malformation; DXA, dual energy X-ray absorptiometry analysis; GI, gastroenterology; HNSCC, head and neck squamous cell carcinomas; HP, human papillomavirus; MRI, magnetic resonance imaging.

[i]Walkup LL, Myers K, El-Bietar J, et al. Xenon-129 MRI detects ventilation deficits in paediatric stem cell transplant patients unable to perform spirometry. *Eur Respir J.* 2019;53(5):1801779. doi:10.1183/13993003.01779–2018.

been evaluated to eliminate the risk for acute and chronic graft-versus-host disease (GVHD) while minimizing risk of graft rejection. There is a strong association with chronic GVHD and SCC; however, it remains unclear if busulfan will improve the incidence or delay development of SCCs. Alternate mismatch donor options have been extensively explored, as has the use of cord blood transplant.[15] T-cell replete grafts with dose-reduced posttransplant cyclophosphamide have been investigated but require dose optimization.[16,17] Future therapy with antibody-based stem cell ablation or gene therapy using gene-corrected autologous hematopoietic stem cells (HSCs)[18,19] may offer a less toxic approach, but long-term data is needed. Gene therapy may be promising, as repaired HSCs may offer selective advantage in vivo and allow for genetic reversion,[20] but it raises the question whether residual nonrevertant stem cells will impact durability of hematopoiesis[21] and lead to risk for MDS/AML, which would necessitate separate screening recommendations.

Outcomes for HCT in FA have improved significantly, and novel approaches may improve long-term toxicity; however, follow-up data are limited, especially in adults. Currently, we are not able to separate the progression of disease phenotypes from those associated or exaggerated by HCT. Endocrinopathies including thyroid dysfunction, glucose intolerance, growth and bone density abnormalities, and gonadal dysfunction[22-24] are common irrespective of transplant status but may be exacerbated by HCT. Disease- and treatment-related complications related to congenital abnormalities, endocrinopathies, and most importantly increased cancer susceptibility make lifelong monitoring imperative (Table 2).

Shwachman-Diamond syndrome (SDS)

SDS is a rare IBMFS classically caused by mutations in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene on centromeric region of chromosome 7 (7p12-7q11), but recently other genes, including *DNAJC21*, *EFL1*, and *SRP54*, have been associated with an SDS-like phenotype. These genes are well defined in ribosomal biogenesis but contribute to the pleiotropic phenotype. SDS is a multisystem disorder characterized by multilineage cytopenias and susceptibility to MDS and AML, skeletal malformations, pancreatic exocrine insufficiency contributing to malnutrition and poor growth, and various dysmorphic features.[25] The prognosis of patients who develop MDS or AML is dismal, and efforts have focused on identifying clonal changes before clinical transformation occurs. Presence of deletion 20q (del(20)(q12)) and isochromosome 7 (i(7)(q10)) are well established as compensatory mechanisms to improve hematopoiesis,[26-28] which in isolation do not necessitate HCT. Other cytogenetic changes and distinct clonal somatic changes in *TP53*[29] confer worse prognosis and can predict progression to myeloid malignancies. Yearly marrow surveillance or earlier in the presence of evolving cytopenias, cytogenetic changes, or molecular aberrations consistent with leukemic clones is recommended such that preemptive HCT can be pursued.

HCT for SDS has been effective before development of MDS or AML, but balancing preexisting organ dysfunction and comorbidities is essential. Myeloablative conditioning (MAC) regimens are required to eliminate the abnormal marrow and facilitate donor engraftment. However, the associated toxicity, specifically cardiac and hepatic, may be too great for patients with SDS,[30-33] suggesting that the defects in ribosomal biogenesis are not only limited to the bone marrow but also impact other organ systems.

Historical HCT outcomes for SDS are poor, with high rates of transplant-related toxicities, increased incidence of graft failure, and infection burden, along with an increased risk for cardiac, liver, and pulmonary complications. Cesaro et al.[34] reported transplant-related mortality (TRM) of 35%, which was higher in those receiving total-body irradiation–containing regimens (~70%) than in those without. Recent updates from the European Society for Blood and Marrow Transplantation[35] indicate that while outcomes for BMF have improved, outcomes with malignancy remain poor, with overall survival (OS) of 71% vs 29%, along with high rates of TRM. Advancements in conditioning regimens should lead to consideration of RIC or reduced-toxicity regimens. Outcomes for RIC regimens report minimal TRM,[36] but long-term follow-up is limited.

Treosulfan, which has a more predictable pharmacokinetic profile, may be better incorporated for SDS regimens to reduce TRM and limit hepatoxicity.[37] Long-term studies are needed to evaluate HCT survivor outcomes, especially in known areas of sensitivity such as cardiac and liver disease, to best tailor long-term monitoring (Table 3).

Patients with SDS may be predisposed to solid tumors, but the exact relative risk is poorly known. Isolated fatal development of solid tumors of the ovary, breast, and esophagus in the fifth decade of life have been reported,[6,7,38] but association with HCT has not been possible given small sample size.

Diamond-Blackfan anemia (DBA)

DBA is a rare congenital red cell aplasia disorder characterized by normocytic or macrocytic anemia, reticulocytopenia in the absence of erythroid precursors in the marrow, and a predisposition to malignancy. It is accompanied by congenital anomalies affecting many systems, including cardiac, renal, musculoskeletal, and craniofacial defects.[39] DBA is primarily inherited in an autosomal dominant fashion, with most mutations involving ribosomal protein subunits; however, 50% of the mutations are de novo. Twenty-five percent of mutations involve the *RPS19* gene, but newer mutations have been recognized in *GATA1* (X-linked) and *TSR2* (X-linked). *ADA2* and biallelic mutations with *EPO* confer a DBA-like phenotype with erythroblastopenia.[40]

The mainstay of treatment for DBA is chronic steroids sufficient to maintain a response at low doses, and chronic transfusions with chelation for those who do not. As outcomes for HCT in DBA improve, a growing number of patients who are unresponsive to steroids are electing to undergo HCT and forgo chronic transfusions with chelation. The OS and event-free survival with MAC regimens have improved post-2010 independent of donor type, likely attributed to improvement in chelation practices and pre-/post-HCT subspecialty care.[41,42] While HCT mitigates the risk of MDS/AML, the increasing use of reduced-intensity HCT[43] may change this with potential for mixed chimerism. Although HCT represents the only curative option to correct the hematological features, posttransplant monitoring is imperative as patients still face concerns for iron overload, endocrinopathies, and solid tumor risk[44,45] (Table 4). It is critical that iron overload be assessed and abated with chelation prior to pursuing HCT[46] and further addressed post-HCT with

phlebotomy to avoid long-term cardiac and hepatic toxicity, as deaths due to cardiac iron overload can occur as late as 5 to 7 years after transplant.[1-3,47] Long-term steroid use may impact bone mineral density and growth and require long-term ophthalmologic follow-up.[1]

Importance must be placed on cancer screening, which is unique to DBA of lung, colorectal, and osteogenic sarcoma. The incidence of malignancies per longitudinal DBA registry data[48] showed an O/E ratio of 4.8 (95% CI 3.2%-6.9%). In a post-HCT subanalysis, the O/E for colorectal cancer (n=2) was 346.9 (95% CI 42.0%-1252.0%) and osteosarcoma (n=1) was 257.8 (95% CI 6.5%-1436.2%). The O/E compared with overall cancer risk may suggest higher risk in the transplanted group, but this is a small sample size and requires further long-term analysis.

Telomere biology disorders/dyskeratosis congenita (TBD/DC)

DC is a rare hereditary multisystem TBD. It is characterized by a triad of oral leukoplakia, reticular skin changes, and abnormal nails, which are now recognized as outward manifestations of a systemic disease process. Patients with DC have shortened telomeres for age (less than first percentile for age) and often present with 1 or a combination of BMF with potential for evolution to MDS/AML, pulmonary fibrosis, liver cirrhosis or fibrosis, esophageal stenosis, enteropathy, avascular necrosis of the joints, and head and neck SCC.[49] To date, more than 18 genes have been identified that account for 70% of the diagnoses with variable expression pattern. By 40 years of age, 50% to 90% of patients have at least single-lineage cytopenia due to accelerated telomere attrition contributing to hematopoietic failure over time.[6,7] HCT is the only curative method to correct the hematological deficiency, but it does not cure underlying tissue involvement and may accelerate it. Temporizing measures with transfusion support is indicated with severe cytopenias but can contribute to allosensitization and iron burden.

HCT regimens for DC historically involved MAC regimens, which resulted in hepatic and pulmonary fibrosis, veno-occlusive disease, and a high early and late mortality rate.[50] Transition to RIC regimens has improved outcomes[51-53] along with the push toward radiation- and alkylator-free approaches.[54,55] Despite the adoption of RIC regimens, several case reports show deaths related to gastrointestinal bleeding post-HCT possibly related to erosive duodenitis, esophageal varices, or ectasia likely secondary to portal hypertension or endothelial dysregulation, highlighting that RIC regimens pose risk for complications.[56,57] A consortium study[58] reported 14 patients with TBD, including several without history of HCT, who were refractory to angiodysplasia treatment, underscoring a propensity for GI bleeding and additional mortality in this patient population.

The early success of RIC regimens should still prompt long-term monitoring, as a review[59] of 109 patients with a combination of MAC and RIC regimens showed 5-year and 10-year survival estimates of 57% and 23%, respectively, highlighting significant late mortality. Early deaths were attributed to infection, nonengraftment, and hemorrhage, whereas deaths >1 year were attributed to pulmonary, liver, and vascular complications. The incorporation of MAC regimens may contribute to the long-term toxicity, or this may indicate that patients with BMF have a worse DC phenotype. Longitudinal studies evaluating RIC-based regimens with the goal of preventing late mortality and identifying cancer risk will take more time; further collaboration is needed given the rarity of the disease.[60]

Cancer predisposition is notable in the DC population, with 20% of patients developing malignancy by age 65 with a higher risk in posttransplant compared with untransplanted patients, 30 vs 4.2.[6,7] The reported O/E ratio was significantly elevated at 11-fold for all cancer sites, with a striking O/E for tongue cancer of 1154-fold. Risk of MDS/AML is substantial at 2500-fold but is largely mitigated by HCT.

Conclusions

IBMFS are a heterogeneous group of rare disorders classified by the overarching hematological manifestations in the setting of extensive disease-specific complexities. HCT remains the only curative treatment option to correct the hematological impairment or halt progression to MDS/AML, but this begs the question of whether we are truly improving patient outcomes or insidiously affecting the natural disease course. We have made significant strides as a transplant community through optimizing HCT regimens, often to prevent graft rejection, minimize acute and chronic GVHD, and enhance short-term OS at the forefront. However, significant limitations remain due to the drought of knowledge of late effects, which is needed to guide prospective trials. Patients with marrow failure or progression to MDS/AML necessitating HCT may inherently have a more severe phenotype, but without cohort studies comparing transplanted versus untransplanted patients, the natural history will be challenging to decipher. The Center for International Blood and Marrow Transplant Research estimates that by 2023, there will be 502000 HCT survivors of which 14% would have been younger than 18 years at the age of transplantation.[61] This emphasizes the need to understand long-term health consequences that patients may face due to the underlying disease process, pre-HCT therapy, the HCT process, or a combination. We propose future directions as guidance for subsequent studies.

Future directions

1. Longitudinal international collaborative efforts are needed to pursue multi-institutional clinical trials with uniform disease-specific approaches and to allow for pooling of retrospective data to lengthen follow-up and establish more information on late effects. With newer regimens proposed, disease-specific long-term outcomes should be prioritized in study aims.
2. Consensus meetings at regular intervals to review new disease-specific data will allow for timely updates on long-term monitoring recommendations. Data-driven publications will assist with bridging care gaps that occur as patients transition from pediatric to adult providers. This will also allow nontransplant community-based physicians to understand the unique and complex health challenges.
3. Further study via international collaboration of large cohorts including both transplanted and untransplanted patients is needed to ascertain whether disease manifestations may mimic late effects or are further exacerbated by the transplant process. This may include genotype-phenotype correlation to further establish long-term guidance tailored to the individual disease processes.
4. Additional future studies should focus on less-toxic conditioning regimens and minimization of toxicity pre-HCT, including gene therapy.

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Off-label drug use

Zahra Hudda: There is discussion of off-label drug use throughout. The majority of pediatric medications are used off-label and many of these transplants are in pediatric patients.

Kasiani C. Myers: There is discussion of off-label drug use throughout. The majority of pediatric medications are used off-label and many of these transplants are in pediatric patients.

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Planning GvHD preemptive therapy: risk factors, biomarkers, and prognostic scores

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Prevention of acute and chronic graft-versus-host disease (aGvHD and cGvHD) is an important objective of allogeneic hematopoietic cell transplantation (HCT). While there is has been significant progress in preventative approaches in the peritransplant period to minimize development of GvHD, no preventative approach has completely eliminated development of either aGvHD or cGvHD. Recently, posttransplant immune biomarker profiling early post-HCT by the Mount Sinai Acute GvHD International Consortium group has resulted in a validated risk assignment algorithm and development of preemptive approaches to decrease aGvHD and mortality in high-risk patients. cGvHD risk assignment algorithms have been developed based on measurements at day 100 and may be used for future preemptive intervention trials to minimize cGvHD. This article discusses the current state of the art in aGvHD and cGvHD preemptive algorithms and therapeutic interventions and what is needed to move these into validated approaches.

LEARNING OBJECTIVES

- Understand the status of risk assignment algorithms for prediction of development of acute and chronic graft-versus-host disease
- Understand the possible design of preemptive trials to prevent future development of acute and chronic graft-versus-host disease

CLINICAL CASE 1

A 11-year-old child with early relapsed B-cell acute lymphoblastic leukemia underwent a bone marrow human leukocyte antigen–matched unrelated donor allogeneic hematopoietic cell transplantation (HCT) with cyclophosphamide and total body irradiation conditioning. Graft-versus-host disease (GvHD) prophylaxis consisted of antithymocyte globulin (ATG, rabbit), tacrolimus, and mycophenolate mofetil. On day +32, the patient began to develop a maculopapular rash on greater than 50% of their body and diarrhea calculated at 34 mL/kg/d with 10 to 15 episodes of nonbloody diarrhea per day without nausea or vomiting. Serum bilirubin was <2 mg/dL. Using the Mount Sinai Acute GvHD International Consortium (MAGIC) criteria, the patient was classified as acute GvHD stage 3 skin, stage 0 upper gastrointestinal tract (GI), stage 3 lower GI, and stage 0 liver. The overall grade was grade 3 acute GvHD. The patient was started on intravenous methylprednisolone at 2 mg/kg/d. After 1 week of treatment, the skin rash had improved to stage 1, but the stool output had only decreased to 30 mL/kg/d. The patient was classified as steroid refractory and was started on ruxolitinib as a second-line agent. Within 2 weeks, the skin had resolved but there was no change in stool output, and it had become bloody and associated with significant cramping abdominal pain. The patient developed gram-negative sepsis and uncontrollable lower GI hemorrhage and died in the pediatric intensive care unit.

Is there a test that could reliably predict the onset and severity of acute GvHD in this patient, allowing the clinician to initiate targeted preemptive therapy?

Advances in GvHD prophylaxis, such as posttransplant cyclophosphamide and TCRαβ/CD19 depletion, expanded the donor pool to include haploidentical donors but have not reduced the overall incidence of GvHD, which remains the most significant nonrelapse complication of pediatric and adult HCT. Furthermore, the intensification of immunosuppression necessary to overcome human leukocyte

Table 1. Acute GvHD risk assignment algorithms that can be used in preemption for aGvHD

	Time measured	Components	Validated	Reference(s)
MAGIC	Day 7	ST2, Reg3alpha	Yes	[1,2]
aGvHD MS-17	14 days before onset of aGvHD	Urine proteome—identified proteins: collagen a-1(II) chain AA[c]; collagen a-1 (XXII) chain; serum albumin, N-term; collagen a-2(I) chain; P-2-microglobulin; collagen a-2(I) chain; collagen a-2(I) chain; CD99 antigen; collagen a-1 (I) chain	Yes	[7,8]
GITMO Prediction of TRM	Day 7	Serum cholinesterase, total protein, blood urea nitrogen, c glutamyl transferase, donor type and cell dose	Yes	[9]

TRM, transplant related mortality.

antigen barriers results in higher rates of opportunistic infections and organ toxicity. There is a critical need for clinically validated biomarkers that accurately predict onset and/or severity of GvHD to allow clinicians to identify high-risk patients who would benefit from intensified immunosuppression to prevent severe GvHD as well as low-risk patients in whom it may be possible to reduce GvHD prophylaxis to maximize graft-versus-leukemia (GvL) effect and reduce risk of infections and toxicity.

Can we predict the onset of acute GvHD?

There have been multiple efforts to develop biomarker panels that could be used to predict the onset of acute GvHD. The MAGIC algorithm probability (MAP) uses the serum concentrations of 2 GI GvHD biomarkers, ST2 and REG3α,[1] to risk stratify patients for nonrelapse mortality (NRM) related to GvHD (Table 1).[2] Patients with a high MAP on day +7 post-HCT were much more likely to die of nonrelapse causes within 6 months (28% vs 7%, $P<.001$), a finding that was reproduced in 2 validation cohorts. Because most of the deaths were from GvHD, MAGIC conducted a pilot trial (n = 30) to prevent GvHD in patients with a high MAP on either day 7 or day 14 post-HCT.[3] Twice-weekly infusions of α1-antitrypsin, a serine protease inhibitor with anti-inflammatory and immunomodulatory properties[4] with few toxicities and promising data as a treatment for steroid-refractory GvHD,[5,6] were administered for 16 doses following the first high MAP. Unfortunately, α1-antitrypsin treatment did not reduce the development of severe GvHD or improve NRM or survival when compared to 90 closely matched controls. Furthermore, the relatively few patients (15% of those screened) eligible for preemptive therapy added significant expense and time. A more selective screening process may be more cost-effective for future trials with different agents but would require a substantially larger pool of patients for screening purposes.

Serial monitoring of a urinary proteomic pattern (aGvHD_MS17) was found to have high sensitivity and specificity for patients at high risk for severe (grade 3/4) GvHD approximately 14 days prior to onset.[7] Although the specific components of this biomarker have not been published, it includes markers of inflammation and T-cell activation. In a multicenter prospective trial, 259 patients were serially monitored for the detection of aGvHD_MS17 for up to 80 days post-HCT; patients who were positive (n = 92) were randomized to preemptive prednisolone therapy or placebo. Patients negative for aGvHD MS-17 were more likely to survive than those who were positive, but it is not clear how much differences in GvHD contributed to these differences (Table 2). Patients positive for aGvHD MS-17 who were randomized to prednisolone experienced less grade 2 GvHD, but there was no reduction in grade 3/4 GvHD or improvement in survival.[8] In another study, 170 patients who were at high risk for severe GvHD on day +7 post-HCT based on a score calculated from blood concentrations of serum cholinesterase, γ-glutamyl transferase, total serum proteins, and blood urea nitrogen were preemptively treated with either 3 doses of ATG totaling 3.75 mg/kg (n = 84) or no treatment (n = 86).[9,10] Patients who were at highest risk and received ATG developed significantly less severe (grade 3/4) GvHD (5% vs 15%, $P = .02$) but survival was not improved, perhaps due to fatal complications related to ATG use.

The endothelial activation and stress index (EASIX) applies a simple mathematical formula to routine laboratory parameters (lactate dehydrogenase [U/L] * creatinine [mg/dL]/platelet count [10^9 cells/L]) to quantify endothelial damage. EASIX predicts mortality from GvHD when calculated at its onset,[11] and a pre-HCT EASIX score can risk stratify patients for a variety of complications prior to HCT, including the development of veno-occlusive/sinusoidal obstructive syndrome,[12] severe organ dysfunction requiring admission to an intensive care unit,[13] and fluid overload.[14] However, neither the pre-HCT EASIX score nor serial monitoring predicts the development of GvHD well[15-18] and thus cannot be used for preemptive intervention. Despite the discovery and validation in cohort studies of aGvHD systemic and organ-specific diagnostic, response, and prognostic biomarkers and biomarker algorithms, GvHD biomarkers for its prediction have not yet led to beneficial preemptive treatments. The ideal strategy for GvHD preemption would effectively prevent severe GvHD while preserving the GvL effect. One strategy to consider is the selective targeting of immune pathways with agents that appear to preserve GvL while reducing GvHD, such as JAK1/2 inhibition.[19] An intriguing approach would be to protect GvHD target organs such as the GI tract from damage with nonimmunosuppressive agents that promote epithelial health such as interleukin 22,[20] RIPK1 inhibition,[21] or manipulation of the GI microbiome.[22] Severe GvHD-free, relapse-free survival, a widely used end point for prophylaxis trials, could also be used to measure the effectiveness of preemptive strategies.[23]

CLINICAL CASE 2

A 15-year-old girl received a haploidentical-related donor transplant for acute myeloid leukemia in first complete remission with posttransplant cyclophosphamide prophylaxis followed by tacrolimus and mycophenolate mofetil GvHD prophylaxis. The patient developed a maculopapular rash at day 21 post-HSCT covering 30% of the body and had no GI or

Table 2. Preemptive trials for aGvHD

ClinicalTrials.gov Identifier:	Age	Risk assignment	No. of patients	Expected completion	Intervention	Outcome to be measured	Improved outcome	Ref.
NCT05368181 Chengdu, Sichuan, China	Adult	High risk by MAP days 7, 14, 21, 28	56	Dec 2024	Methylprednisolone starts with the dose of 2 mg/kg for 5 days	High Risk Patients Who Develop Grade III-IV aGvHD by day 100	Pending	NA
NCT03459040 MAGIC consortium	Adults	High risk by day 7 or 14	30	Completed	α1-Antitrypsin	Number of High Risk patients who develop steroid refractory GvHD by day 100	No	3
EudraCT number: 2008-005862-30. aGvHD MS-17	Adults	High risk 14 days before onset	92	Completed	Prednisolone 2–2.5 mg/kg for 5 days followed by a taper of 19 days in the absence of aGvHD	Incidence of aGvHD II-IV between randomization and day +100 after HSCT	No	8
Bagialupo	Adults	High risk by day 7	170	Completed	ATG 1.25 mg/kg intravenously on days 7 and 9	Primary end point of the study was TRM Secondary end point was acute GvHD grades 3 to 4.	Decreased aGvHD	10
Storek	Adults	High risk on day 7	68	Completed	ATG day 8	Reduction in high risk GvHD (both aGvHD and cGvHD)	No	42

NA, not available; TRM, transplant related mortality.

liver manifestation. The patient was classified as skin stage 2, GI stage 0, and liver stage 0 and an overall MAGIC aGvHD grade of 1. She was treated with a short course of prednisone at 2 mg/kg/d with rapid resolution of the skin rash and was tapered off steroids by day 56 posttransplant. Because of high risk of relapse, all immune suppression was withdrawn by day 120. On day 125 after HCT, the patient developed skin changes with lichen planus–like features involving 19% to 50% of the body surface area and complained of dry, gritty eyes and dry mouth. Examination of the mouth had lichen planus–like features. There were no nail changes, GI symptoms, liver abnormalities, or restriction of mobility or muscle pain. Pulmonary function testing revealed a normal forced expiratory volume in 1 second (85%). The overall grading of the patients as per 2014 chronic GvHD (cGvHD) diagnostic criteria was moderate cGvHD. The patient was started on prednisone 1 mg/kg/d and reevaluated in 1 month. At the 1-month evaluation, the skin had changed to superficial sclerotic features (able to pinch and still involved between 19% and 50% of the skin). Also noted were dystrophic nail changes. The patient also complained of increasing shortness of breath when going up the stairs and had a drop in FEV1 to 70%. A high-resolution inspiratory and expiratory computed tomography (CT) confirmed air trapping and small airway thickening upon expiration. A bronchoscopy confirmed no evidence of an active infection. The findings were consistent with the diagnosis of severe cGvHD. Fluticasone, azithromycin, and montelukast therapy and ruxolitinib were added to prednisone therapy, and the skin, eyes, and mouth demonstrated improvement. However, her lung GvHD progressed worsening over the next number of months, leading eventually to death from respiratory failure.

The need for late acute and chronic GvHD risk biomarkers

The later onset of cGvHD post-HCT potentially makes it make more amenable to preemptive intervention; the earliest onset of cGvHD usually is about 80 days after HCT.[24] A number of strategies have been shown to decrease the risk of cGvHD onset, including posttransplant cyclophosphamide, ATG, alemtuzumab, umbilical cord blood donor, marrow donor, TCRαβ/CD19, and CD45RA depletion of grafts.[25,26] While these have decreased the frequency of cGvHD after HCT, cGvHD still remains a major cause of lifelong morbidity and mortality, especially in children given their longer life expectancy. Risk assignment biomarkers that reliably predict the development of cGvHD measured prior to 100 days post-HCT are optimal as it allows for development of an intervention that minimizes both potential onset of late acute GvHD (aGvHD) and cGvHD in high-risk patients as well as potentially allowing early withdrawal of immune suppression in those patients who are at lower risk of cGvHD. This will allow optimal development of post-HCT antiviral immunity and the GvL effect.[27]

Several studies have identified plasma-based proteomic markers at 100 days post-HCT that can predict the development of cGvHD, including ST2, CXCL9, and α-ketoglutaric acid,[28,29] but at least 10% of cGvHD cases occur before 100 days (Table 3).[19] Furthermore, none of these markers has been validated by more than 1 study, as recommended by the National Institutes of Health (NIH) cGvHD consensus group.[30] Like aGvHD, there is at present no validated risk biomarker algorithm for cGvHD. Due to the complex pathophysiology underlying cGvHD, it has been hypothesized that a polyomic approach to biomarker discovery is necessary to accurately predict cGvHD onset.

Table 3. Chronic GvHD risk assignment algorithms for preemptive therapy

	Time measured	Age group	Components	ROC AUC, PPV, NPV	Validated	Reference
cGvHD MS-14	Days 100, 180, 280, 365	Adult	Successfully sequenced 6 of the 14 cGvHD naturally occurring peptides. In patients with cGvHD, increased thymosin β-4, eukaryotic translation initiation factor 4γ2, fibrinogen β-chain, and specific fragments of collagen, 1 peptide derived from collagen α-1(I) and another derived from collagen α-2(V), and collagen α-1(III) fragment decreased.	0.83–0.88 ROC AUC = 0.88 PPV = NA NPV = NA	Yes	[43]
ABLE	Day 100	Pediatric	Polyomic (immune phenotyping, cytokines, metabolome, clinical)	ROC AUC = 0.80 PPV = 0.75 NPV = 0.74	No	Unpublished
	Day 100	Adult	ST2, CXCL9, matrix metalloproteinase 3, and osteopontin	ROC AUC = 0.65–0.69 PPV = 0.22–0.32 NPV = 0.88–0.92	Yes	[44]

AUC, area under the curve; NA, not available; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operator curve.

In 2012, the ABLE Team established a pediatric cGvHD biomarker study network consisting of 1 European, 6 Canadian, and 20 US HCT centers. The initial ABLE 1.0 study enrolled 302 children with more than a 1000 highly annotated samples; a subsequent validation pediatric study, ABLE 2.0/PTCTC 1901, opened in November 2020 with the goal of accruing another 350 children. The ABLE studies evaluated peripheral blood immune cell markers, clinical factors, and plasma samples after HCT and linked the results with thoroughly adjudicated NIH cGvHD consensus criteria (NIH-CC). The biomarker categories included clinical HCT parameters, cell populations, cytokines/chemokines, and metabolomics, all corrected to post-HCT controls at 3, 6, and 12 months to account for the influence of immune reconstitution on biomarker interpretation. Firstly, the ABLE study identified a significant number of late aGvHD cases after day 100 in addition to 10% of cGvHD cases occurring before day 100.[24] Second, based on day 100 analyses, we identified cytokine, chemokine, and cell populations patterns associated with development of cGvHD after day 114 and that late aGvHD had a distinctly different pattern than cGvHD.[29] Last, we found that there were distinct metabolomic patterns at day 100 associated with the later development of cGvHD.[28] Recently, we were able to apply a machine learning approach based on a polyomic evaluation of this population that included a broad analysis of immune cell populations, cytokines, chemokines, metabolites, and clinical factors to achieve a diagnostic algorithm with a receiver operator curve of 0.89.[31] We have now taken that same approach to develop a day 100–based risk assignment algorithm to predict the later development of cGvHD after day 114 (unpublished data). This risk assignment algorithm is being validated in the multicenter open pediatric ABLE 2.0 study (N = 350) and open adult ABLE 3.0 study (N = 320). In addition, since 10% of cGvHD cases occur before day 100, we are also attempting to modify the study to a day 60 algorithm. Because late aGvHD appears to be biologically different from cGvHD,[29] it will most likely require a separate risk assignment algorithm. Furthermore, it is necessary to account for atypical presentations of cGvHD that do not meet current NIH diagnostic criteria.[32] To date, we have not been able to identify a difference in the biomarker patterns between atypical cGvHD and cGvHD that meets current NIH diagnostic criteria,[30] but this still requires confirmation before they need to be considered separately. We have been able to identify at least 2 cGvHD biology patterns and a pattern associated with the development of immunotolerance after HCT.[33,34] It is further possible that certain biological patterns may emerge that are uniquely associated with organ-specific manifestations of cGvHD, ideally enabling more targeted organ-specific therapy.

Risk assignment in the future may also be performed using clinical algorithms and other nonimmune assays. A cGvHD risk algorithm was developed based on clinical indicators that can predict GvHD-free relapse-free survival[35] as well as another for mortality.[36] Pulmonary testing using pulmonary function testing, multiple breath washout, and xenon magnetic resonance imaging may also be used to assign risk, but none are validated at present for this application.[37,38] Stool microbiome analysis may also be useful in the future as well.[39,40]

The way forward for preemptive trial design

One of the potential issues with all-inclusive prophylactic therapy trials in GvHD is that a significant proportion of patients are unnecessarily exposed to an immunosuppressant agent because they are at low risk for developing GvHD. Preemption of aGvHD is an attractive therapeutic strategy as it limits intensified immunosuppression to patients most likely to benefit from such an approach and avoids potential toxicity in patients likely to do well with standard approaches. Several biomarkers are being developed to predict severe aGvHD and mortality, but clinical trials have yet to show a benefit from preemptive treatment. The role of preemptive therapy for cGvHD could be based on applying interventions that were previously successful in prophylactic trials, but the benefit of a preemptive trial is that only patients with the highest risk would receive the intervention. Prophylactic approaches such as posttransplant cyclophosphamide, ATG, alemtuzumab, and ex vivo or in vivo depletion of pathogenic T-cell populations have primarily been used in the peritransplant setting and could not be used in the

post-HCT setting. One successful prophylactic trial using rituximab showed an ability to decrease cGvHD.[41] There is some suggestion that prolonged administration of abatacept may also decrease cGvHD. More likely, a preemptive trial will incorporate established therapeutic drugs such as ruxolitinib, ibrutinib, or belumosudil in high-risk patients identified by a risk assignment algorithm as early as day 60 post-HCT. Conversely, the identification of low-risk patients for development of acute or chronic GvHD may allow us to minimize the toxicity of GvHD prophylaxis; this is especially relevant for nonmalignant conditions that traditionally require prolonged GvHD prophylaxis post-HCT. Last, it would allow optimal development of a GvL effect for patients with malignant conditions. Barriers to preemption include the need for more sensitive and specific diagnostic tests than currently available, the costs associated with biomarker assays, and the lack of an identified agent that can alter the outcome in high-risk patients after they have been identified. Future research in this area remains a critical need.

Conflict-of-interest disclosure

Jacob Rozmus: no competing financial interests to declare.
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Off-label drug use

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Novel approaches to acute graft-versus-host disease prevention

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The field of graft-versus-host disease (GvHD) has experienced significant growth, with increased number of clinical trials and the approval of several agents by the US Food and Drug Administration for both acute and chronic GvHD treatment. In addition, the development of prognostic biomarker algorithms has enabled risk stratification in acute GvHD. However, prevention remains the cornerstone of GvHD management. Notable recent changes include the expansion of donor options with the increased use of haploidentical donor and unrelated donor transplantation, the development of ex vivo selective T-cell depletion strategies, recent approval by the Food and Drug Administration of abatacept for GvHD prevention, and the application of posttransplant cyclophosphamide in matched and mismatched donor settings. In this article, we review the results of recent clinical trials in GvHD prophylaxis and discuss the changes in clinical practice and promising emerging strategies driving the field forward.

LEARNING OBJECTIVES

- The field of graft-versus-host disease prophylaxis is changing, with expanded options and effective approaches in matched and alternate donor transplantation
- The choice of graft-versus-host disease prophylaxis should consider factors including donor availability, primary disease characteristics, and comorbidities

Introduction

The landscape of graft-versus-host disease (GvHD) prevention is undergoing significant changes from the standard previously accepted calcineurin inhibitor and methotrexate regimens. Posttransplant cyclophosphamide (PTCy) and the incorporation of novel agents have gained broad use across various donor types. Innovative graft manipulation techniques beyond $CD34^+$ selection and $CD3^+$ depletion now allow for GvHD prevention with more robust immune reconstitution. Furthermore, there has been an increase in haploidentical transplants and matched unrelated donor (MUD) options.[1] This expansion of donor options, coupled with the introduction of new regimens for disease control and advancements in supportive care, has allowed a greater number of patients to undergo hematopoietic stem cell transplantation (HCT). This review primarily focuses on the evolving landscape of acute GvHD (aGvHD) prevention while acknowledging the vital role of donor selection in minimizing GvHD risk.[2]

CLINICAL CASE 1

A 61-year-old man with $FLT3^+$ acute myeloid leukemia in complete remission is referred for HCT. He has no matched sibling donor options. There are several matched unrelated donors available. He underwent HCT with fludarabine and melphalan reduced intensity conditioning and PTCy, tacrolimus, and mycophenolate mofetil with a 10/10 matched peripheral blood stem cell (PBSC) graft. He did not develop acute GvHD and developed mild oral chronic GvHD at 8 months posttransplant, which was treated with topical steroids. He developed recurrent cytomegalovirus reactivation requiring antiviral therapy between days 60 and 150 posttransplant. At 1 year posttransplant, he remains in remission with no evidence of GvHD.

Posttransplant cyclophosphamide

The use of PTCy, paired with mycophenolate mofetil (MMF) and tacrolimus, has broadly expanded access to

haploidentical donor grafts. In nonmyeloablative and reduced intensity conditioning (RIC) HCT, low rates of grade 3 to 4 aGvHD (7%-9%) and chronic GvHD (cGvHD; 26%-27%) have recently been observed with relapse rates ranging from 42% to 48%.[3,4] Myeloablative conditioning (MAC) approaches have also been used with similarly low GvHD rates.[5-7] In a prospective multicenter trial in children undergoing MAC haploidentical HCT with bone marrow grafts for hematologic malignancies, no grade 3 to 4 aGvHD was observed, and the incidence of moderate to severe cGvHD was 4%. However, there was a high rate of graft failure (16%), possibly linked to lower cell doses.[8]

Given the success in haploidentical donor transplants, PTCy has been examined in matched and mismatched donor HCT (Table 1). The Blood and Marrow Transplant Clinical Trials Network conducted a phase 2 randomized study, using RIC and incorporating 3 arms for GvHD prophylaxis including PTCy, with each arm compared to a contemporaneous control cohort using tacrolimus/methotrexate (Tac/MTX) prophylaxis.[9] Results for the PTCy arm were superior, and thus the approach was tested in the phase 3 study (BMTCTN 1703) using RIC for hematologic malignancies. Most patients received a matched related or unrelated PBSC graft.[10] Patients were randomized to receive either PTCy/MMF/tacrolimus or Tac/MTX GvHD prophylaxis. The PTCy arm had significantly lower grade 3 to 4 aGvHD (6% vs 15%) and 1-year cGvHD (22% vs 35%). The primary end point, GvHD (including grade 3-4 aGvHD and cGvHD requiring systemic immune suppression) and relapse-free survival at 1 year, was significantly improved with PTCy (53% vs 35%). Of note, lymphocyte count >1000 by 1 year was lower in the PTCy arm, with a significantly increased incidence of infections compared to Tac/MTX, consistent with previous studies showing delayed T-cell immune reconstitution[11] and increased viral reactivations.[12] While increased infections were observed with PTCy, the difference was driven by grade 2 and not grade 3 infections. In a phase 3 trial using MAC for hematologic malignancies with matched related or unrelated donors, patients were randomized to CD34$^+$ selected PBSC graft, PTCy with bone marrow graft, and Tac/MTX with bone marrow graft as options for GvHD prevention (CTN 1301).[13] Rates of severe aGvHD and cGvHD in with PTCy were 10% and 27%, respectively, comparable to the control arm. In a phase 2 single-center trial in adults and pediatrics, with PTCy/MMF/tacrolimus prophylaxis, patients underwent total body irradiation–based MAC, and the rate of severe aGvHD and cGvHD requiring systemic treatment were low at 6% and 6%, respectively. However, the overall rate of relapse was 39% (and higher in children).[14]

A phase 2 study also recently evaluated PTCy in mismatched unrelated donor (MMUD) transplant (including <7/8 human leukocyte antigen [HLA] matched donors) where it was added to sirolimus and MMF in patients receiving MAC or RIC. Grade 2 to 4 and 3 to 4 aGvHD was 47.5% and 20% in the MAC setting and 35% and 2.5% in the RIC, respectively. One-year cGvHD incidence was 36% and 18% in MACs and RICs, respectively.[15] The follow-up study (NCT04904588) in MMUD transplant (≤7/8 HLA matched) examines this approach in adults receiving PBSC graft following MAC, adults receiving a PBSC graft following a nonmyeloablative or RIC regimen, and children receiving bone marrow grafts following a MAC regimen.

Alternate donor and GvHD prophylaxis considerations

In the absence of a matched donor, there are 2 primary options, MMUD or haploidentical donors. The choice between the options must be considered within the context of planned GvHD prophylaxis and should take into consideration other donor and recipient characteristics, an overall assessment of GvHD and disease relapse risks, and center resources and experience, including the capabilities for graft selection, and the availability of clinical trials.

A haploidentical donor with PTCy is an option that is favored by many centers and is associated with low rates of GvHD but has been associated with delayed immune reconstitution and increased infection risk. Abatacept was recently approved by the US Food and Drug Administration (FDA) for GvHD prevention in MUD and MMUD HCT and is associated with low rates of severe aGvHD, robust immune reconstitution, and engraftment but is not effective in preventing cGvHD. Antithymocyte globulin (ATG) has been a commonly used approach in the prevention for GvHD but appears to have a greater impact on cGvHD than aGvHD (Table 1). Graft manipulation is an option and is associated with the low rates of GvHD, but few centers have the expertise or resources needed. There are also several emerging strategies that have shown promise in GvHD prevention offered in the context of a clinical trial.

CLINICAL CASE 2

A 17-year-old girl with acute myeloid leukemia and end of induction minimal residual disease (MRD) positive is referred for HCT. Her mother is 44 years old and is in good health. She has no MUD options, but several 9/10 MMUDs <35 years of age. She has good organ function and has tolerated therapy well. Disease evaluation was positive for 0.01% MRD prior to HCT. The patient underwent busulfan and fludarabine myeloablative conditioning and GvHD prophylaxis with tacrolimus, methotrexate, and abatacept and a 9/10 HLA-matched unrelated bone marrow graft. She developed stage 1 skin aGvHD treated with topical triamcinolone. She was diagnosed with moderate cGvHD of the skin at 8 months post-HCT and required systemic therapy with sirolimus and short-course prednisone. She is 1 year post-HCT in MRD-negative remission, GvHD is quiescent, and she remains on sirolimus.

Abatacept

T-cell costimulation pathways play an integral role in T-cell activation and the GvHD response. Abatacept blocks the CD28-CD80/86 interaction and prevents T-cell activation. The efficacy of abatacept for prevention of severe aGvHD was tested in a phase 2 multicenter trial in MUD and MMUD transplantation. In MMUD HCT, the addition of abatacept to calcineurin inhibitor and methotrexate in patients with hematologic malignancies receiving MAC or RIC conditioning was effective in preventing severe aGvHD (with a low observed incidence of 2%) and improved day 180 severe aGvHD-free survival of 98%, both significantly lower than control cohorts receiving calcineurin inhibitor/methotrexate alone or with ATG.[16] Day 180 severe aGvHD-free survival was significantly better in MUD HCT with abatacept compared to placebo (93% vs 82%), with lower incidence of severe aGvHD in

Table 1. Representative GvHD prophylaxis trials using posttransplant cyclophosphamide, abatacept, and ATG in unrelated donor transplantation

Trial/Reference	Study design	GvHD prophylaxis	Acute GvHD	Chronic GvHD	Relapse	Graft failure	Survival
Phase II Trial of Costimulation Blockade With Abatacept for Prevention of Acute GVHD (ABA2) Ref.[16]	Phase: 2 N: MUD (142), MMUD (43) Recipient Age: >6 years Donor: MUD, MMUD Graft Source: PBSC, BM Conditioning: MAC or RIC	CNI/MTX Randomized± abatacept (MUD) CNI/MTX/ abatacept (MMUD)	MUD Grade 2–4: 43% Grade 3–4: 7% MUD Control Grade 2–4: 62% Grade 3–4: 15% MMUD Grade 2–4: 40% Grade 3–4: 2%	MUD Mild-Sev: 52% Mod-Sev: 45%, MUD control Mild-Sev: 45% Mod-Sev: 36% MMUD Mild-Sev: 22% Mod-Sev: 58%	2-y Relapse: MUD 22% MMUD 9% MUD control 24%	Secondary graft failure N=1 (MUD)	MUD 2-y NRM: 13% 2-y EFS: 66% 2-y OS: 74% MUD control 2-y NRM: 16% 2-y EFS: 60% 2-y OS: 64% MMUD 2-y NRM: 17% 2-y EFS: 74% 2-y OS: 74%
Addition of Anti-Thymocyte Globulin to Standard Graft-Versus-Host Disease prophylaxis Versus Standard Treatment Alone in Patients With Haematological Malignancies Undergoing Transplantation From Unrelated Donors: Final Analysis of a Randomized, Open-Label, Multicentre, Phase 3 Trial Ref.[33]	Phase: 3 N: 203 Recipient Age: 16–70y Donor: MUD, MMUD Graft Source: PBSC, BM Conditioning: MAC or RIC	CNI/MTX Or CNI/MMF + Randomized±ATG	ATG Grade 2–4: NA Grade 3–4: 28% Control Grade 2–4: NA Grade 3–4: 28%	ATG Mild-Sev: 26% Mod-Sev: NA Control Mild-Sev: 40% Mod-Sev: NA	2-y Relapse: ATG 16% Control 18%	NA	ATG 2-y NRM: 21% 2-y EFS: NA 2-y OS: 71% Control 2-y NRM: 31% 2-y EFS: NA 2-y OS: 53%
Post-Transplantation Cyclophosphamide-Based Graft-Versus-Host Disease Prophylaxis (CTN 1703) Ref.[10]	Phase: 3 N: 431 Recipient Age: >18y Donor: MSD, MUD, MMUD Graft Source: PBSC Conditioning: RIC	PTCy/Tac/MMF Or Tac/MTX	PTCy/Tac/MMF Grade 2–4: 54% Grade 3–4: 6% Tac/MTX Grade 2–4: 52% Grade 3–4: 15%	PTCy/Tac/MMF Mild-Sev: 22% Mod-Sev: NA Tac/MTX Mild-Sev: 35% Mod-Sev: NA	PTCy/Tac/MMF 1-y Relapse: 21% Tac/MTX 1-y Relapse: 20%	PTCy/Tac/MMF 3% Tac/MTX	PTCy/Tac/MMF 1-y NRM: 12% 1-y EFS: 67% 1-y OS: 77% Tac/MTX 1-y NRM: 17% 1-y EFS: 62% 1-y OS: 72%
National Marrow Donor Program-Sponsored Multicenter, Phase II Trial of HLA-Mismatched Unrelated Donor Bone Marrow Transplantation Using Post-Transplant Cyclophosphamide (15-MMUD) Ref.[15]	Phase: 2 N: 40 MAC, 40 RIC Recipient Age: 18–70 Donor: MMUD Graft Source: BM Conditioning: MAC or RIC	PTCy/Sirolimus/ MMF	Grade 2–4: 48% MAC, 35% RIC Grade 3–4: 20% MAC, 3% RIC	Mild-Sev: 36% MAC, 18% RIC Mod-Sev: NA	1-y Relapse: 30% MAC, 23% RIC	0% MAC, 8% RIC	1-y NRM: 8% MAC, 10% RIC 1-y EFS: 62% MAC, 68% RIC 1-y OS: 72% MAC, 79% RIC
Three Prophylaxis Regimens (Tacrolimus, Mycophenolate Mofetil, and Cyclophosphamide; Tacrolimus, Methotrexate, and Bortezomib; or Tacrolimus, Methotrexate, and Maraviroc) Versus Tacrolimus and Methotrexate for Prevention of Graft-Versus-Host Disease With Haemopoietic Cell Transplantation With Reduced-Intensity Conditioning: A Randomised Phase 2 Trial With a Non-Randomised Contemporaneous Control Group (BMT CTN 1203) Ref.[9]	Phase: 2 N: 273 (92 PTCy) Recipient Age: 18–75y Donor: MSD, MUD, MMUD Graft Source: PBSC Conditioning: RIC	PTCy/Tac/MMF Or Tac/MTX/ Bortezomib Or Tac/MTX/ maraviroc	PTCy/Tac/MMF Grade 2–4: 27% Grade 3–4:2% Tac/MTX Grade 2–4: 30% Grade 3–4: 13%	PTCy/Tac/MMF Mild-Sev: 28% Mod-Sev: NA Tac/MTX Mild-Sev: 38% Mod-Sev: NA	PTCy/Tac/MMF 1-y Relapse: 28% Tac/MTX 1-y Relapse: 25%	PTCy/Tac/MMF 4% Tac/MTX 0	PTCy/Tac/MMF 1-y NRM: 11% 1-y EFS: 60% 1-y OS: 71% Tac/MTX 1-y NRM: 16% 1-y EFS: 56% 1-y OS: 71%

Table 1. Representative GvHD prophylaxis trials using posttransplant cyclophosphamide, abatacept, and ATG in unrelated donor transplantation (*Continued*)

Trial/Reference	Study design	GvHD prophylaxis	Acute GvHD	Chronic GvHD	Relapse	Graft failure	Survival
Randomized Phase 3 BMT CTN Trial of Calcineurin Inhibitor–Free Chronic Graft-Versus-Host Disease Interventions in Myeloablative Hematopoietic Cell Transplantation for Hematologic Malignancies (CTN 1301) Ref.[13]	Phase: 3 N: 346 Recipient Age: <65y Donor: MSD, MUD Graft Source: BM, PBSC (CD34⁺ selection) Conditioning: MAC	Tac/MTX or PTCy Or none (CD34⁺ selection)	Tax/MTX Grade 2–4: 30% Grade 3–4:4% PTCy Grade 2–4: 38% Grade 3–4: 10% CD34⁺ selection Grade 2–4: 16% Grade 3–4: 3%	Tax/MTX Mild-Sev: NA Mod-Sev: 34% PTCy Mild-Sev: NA Mod-Sev: 27% CD34⁺ selection Mild-Sev: NA Mod-Sev: 9%	Tax/MTX 2-y Relapse: 26% PTCy 2-y Relapse: 14% CD34⁺ selection 2-y Relapse: 21%	Secondary graft failure Tax/MTX 1% PTCy 0% CD34⁺ selection 3%	Tax/MTX 2-y NRM: 8% 2-y EFS: 67% 2-y OS: 76% PTCy 2-y NRM: 16% 2-y EFS: 70% 2-y OS: 76% CD34⁺ selection 2-y NRM: 22% 1-y EFS: 57% 2-y OS: 60%
Phase II Study of Myeloablative 7–8/8-Matched Allotransplantation With Post-Transplantation Cyclophosphamide, Tacrolimus, and Mycophenolate Mofetil Ref.[14]	Phase 2 N: 125 Recipient Age: median 39y Donor: MUD, MMUD Graft Source: BM, PBSC Conditioning: MAC	PTCy/Tac/MMF	Grade 2–4: 17% Grade 3–4: 6%	Mild-Sev: NA Requiring IST: 6%	2-y Relapse: 39%	NA	2-y NRM: 10% 2-y EFS: 2-y OS: 74%

BM, bone marrow; CNI, calcineurin inhibitor; EFS, event-free survival; IST, immunosuppressive treatment; Mod, moderate; MSD, matched sibling donor; NA, not available; NRM, nonrelapse mortality; OS, overall survival; Sev, severe.

patients receiving abatacept (15% vs 7%). The additional immune suppression did not result in increased relapse or severe infections or delay in immune reconstitution. The abatacept phase 2 study findings were validated in real-world analyses, comparing abatacept to calcineurin inhibitor/MTX in MUD and in MMUD.[17,18] The results for MMUD transplantation using abatacept for prophylaxis were similar to MUD using standard calcineurin inhibitor and methotrexate (compared among trial arms and in real-world analysis) in all survival outcomes, including nonrelapse mortality, overall survival, relapse-free survival, and GvHD-free survival.[18,19] Studies incorporating abatacept in GvHD prophylaxis for nonmalignant hematologic diseases were recently reported, with similarly promising results.[20,21] The 4-dose regimen of abatacept did not effectively prevent the development of cGvHD, and ongoing trials (NCT03924401, NCT04380740) are examining the use of extended-dose abatacept for this purpose.

Ex vivo graft manipulation

The 3- to 4-log reduction in T cells through ex vivo CD34⁺ cell enrichment effectively prevents GvHD in related and unrelated donor transplantation. Earlier approaches with CD34⁺ selection or CD3⁺ depletion were hampered by increased infections and increased nonrelapse mortality. In BMTCTN1301, moderate to severe cGvHD-free, relapse-free survival was 50% for CD34⁺ selection, 48% for PTCy, and 41% for Tac/MTX. While CD34⁺ selection had lower rates of GvHD, nonrelapse mortality was inferior, attributed to increased infection and organ failure.[13]

Selective ex vivo depletion of specific T-cell populations has been investigated as a strategy for preventing GvHD while improving immune reconstitution (Table 2).[22-28] Alpha beta T-cell receptor depletion can protect against GvHD, while retaining gamma delta T cells and natural killer cells, resulting in a low incidence of GvHD. ATG is usually included in the conditioning regimen. The approach is combined with CD19⁺ depletion, which may further prevent GvHD and also protect against posttransplant lymphoproliferative disorder. In a recent study in children and adults,[24] the incidence of aGvHD was 10%, and moderate to severe cGvHD was 21%, with findings reproduced in multicenter experiences.[29] Alternatively, naive T-cell (CD45RA⁺) depletion offers another approach that selectively removes naive T cells that contribute to GvHD development while retaining memory T cells, which enhance antiviral immunity. In a single-center study, CD45RA⁺ depletion combined with CD34⁺ selection in adults with hematologic malignancies had low severe acute and chronic GvHD (4% and 7%, respectively) and rapid T-cell recovery, with similar results for patients receiving matched sibling and MUD grafts.[25] The generalizability of these techniques is limited by the required resources, including cost, equipment, staff training, and regulatory burden.[26] In addition, in adults, cell dose constraints often require an additional CD34⁺ selected graft infusion, further adding to the donor burden and center resource requirements. The success of ex vivo selective T-cell depletion has been the basis for further donor graft engineering, with the aim to enrich immunoregulatory populations. A recent study used a combined approach of CD34⁺ selected cell infusion with a high-purity Treg infusion, followed by a conventional T-cell infusion with set Treg/Tcon ratios (Orca-T) in MRD and MUD. The results from the single-institution and multi-institution studies demonstrate low risk of both severe aGvHD and moderate to severe cGvHD at 5% and 6%, respectively.[30,31]

Table 2. Representative graft manipulation trials for GvHD prophylaxis in patients undergoing stem cell transplantation for hematologic malignancies

Approach	Study design	GvHD prophylaxis	Acute GvHD	Chronic GvHD	Relapse	Graft failure	Survival
Tn-depleted PBSC + CD34⁺-selected PBSC Ref.[25]	Phase: 2 N: 138 Recipient Age: 1–60y Donor: MRD, MUD Conditioning: MAC	Tac Tac/MTX Tac/MMF	Grade 2: 71% Grade 3–4: 4%	Mild-Sev:6% Mod-Sev:1%	3-y Relapse: 23%	Primary graft failure: 0 Secondary graft failure: N=2	3-y NRM: 8% 3-y EFS: 69% 3-y OS: 77%
TCRab-depleted/ CD19-depleted PBSC Ref.[27]	Phase: 2 N: 60 Recipient Age: >20y Donor: Haplo Conditioning: MAC	CSA	Grade 2–4: 34% Grade 3–4: N=18	Mild-Sev: 25% Mod-Sev: N=12	Median follow-up 28 months Relapse 27%	Primary graft failure N=4	Median follow-up 28 months NRM: 23% EFS: 52% OS: NA
ORCA-T Manufactured cellular therapy with stem cells and Tregs Ref.[31]	Phase: 1b, 2 N: 127 Recipient Age: 19–69y Donor: MRD, MUD Conditioning: MAC	Tac or sirolimus	Grade 2–4: NA Grade 3–4: 5%	Mild-Sev: NA Mod-Sev: 6%	Relapse: NA	Primary graft failure 2%	1-y NRM:5% 1.5-y EFS: 81% 1.5-y OS: 86%
CD34⁺-selected PBSC graft + Tn-depleted PBSC graft Ref.[23]	Phase: NA N: 25 Recipient Age: 2–17y Donor: Haplo Conditioning: MAC	CSA=3 CSA/MTX=1 MMF=21	Grade 2–4: 39% Grade 3–4: 33%	Mild-Sev: 22% Mod-Sev: N=2	2.5-y Relapse: 17%	Primary graft failure: 0 Secondary graft failure: N=2	2.5-y NRM: 22% EFS: NA 2.5-y OS: 58%
TCRab-depleted PBSC graft Ref.[28]	Phase: 1/2 N: 35 Recipient Age: 19–69y Donor: MRD, MUD, MMUD Conditioning: MAC	ATG MMF	Grade 2–4: 26% Grade 3–4: 14%	Mild-Sev: 23% Mod-Sev: 17%	2-y Relapse: 29%	Primary graft failure: 0 Secondary graft failure: N=1	2-y NRM: 32% 2-y EFS: 40% 2-y OS: 54%
TCRab-depleted/ CD19-depleted PBSC graft Ref. [22]	Phase: NA N: 60 Recipient Age: 1–23y Donor: MUD, MMUD Conditioning: MAC	ATG=22	Grade 2–4: NA Grade 3–4: 13%	Mild-Sev: 26% Mod-Sev: NA (Extensive 11%)	Median follow-up 3.1y Relapse: 21%	Primary graft failure: 1	3-y NRM: 15% 4-y EFS: 64% 4-y OS: 69%
TCRab-depleted/ CD19-depleted PBSC graft Ref.[24]	Phase: 1/2 N: 60 Recipient Age: 1–63y Donor: Haplo Conditioning: RIC	MMF±ATG	Grade 2–4: 10% Grade 3–4: 0%	Mild-Sev: 31% Mod-Sev: 21%	2-y Relapse: 21%	Primary graft failure: 9	2-y NRM: 17% 2-y EFS: 50% 2-y OS: 63%

CSA, cyclosporine; haplo, haploidentical; Tn, naive T cells; TCRab, αβ + T cells; Tregs, regulatory T cells.

Antithymocyte globulin

ATG results in antibody-mediated destruction of T cells and has been widely used for the prevention of GvHD when combined with a calcineurin inhibitor and methotrexate or MMF. There are multiple preparations of ATG (horse and rabbit derived) with differing degrees of lymphodepletion and half-life, with rabbit ATG being the most commonly used for GvHD prevention.[32] A recent randomized phase 3 trial of adults receiving MUD or MMUD transplant with rabbit ATG, calcineurin inhibitor, and methotrexate or MMF showed no difference in grade 3 to 4 aGvHD, but a significant improvement was observed in cGvHD (26% with ATG vs 41% with standard prophylaxis). There was no difference in relapse and nonrelapse mortality at 16% vs 17% and 21% vs 31% in the ATG and standard prophylaxis groups, respectively.[33] A retrospective analysis from the European Society for Blood and Marrow Transplantation registry comparing ATG with PTCy in haploidentical transplant did not show a difference in GvHD, but the ATG group had an increased risk of relapse and lower leukemia-free survival and overall survival compared to ATG.[34] Alemtuzumab (humanized anti-CD52 antibody) also results in antibody-mediated destruction of lymphocytes and has been shown to be effective in the prevention of acute and chronic GvHD.[35]

Emerging strategies and ongoing trials

New approaches to the prevention of aGvHD have recently shown promise, with several ongoing trials (Table 3). These trials encompass repurposing of drugs from other diseases and indications, including FDA-approved agents such as sitagliptin and alpha-1 antitrypsin, the extension of drugs with efficacy in GvHD treatment to prevention such as ruxolitinib, and testing of novel combinations to optimize the prevention of both acute and chronic GvHD such as combinations of abatacept and PTCy.

Table 3. Ongoing phase 2/3 clinical trials for GvHD prophylaxis

Trial	Agents/approach	Study design	Study population
A Randomized Double-Blind Trial of Abatacept Extended Dosing Versus Abatacept Short-Term Dosing for GVHD Prophylaxis (ABA3) NCT04380740	**Abatacept**, CNI/MTX Extending dosing of abatacept for GvHD prevention	Phase: 2 N: 160	Disease: Malignant Recipient Age: >2y Donor: MUD/MMUD Graft Source: PBSC or BM Conditioning: MAC/RIC
Acute GVHD Suppression Using Costimulation Blockade to Expand Non-malignant Transplant (ASCENT) NCT03924401	**Abatacept**, CNI/MTX Extended dosing of abatacept for GvHD prevention	Phase: 2 N: 30	Disease: Nonmalignant Recipient Age: 0–20y Donor: MUD/MMUD Graft Source: PBSC or BM Conditioning: MAC/RIC
Cyclophosphamide, Abatacept, and Tacrolimus for the Prevention of GVHD NCT05621759	**PTCy, Abatacept**, short-course tacrolimus	Phase: 2 N: 92	Disease: Malignant Recipient Age: >18y Donor: Haploidentical Graft Source: PBSC Conditioning: MAC/RIC
Optimizing PTCy Dose and Timing NCT03983850	**PTCy**, Sirolimus, MMF Reduced-dose PTCy	Phase: 1/2 N: 400	Disease: Malignant Recipient Age: >12y Donor: Haploidentical Graft Source: BM or PBSC Conditioning: MAC
HLA-Mismatched Unrelated Donor Hematopoietic Cell Transplantation With Post-Transplantation Cyclophosphamide (ACCESS) NCT04904588	**PTCy**, Tacrolimus, MMF	Phase: 2 N: 300	Disease: Malignant Recipient Age: >1y Donor: MMUD Graft Source: PBSC, BM Conditioning: MAC, RIC, NMA
A Randomized Pilot Trial Comparing Anti-Thymocyte Globulin (ATG) With ATG Plus Post Transplant Cyclophosphamide (PTCy) for Prophylaxis Against Acute and Chronic Graft Versus Host Disease NCT04202835	**ATG ± PTCy**	Phase 2 N: 80	Disease: Malignant Recipient Age: 16–70y Donor: MRD, MUD Graft Source: PBSC Conditioning: MAC, RIC
A Phase II Pediatric Study of GVHD Prophylaxis Regimen With No Calcineurin Inhibitors After Day +60 Post First Allogeneic Hematopoietic Cell Transplant for Hematological Malignancies NCT05579769	CNI/MTX, **Ruxolitinib** ± ATG	Phase: 2 N: 32	Disease: Malignant Recipient Age: >12y Donor: MRD, MUD Graft Source: BM Conditioning: MAC
Bendamustine With or Without Cyclophosphamide in Preventing GVHD in Patients Undergoing Stem Cell Transplant NCT04022239	**Bendamustine**, Tac, MMF,±PTCy Posttransplant bendamustine	Phase: 1/2 N: 40	Disease: Malignant Recipient Age: 18–70y Donor: MMUD, Haploidentical Graft Source: NA Conditioning: RIC
Tocilizumab for the Prevention of Graft Failure and GVHD in Haplo-Cord Transplantation NCT04395222	**Tocilizumab**,±ATG Tocilizumab day −1	Phase: 2 N: 70	Disease: Malignant Recipient Age: >18y Donor: Haplo Graft Source: Haplo NA + UCB Conditioning: RIC
Haplo-identical Transplantation for Severe Aplastic Anemia, Hypo-plastic MDS and PNH Using Peripheral Blood Stem Cells and Post-transplant Cyclophosphamide for GVHD Prophylaxis NCT03520647	**PTCy**	Phase: 2 N: 56	Disease: Nonmalignant Recipient Age: 4–75y Donor: Haploidentical Graft Source: PBSC Conditioning: NA
The Safety and Efficacy of Alpha-1 Antitrypsin (AAT) for the Prevention of Graft-Versus-host Disease (GVHD) in Patients Receiving Hematopoietic Cell Transplant (MODULAATE) NCT03805789	**Alpha-1 Antitrypsin**	Phase: 2/3 N: 310	Disease: Malignant Recipient Age: >12y Donor: MUD, MMUD Graft Source: PBSC and BM Conditioning: MAC
Comparison of Triple GVHD Prophylaxis Regimens for Nonmyeloablative or Reduced Intensity Conditioning Unrelated Mobilized Stem Cell Transplantation NCT03246906	CNI, sirolimus±MMF,±**PTCy**	Phase: 2 N: 160	Disease: Malignant Recipient Age: >18y Donor: MUD, MMUD Graft Source: PBSC Conditioning: NMA, RIC

Table 3. Ongoing phase 2/3 clinical trials for GvHD prophylaxis (*Continued*)

Trial	Agents/approach	Study design	Study population
GvHD Prophylaxis in Unrelated Donor HCT: Randomized Trial Comparing PTCY Versus ATG (GRAPPA) NCT05153226	**PTCy** Or **ATG**	Phase: 3 N: 540	Disease: Malignant Recipient Age: >18y Donor: Haploidentical Graft Source: PBSC Conditioning: NA
High-Dose Post-Transplant Cyclophosphamide, Bortezomib and Abatacept for the Prevention of Graft-versus-Host-Disease (GvHD) Following Allogenic Hematopoietic Stem Cell Transplantation (HSCT) Study NCT05289167	**PTCy, Bortezomib, Abatacept**±ATG	Phase: 1/2 N: 74	Disease: Malignant Recipient Age: >18y Donor: MRD, MUD, MMUD Graft Source: PBSC Conditioning: NA
Tildrakizumab for Prevention of Acute Graft-Versus-Host Disease NCT04112810	**Tildrakizumab** (anti–IL-23 antibody), CNI/MTX	Phase: 2 N: 55	Disease: Malignant Recipient Age: >18y Donor: MRD, MUD Graft Source: PBSC Conditioning: MAC
Ustekinumab for the Prevention of Acute Graft-Versus-Host Disease After Unrelated Donor Hematopoietic Cell Transplant NCT04572815	**Ustekinumab**	Phase: 2 N: 116	Disease: Malignant Recipient Age: 18–70y Donor: MRD, MUD Graft Source: PBSC Conditioning: MAC, RIC
Adding Itacitinib to Cyclophosphamide and Tacrolimus for the Prevention of Graft Versus Host Disease in Patients Undergoing Hematopoietic Stem Cell Transplants NCT05364762	**Itacitinib, PTCy**, and Tacrolimus	Phase: 2 N: 50	Disease: Malignant Recipient Age: 0–80y Donor: MRD, MUD Graft Source: PBSC Conditioning: RIC
The Lowest Effective Dose of Post-Transplantation Cyclophosphamide in Combination With Sirolimus and Mycophenolate Mofetil as Graft-Versus-Host Disease Prophylaxis After Reduced Intensity Conditioning and Peripheral Blood Stem Cell Transplantation NCT05436418	**PTCy**, Sirolimus, MMF Reduced dose PTCy	Phase: 1/2 N: 220	Disease: Malignant Recipient Age: >12y Donor: Haploidentical, MRD, MUD Graft Source: PBSC Conditioning: RIC
Vorinostat for GVHD Prevention in Children, Adolescents and Young Adults Undergoing Allogeneic Blood and Marrow Transplantation NCT03842696	**Vorinostat**, CNI, MTX,±PTCy	Phase: 1/2 N: 49	Disease: Malignant Recipient Age: 3–39y Donor: Haploidentical, MRD, MUD Graft Source: PBSC, BM Conditioning: MAC/RIC
PTCy + Sirolimus/VIC-1911 as GVHD Prophylaxis in Myeloablative PBSC Transplantation NCT05120570	PTCy, sirolimus combined with **VIC-1911** (Aurora Kinase A inhibitor)	Phase: 1/2 N: 75	Disease: Malignant Recipient Age: >18y Donor: MRD, MUD Graft Source: PBSC Conditioning: MAC
High Dose Thymoglobulin Instead of Cyclosporine With a Low Dose of Thymoglobulin for GVHD Prophylaxis (ATG2017) NCT03456817	**ATG**, CSA, MTX High-dose ATG with low-dose CSA	Phase: 2 N: 200	Disease: Malignant Recipient Age: >18y Donor: MSD, MUD, MMUD Graft Source: PBSC Conditioning: MAC
Naive T Cell Depletion for Preventing Chronic Graft-Versus-Host Disease in Children and Young Adults With Blood Cancers Undergoing Donor Stem Cell Transplant NCT03779854	**Naive T-cell depletion**, CNI/MTX	Phase: 2 N: 68	Disease: Malignant Recipient Age: 6 mo to 22y Donor: MRD, MUD Graft Source: PBSC, BM Conditioning: MAC

Study agent/approach in bold.

Noncomprehensive list of actively recruiting trials extracted from ClinicalTrials.gov.

UCB, umbilical cord blood.

Table 4. Comparison of select GvHD prevention approaches in transplant for hematologic malignancies

Approach	Severe aGvHD	cGvHD	Graft failure	Relapse	Immune reconstitution/ viral infections	Optimal use
Abatacept	Decreased	No impact	No impact	No impact observed	No impact/increased reactivation without severe disease	Unrelated donor (especially mismatched)
Posttransplant cyclophosphamide	Decreased	Decreased	Possibly increased	Increased in some studies	Delayed in some studies/ increased grade 2 infections	Unrelated, haploidentical
Selective ex vivo graft manipulation	Decreased	Decreased	Increased	No impact observed	Varies by approach, enhanced related to other T-cell depletion approaches	Unrelated, haploidentical (clinical trial)

Sitagliptin is a dipeptidyl peptidase 4 inhibitor that was evaluated in a multicenter phase 2 nonrandomized trial of 36 patients receiving matched related or unrelated donors for hematologic malignancies, combined with tacrolimus and sirolimus.[36] They observed low rates of grade 2 to 4 and 3 to 4 aGvHD at 5% and 3%, respectively. The 1-year cGvHD rate was 37%. These early results are promising and should be validated in a larger cohort of patients.

Ruxolitinib is a Janus kinase 1/2 inhibitor with efficacy in GvHD treatment. An analysis of GvHD outcomes from 2 trials using ruxolitinib maintenance after transplantation in acute myeloid leukemia (NCT03286530) and starting prior to transplant and continued as maintenance for myelofibrosis (NCT03427866) showed a low incidence of grade 2 to 4 aGvHD (24%) with no grade 3 to 4 disease, and low incidence of cGvHD (21%), including only 3.8% with moderate to severe disease.[37]

Vorinostat is a histone deacetylase inhibitor that has been evaluated as GvHD prophylaxis in combination with Tac/MTX.[38] In patients receiving MAC for hematologic malignancies using MUD, vorinostat was given from day −10 through day +100. The grade 2 to 4 aGvHD rate was 22%, grade 3 to 4 was 8%, and cGvHD was 29%. The follow-up multicenter study is ongoing (NCT03842696).

Vedolizumab is a humanized monoclonal antibody that inhibits α4β7 integrin adhesion to MAdCAM-1 on gut endothelial cells. A phase 1b study evaluated vedolizumab combined with Tac/MTX for GvHD prophylaxis, reporting a 19% incidence of grade 2 to 4 aGvHD, with 5% grade 3 to 4 aGvHD. Results of the subsequent phase 3 randomized double-blind placebo-controlled study in unrelated donor HCT were recently presented.[39] The primary end point, lower gut aGvHD-free survival, was significantly improved in patients receiving vedolizumab compared to placebo (86% vs 71%), with a decrease in lower gut aGvHD (7% with vedolizumab vs 19% with placebo).

The combination of PTCy with other agents has been evaluated in single-center studies. In a phase 1b to 2 study of patients undergoing haploidentical transplant with abatacept, PTCy, and short-course tacrolimus, grade 3 to 4 aGvHD and moderate to severe cGvHD were 5.1% and 17.1%, respectively.[40] PTCy has also been combined with ATG in the MRD, unrelated donor, and haploidentical settings with encouragingly low rates of acute and chronic GvHD.[41,42] Both approaches, as well as other combinations, require further study.

Conclusions

The field of GvHD prophylaxis is rapidly changing with many approaches showing superiority to standard Tac/MTX. With the many new promising options, each carrying their own distinct risks and benefits, there is not a "one-size-fits-all" approach to GvHD prevention (Table 4).

Comparing results of clinical trials is hindered by differences in patient populations, conditioning intensity, outcomes reported, and variable follow-up. One-year end points are more feasible, but longer follow-up is imperative to assess cGvHD, relapse, and immune reconstitution. Additionally, understanding the interactions between prophylaxis regimens and donor characteristics, including degree of matching and donor age, is also important. Only by integrating all this information can we make personalized treatment decisions for patients, taking into account their donor options, disease characteristics, comorbidities, and long-term toxicities risk.

In the current era where GvHD prevention is both feasible and safe, the expectations have been raised. There is a need to continue refining existing and emerging therapies, with the aim of preventing GvHD and associated toxicities, including infections, while optimizing primary disease control and minimizing long-term morbidities.

Conflict-of-interest disclosure

Benjamin Watkins: no competing financial interests to declare.
Muna Qayed: Honoraria, Novartis, and Vertex.

Off-label drug use

Benjamin Watkins: All the drugs discussed for GVHD prophylaxis are used off-label (except Abatacept).
Muna Qayed: All the drugs discussed for GVHD prophylaxis are used off-label (except Abatacept).

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Chronic GVHD: review advances in prevention, novel endpoints, and targeted strategies

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Allogeneic hematopoietic cell transplantation (allo-HCT) is a curative therapy for many malignant and non-malignant hematologic disorders. Chronic graft-versus-host (cGVHD) disease remains a significant hurdle for long-term survival in patients post allo-HCT, and it remains the leading cause of late non-relapse mortality. The risk factors for development of cGVHD include degree of human leukocyte antigen (HLA) disparity, increasing recipient age, use of peripheral blood stem cells as a source, myeloablative conditioning regimens, prior acute GVHD (aGVHD), and female donor to male recipient. Our biological understanding of cGVHD is mostly derived from transplantation mouse models and patient data. There are three distinct phases in the development of cGVHD. Approaches to prevent GVHD include pharmacologic strategies such as calcineurin inhibitors (cyclosporine, tacrolimus) combined with methotrexate or mTOR inhibitors (sirolimus),and IMP dehydrogenase inhibitors (mycophenolate mofetil). Increasingly, posttransplant cyclophosphamide is emerging as a promising strategy for GVCHD prevention especially in a setting of reduced intensity conditioning. Other approaches include serotherapy (ATG, Campath) and graft manipulation strategies. A significant obstacle to evaluating the response of novel GVHD-directed therapies has been standardized response assessments. This has functioned as a barrier to designing and interpreting clinical trials that are structured around the treatment of cGVHD. Novel endpoints including failure-free survival, Graft-versus-host disease-free, relapse-free survival (GRFS), and current GVHD-free, relapse-free survival (CGRFS) may create a clearer picture for post-HCT outcomes. Targeted therapies including Bruton's tyrosine kinase inhibition, JAK1/2 inhibition, and ROCK2 inhibitors have improved cGVHD therapy, especially in the steroid refractory setting. Continued improvement in prophylactic strategies for cGVHD, identification of accurate cGVHD treatment endpoints, and access to novel therapeutic agents are expected to improve cGVHD outcomes.

LEARNING OBJECTIVES

- Be able to recognize FDA-approved targeted therapies in cGVHD
- Understand the basic strategy of prevention techniques of cGVHD

Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a curative therapy for many malignant and non-malignant hematologic disorders. Chronic graft-versus-host (cGVHD) disease remains a significant hurdle for long-term survival in patients post allo-HCT, and it remains the leading cause of late non-relapse mortality (NRM). The most recent data from the Center for International Blood and Marrow Transplant Research (CIBMTR) suggest that approximately 15% of post-allogeneic stem cell transplant mortality beyond day +100 is related to GVHD.[1] Patients with cGVHD also have increased morbidity, poor quality of life, and increased resource utilization, resulting in higher healthcare costs and patient dissatisfaction rates.[2,3] Despite many advances in HCT, the incidence of cGVHD is predicted to rise, given demographic changes in patients undergoing allo-HCT and the increasing use of peripheral blood stem cell grafts.[4] CIBMTR data suggest that utilization of matched unrelated donors (MUDs) is increasing with time, and a majority of HCTs in adults are now being done using peripheral blood stem cell (PBSC) grafts. Patients above the age of 65 comprise 25% of patients undergoing allo-HCT and are the fastest-growing demographic based on the recent CIBMTR database. Based on the factors described above, the incidence of chronic GVHD will continue to rise as allo-HCT becomes accessible to more patients.[5] The incidence of cGVHD is estimated to be in around 40-70% of patients after undergoing allo-HCT.[6,7] In 2016, the prevalence of cGVHD was predicted to be 14,000 patients based on the Medicare fee-for-service database. In this data set, 40% of

patients developed chronic GVHD within three years of undergoing allo-HCT, with 70% requiring second-line therapy after failing corticosteroids.[6] Chronic GVHD is a multisystem disorder involving multiple organ systems. The most involved organs include the skin, eyes, mouth, gastrointestinal (GI) tract, and liver. Approximately 40% of patients who develop cGVHD have severe disease, and 42% of patients have four or more organs involved. With improvements in supportive care practices and the use of reduced intensity conditioning (RIC) regimens in older patients, there has been a decline in NRM, but with an increase in the incidence of cGVHD as patients are surviving longer post HCT (Odds ratio [OR] 1.19, $P<0.0001$).[4] As our overall population ages and more patients have access to HCT, the incidence and burden of cGVHD will continue to rise. Hence, novel strategies are needed to prevent and treat cGVHD.[7]

The NIH consensus criteria were developed to assess the severity of cGVHD and classified into either mild (one to two organs, each with an organ score of 1), moderate (≥3 organs with a score of 1, or at least one organ with a score of 2), or severe cGVHD (at least one organ with a score of 3 or lung score of 2).[8,9] Involved organs (eyes, mouth, lungs, GI tract, liver, joints fascia, genital tract) in the 2014 NIH grading system are scored for severity (0 to 3) of GVHD manifestations. This system predicts overall survival (OS); for patients diagnosed with moderate or severe cGVHD, the OS and NRM are worse compared to patients classified with mild cGVHD.[10] The NIH classification is a significant advancement in the field because it allows uniform assessment of GVHD target organs and because response to treatment can be accurately evaluated longitudinally in patients, thereby removing interobserver bias. The widespread adoption of NIH consensus criteria has facilitated clinical trial development in cGVHD patients, resulting in the approval of three novel agents for treating patients with cGVHD.

Pathophysiology

The risk factors for the development of cGVHD include the degree of HLA disparity, increasing recipient age, use of PBSC as stem cell source, myeloablative conditioning regimens, prior aGVHD, and female donor to male recipient. Chronic GVHD is primarily thought to be a Th2-mediated T-effector cell response with a relative deficiency of regulatory T cells. Increasingly, the role of B cells, antigen-presenting cells, and macrophages is being understood in the pathogenesis of cGVHD, and therapies targeting these cell types are being tested in clinical trials. Proinflammatory cytokines such as IL-21, IL-17, TGF-β, IL-6, IL-12, IL-1, IFN gamma, TNF, and BAFF are essential mediators for graft-versus-host disease, and these mediators lead to tissue damage and, eventually, fibrosis.

Our biological understanding of cGVHD mainly derives from transplantation mouse models and patient data.[10] There are three distinct phases in the development of cGVHD. The early phase is related to acute inflammation and tissue injury. Conditioning-related tissue damage leads to the activation of donor T cells on contact with antigen-presenting cells, which then upregulate co-stimulatory molecules.[11] Epithelial damage from conditioning stimulates the release of soluble inflammatory mediators that activate antigen-presenting cells that present host antigens to donor T cells.[12] Endothelial damage reduces microvascular density due to intimal arteritis and, subsequently, fibrosis.

In the second phase of cGVHD, hallmarks are chronic inflammation and dysregulated immunity. The tissue injury from phase 1 causes the expansion of alloreactive B and T cells primed by antigen-presenting cells to proliferate into Type-1, Type-2, and Type-17 helper T cells.[13,14] This leads to increased cytokine production by CD4 positive T cells that have previously escaped immune regulation and deletion in the thymus due to thymic injury from conditioning. Within lymphoid follicles, T follicular helper cells produce inflammatory cytokines that lead to the expansion of B cell clones. Thymic epithelial damage due to conditioning leads to loss of regulatory T and B cells and peripheral tolerance.[15]

In the final phase, which is characterized by aberrant tissue repair and fibrosis, platelet-derived growth factor alpha activates fibroblasts, and the production of collagen by transforming growth factor beta secreted by macrophages leads to sclerotic cGVHD.[16]

Prevention

Approaches to prevent GVHD include pharmacologic strategies such as calcineurin inhibitors (cyclosporine, tacrolimus) combined with methotrexate or mTOR inhibitors (sirolimus) and IMP dehydrogenase inhibitors (mycophenolate mofetil). Increasingly, posttransplant cyclophosphamide is emerging as a promising strategy for GVHD prevention, especially in the RIC setting. Other approaches include serotherapy (ATG, Campath) and graft manipulation strategies.

Calcenurin inhibitor (CNI); with four doses of methotrexate (45 mg/m^2) has been the standard of care (SOC); for GVHD prophylaxis in patients undergoing RIC or myeloablative conditioning (MAC), but the field is evolving. Allo-HCT based on studies done at Seattle[17] shows that the combination is more effective in controlling aGVHD than CNI alone. The combination, however, is associated with significant mucositis, delay in engraftment, and interstitial pneumonitis, prompting efforts to look at alternative prophylactic strategies. Sirolimus has immunosuppressive properties by virtue of its FKBP12 binding and mTOR inhibition, which leads to multiple downstream effects and regulatory T cell expansion.[18-20] In combination with CNI and methotrexate (MTX), sirolimus was first used in a phase 1/2 study of alternative donor transplants (mMRD and MUD) in patients receiving TBI-based MAC-allo-HCT. The combination tolerated and effectively controlled acute and chronic GVHD.[21] Subsequent studies showed acceptable GVHD rates with Tacrolimus/sirolimus (T/S) alone in matched related donor (MRD) undergoing MAC[22] and RIC allo-HCTs using Flu/Bu regimen.[23] Based on these promising early results, we were the first group to study the combination of Flu/Mel (n=46) conditioning with Tacrolimus/sirolimus (T/S)-based GVHD prophylaxis in a pilot phase 2 study in 85 patients who received sibling HCT (n=46 received Bu/Cy and n=28 received FTBI/VP16). All patients in this study were engrafted, and the incidence of Gd 2-4 and 3-4 aGVHD was 43% and 19%, respectively, and the two-year incidence of cGVHD was 46%. Higher rates of thrombotic microangiopathy were seen in the Bu/Cy group.[24,25] This regimen has been successfully used in multiple other disease subtypes, including acute lymphoblastic leukemia[26] myelofibrosis,[27] and myelodysplastic syndrome (MDS).[28]

Based on predictable engraftment, low incidence of mucositis, and reasonable control of aGVHD, we have used T/S-based GVHD prophylaxis as our standard for allo-HCTs in patients across multiple disease subtypes since 2005 in both RIC and

MAC setting.[29] A few studies directly compare T/S versus Tac/MTX in the RIC setting. Pidala et al reported in the long-term follow-up of their randomized phase 2 study significantly lower rates of NIH moderate-severe cGVHD in favor of the T/S arm in contrast to Tac/MTX.[30] Another randomized study comparing T/S to CNI/MTX found no differences in key outcomes of aGVHD, NRM, or five-year OS between the two groups.[31] Cutler et al reported clinical outcomes of T/S vs Tac/MTX in acute myeloid leukemia (AML)/MDS patients undergoing allo-HCT after MAC. They did not see any differences between aGVHD or OS between the two groups.[32]

PROGRESS 1 and PROGRESS 2 are two large randomized controlled trials evaluating novel regimens with intriguing results. PROGRESS 1 was a randomized phase 3 study evaluating GVHD prophylaxis interventions with myeloablative conditioning regimens. The three study arms were (1) PTCy from BM graft, (2) control Tac/MTX with BM graft, and (3) CD34 selected T-cell depleted PBSC. Among the 346 patients randomly assigned, the two-year incidence of cGVHD and chronic GVHD, relapse-free survival (CRFS) was no different between the three arms. There was a noted reduction in OS in the CD34 selected PBSC arm (60%; hazard ratio [HR], 1.74; 1.09 to 2.80; P=.02) compared to the control (76.1%) and PTCy (76.2%; HR, 1.02; 0.60 to 1.72; P=.95). CD34 selection was associated with lower moderate to severe cGVHD (HR 0.25; p=0.02).[33] Currently, CNI with methotrexate remains SOC for patients undergoing allo-HCT with MAC regimens.

PROGRESS 2 is a phase 2 multicenter trial in allo-HCT patients who received an RIC regimen and who were randomized to (i) TAC/MMF/PTCy, (ii) TAC/MTX/BOR, (iii) TAC/MTX/Maraviroc and compared to a nonrandomized prospective standard of care cohort TAC/MTX. In all, 273 patients were randomized to the three study arms, and 224 received control, and the composite endpoint GRFS revealed improved outcomes with TAC/MMF/PTCy (HR 0.72; 90% CI 0.54-0.94, p=0.04).[34]

Posttransplant cyclophosphamide (PTCy) in conjunction with tacrolimus and mycophenolate mofetil has been used for many years in haploidentical transplantation with a low incidence of cGVHD,[35] in addition to manageable cGVHD rates in the mismatch unrelated donor (MMUD) setting.[36] There is emerging data showing that patients with HLA-matched unrelated donors using PTCy for GVHD prophylaxis effectively reduce the incidence of GVHD without any substantial changes in relapse and overall survival.[37] PROGRESS 3 is a phase 3 trial evaluating GVHD prophylaxis TAC/MMF/PTCy (experimental) compared to TAC/MTX (standard) in allo-HCT patients receiving either an HLA-matched donor (related or unrelated) or a mismatched (7/8) donor. There were 214 patients in the experimental arm and 217 patients in the standard arm with GRFS as a primary endpoint. The experimental arm had improved GRFS compared to standard prophylaxis (52.7% compared to 34.9%). In addition, there were reduced rates of cGVHD at one year with the experimental prophylaxis group (21.9%) compared to the standard (35.1%).[37]

Abatacept has shown promising results when used as a prophylaxis in combination with Tac/MTx and has been approved for GVHD prophylaxis since December 2021. It is a cytotoxic T-lymphocyte-associated antigen 4 directed monoclonal antibody. In trial ABA2, it was noted that abatacept showed a decrease in D100 grade 3-4 aGVHD rates without any improvement in cGVHD rates.[38] A multicenter phase II randomized controlled trial ABA3 will be performed to evaluate whether an extended abatacept dose compared to a short-term dose can prevent cGVHD (NCT04380740).

Graft manipulation strategies are emerging as promising strategies for preventing GVHD in early clinical trials. These strategies involve the removal of specific T-cell subsets from a PBSC graft or decreasing the inoculum of conventional T cells (Tcons) after infusion with regulatory T cells (Tregs).

Investigators at the University of Perugia pioneered the strategy of using T-cell-depleted stem cell grafts from haploidentical donors in patients with high-risk leukemia. They used a strategy of T-cell depletion by soybean agglutination, E-rosseting, and CD34 positive selection. Using the strategy, they minimized regimen-related toxicity and incidence of graft-versus-host disease.[39] However, relapse-related mortality remained problematic, and this approach was further refined by developing T-cell adoptive immunotherapy wherein patients received myeloablative conditioning followed by co-infusion of regulatory T cells and conventional T cells. This approach achieved complete donor chimerism with a low incidence of acute GVHD and relapse in most patients. The graft versus leukemia effect and low relapse were mainly due to unopposed Tcon alloantigen recognition in the bone marrow.[40,41] Similarly, studies done in patients with high-risk hematologic malignancies using matched donors showed promising results. Patients received HLA-matched Tregs and CD34-selected Hematopoietic progenitor cells (HPC) followed by infusion of equal ratio Tcons after myeloablative conditioning.[42] In matched donor settings, low rates of acute graft-versus-host disease were noted without the use of posttransplant immunosuppression. Thus, the approach of using ex vivo engineered graft after myeloablative conditioning in young patients successfully allows engraftment and is associated with low rates of relapse and known relapse mortality.[43]

Orca-T is a cellular infusion product of purified donor regulatory T cells, and utilization of this product augments alloreactive immune responses. In a phase I/II study with Orca-T patients who received myeloablative conditioning, Orca-T and a single agent prophylaxis of either sirolimus or tacrolimus had low rates of moderate/severe GVHD (6% at one year).[43] Low relapse rates with the loss of control of GVHD have also been shown in haploidentical stem cell transplantation.[40,41]

Naive T cells (CD45RA+) have been shown to cause severe GVHD in murine models. A prospective study evaluated naive T-cell-depleted allo-HCT grafts. The three-year cumulative incidence of mild, moderate, and severe cGVHD were 6%, 1%, and 0%, respectively, cGVHD without any increase in relapse or infections.[44] Studies are currently in progress to evaluate the efficacy of naive T-cell depletion from a PBSC graft in a haploidentical and matched donor setting (NCT03802695)

Novel endpoints

A significant obstacle to evaluating the response of novel GVHD-directed therapies has been standardized response assessments. This has functioned as a barrier to designing and interpreting clinical trials structured around the treatment of cGVHD. Previously, overall survival or survival with the resolution of cGVHD were endpoints used for cGVHD clinical trials, but collection of these data is less than ideal in early phase studies. A consensus criterion was developed in 2005 by the NIH Consensus Conference with quantitative measurements to capture

responses better.[45] A multicenter prospective study of the incidence and prevalence of cGVHD requiring systemic therapy did not show that these response criteria correlated with survival (adjusted HR, 0.6; 95% CI 0.2-1.4; *P*=.20).[46]

Failure-free survival (FFS) is a composite endpoint defined as the absence of treatment change, NRM, and recurrent malignancy during initial systemic therapy.[47] FFS rates were 54% at 12 months at first-line immunosuppression[47] and 45% at second-line.[48] A prospective observational study identified variables associated with lower FFS: higher NIH skin score, higher NIH GI score, worse range of motion score, lower forced vital capacity (%), bronchiolitis obliterans syndrome (BOS), worse health-related quality of life (HRQOL), moderate to severe hepatic dysfunction, absence of treatment for gastric acid, female donor for male recipient, and prior grade II-VI aGVHD.[49] In landmark analyses by the Chronic GVHD Consortium, FFS at one year with a complete response (CR)/partial response (PR) (20%) has been associated with clinical benefit, including lower burden of disease, shorter time to end of the systemic treatment, and better survival.[50] Treatment of cGVHD that incorporates glucocorticoid treatment initially has clinical improvement, but those who have sustained responses and FFS at one year are less than 20%.[51]

The NIH Consensus 2020 Treatment of Chronic GVHD report recommends FFS as a key secondary endpoint to be used in phase 2 cGVHD studies.[51] A few pivotal studies have used FFS as an endpoint.

A large cohort of 745 patients from three observational studies evaluated the effect of initial therapy for cGVHD on FFS. Initial therapies were no prednisone (*n*=137), prednisone alone (*n*=411), or prednisone plus other therapy (*n*=197). There were no associations noted with FFS in regard to the type of initial therapy, the dose of steroids, or the overall cGVHD severity.[52] This may signal that lower doses of prednisone or prednisone-free therapies to treat cGVHD may be on the horizon, but we will need prospective studies to clarify this.

FFS was also utilized as a secondary endpoint in REACH3, a phase 3, open-label, randomized study evaluating ruxolitinib vs best available therapy (BAT) in steroid-refractory/dependent cGVHD, which showed significant improvement in the overall response rate (ORR) ($p<0.0001$), more prolonged FFS ($p<0.0001$), and greater symptom improvement. However, 50% of patients enrolled in RUX discontinued it either because of lack of efficacy (15%), adverse effects (17%), or relapse (5%). FFS was significantly longer in the RUX-treated patients (median FFS not reached vs 5.7 months, HR 0.370; *P*<0.0001).[53]

BMT CTN 0801 evaluated prednisone/sirolimus with or without calcineurin inhibitor calcineurin inhibitor for the treatment of cGVHD and evaluated failure-free survival rates between two and three-drug regimens and failed to show any difference in benefit between a three-drug regimen compared to two-drug regimen.[54]

A phase 2 study evaluated the combination of prednisone and Ofaftumab as initial therapy for cGVHD. This study had 53% FFS at 12 months. This was statistically superior to the landmark Martin et al study.[50] The 12-month FFS with CR/PR compared to the 12-month FFS without CR/PR had a higher likelihood of completely discontinuing steroids by 24 months (OR 8; $p=0.025$).[55] Though the study did not meet its primary endpoint of hypothesized ORR, the secondary endpoint of FFS revealed promising results.

We are still looking for novel therapies that can improve rates of complete/partial responses and failure-free survival. Though FFS can be a helpful endpoint, it does not quantify the extent of organ involvement or the severity of symptoms. Thus, for clinicians, it is unclear how this endpoint may dictate the clinical management of patients.

GVHD-free/relapse-free survival (GRFS) is a composite endpoint that includes grade 3-4 acute GVHD, chronic GVHD requiring systemic therapy, relapse, or death in the first post-HCT year. BMTCTN proposed GRFS as a more effective endpoint in capturing the effectiveness of GVHD prophylaxis. In 907 HCT recipients with tacrolimus and methotrexate as GVHD prophylaxis, one-year GRFS was 31%, with a one-year OS at 63%.[56] These results suggested survival may not completely capture those with suboptimal results. An extensive registry analysis of 5059 HCT recipients with AML evaluated GRFS incidence in MUD and match sibling donor (MSD) recipients. MDS had better GRFS outcomes (HR 1.19, CI 1.07-1.31, $p<0.01$), which may be related to greater extensive cGVHD in MUD recipients (HR 1.42, CI 1.19-1.69, $p<0.01$).[57]

Since mild cGVHD can receive systemic immunosuppression to treat their cGVHD attempts have been made to improve the original GRFS composite endpoint. An extensive European Society for Blood and Marrow Transplantation (EBMT) analysis refined GRFS by replacing cGVHD requiring systemic therapy with the occurrence of severe cGVHD. They analyzed 20 937 patients with AML who received HCT with three-year modified GRFS at 40.1%, with severe cGVHD making up 26%. Of those noted to have severe cGVHD, 86% still had severe cGVHD at the last follow-up, with 14% limited.[58] It has been indicated that moderate to severe cGVHD is associated with inferior survival.[3] Since mild cGVHD can receive systemic immunosuppression to treat their cGVHD. A single institution study attempted to adequately capture the development of NIH-grade moderate to severe cGVHD in a modified GRFS endpoint. The retrospective study evaluated 613 HCT patients after an MRD, MUD, or haplo donor source. It replaced cGVHD requiring systemic immunosuppression in GRFS with the development of NIH-grade moderate or severe cGVHD. One-year modified GRFS was 36% compared to the traditional GRFS of 33%, with moderate/severe cGVHD being the most common (38%) reason for failing at one year.[59] GRFS may not adequately capture the dynamic nature of cGVHD because this endpoint captures GVHD in a binary fashion, and its endpoint does not indicate the resolution of GVHD.

Current GVHD-free, relapse-free survival (CGRFS) is a composite endpoint to help address some of the issues associated with GRFS. At any time posttransplant, it is defined as the probability of being alive, in remission, and without clinically significant chronic GVHD, defined as moderate to severe.[60] This is a natural extension of Pidala et al's analysis—a single institution analysis of 422 allo-HCT patients using MRD, MUD, or Haplo donor sources. Solomon et al noted that CGRFS occurrence after one, two, three, and four years was 45%, 46%, 47%, and 49%, respectively. At year 4, less than a quarter of patients were captured by GRFS, but nearly half were captured by CRFS, effectively demonstrating CGRFS as a better endpoint for capturing success without GVHD. In addition, there has been a steady improvement in outcomes over time. The treatment of cGVHD as a dynamic outcome as opposed to a binary one may create a clearer picture of

Table 1. Clinical reports of JAK inhibitor treatment for cGVHD

Reference	JAK inhibitor	Study type	GVHD severity	Patients	Prior treatments, median (range)	Follow-up duration, median (range)	Response	OS (95% CI)
cGVHD								
Khoury et al[67]	Ruxolitinib	Retrospective	Severe	19	NA	18 (6–27) mo	ORR, 89%	NA
Zeiser et al[68–69]	Ruxolitinib	Retrospective	Moderate or severe	41	3 (1–10)	22.4 (3–135) wk	ORR, 85% (CR, 7%)	6 mo, 97% (92%-100%)
						24 (NA) mo	Ongoing, 24%	12 mo, 93% (85%-100%)
Spoerl et al[70]	Ruxolitinib	Pilot	Grade 3	2	4 (3–5)	23.5 (10–37) wk	Response, 100%	NA
Mori et al[71]	Ruxolitinib	Retrospective	Severe	3	2 (1–2)	NA	ORR, 100% (CR, 57%)	NA

post-HCT outcomes. A large number of trials evaluated the endpoints of GRFS/cGRFS at one year, and it is essential to note that though the median time to onset is four to six months after HCT, up to 10% are diagnosed beyond one year with treatment for a median of two to three years.[61] These studies of short duration may under/overestimate the significance of cGVHD in alloHCT in their time-to-event endpoint analysis.

Targeted therapies

Ibrutinib targets Bruton's tyrosine kinase (BTK) pathway in B cells and IL-2 inducible T cell kinase (ITK) in T cells and was the first FDA-approved agent in cGVHD. Its approval was based on a multicenter, open-label, phase 1b/2 study in patients with active cGVHD who were steroid-dependent/refractory. The median follow-up was 14 months, and the overall response rate was 67% (CR 21% and PR 45%), with 71% of responders having a durable response (>20 weeks). Responses were seen in all organs. The update follow-up (median follow-up of 26 months) published two years after the initial publication revealed ORR 69% and CR 31% with sustained responses >44 weeks at 55%. The most common grade 3 adverse effects (AEs) were pneumonia, fatigue, and diarrhea.[62]

JAK1-JAK2 signaling is vital to inflammation and tissue damage in acute and chronic GVHD. Ruxolitinib, a JAK1/2 inhibitor, was evaluated in a phase 3 open-label study in patients with steroid-refractory cGVHD, comparing ruxolitinib 10mg twice daily to investigators' choice (REACH 3). The overall response (CR + PR) at week 24 was 49.7% in the ruxolitinib arm compared to 25.6%. Those randomized to the ruxolitinib arm had longer FFS compared to controls (18.6 vs 5.7 months; $p<0.001$)[63] (see Table 1). A phase I/II study evaluating pacritinib, a novel selective JAK2/IRAK inhibitor in refractory chronic GVHD, is ongoing (NCT05531786).

Belumosodil is an oral selective rho-associated coiled-coil-containing protein kinase 2 (ROCK2) inhibitor. ROCK2 acts on the dysregulated adaptive immune system and fibrosis due to aberrant tissue repair.[64] ROCKstar was a phase 2 multicenter registration study in cGVHD patients who previously received two to five lines of therapy. High response rates (ORR 74% and 77% for 200 mg daily and 200 mg twice daily, respectively) were seen in all organs, including high levels of CR, and responses were seen in all subgroups in addition, including those who previously received ruxolitinib, which was 68%, and ibrutinib, which was 74%. Responses were also generally rapid, with a median response time of five weeks. AEs were seen in 54% of patients.[64] Belumosodil was approved in July 2021.

Colony-stimulating factor 1 receptor (CSF-1R) dependent macrophages promote inflammation and tissue injury, leading to cGVHD fibrosis. Axatilimab is an IgG4 monoclonal antibody with a high affinity for CSF-1R, leading to impaired CSF-1R signaling via two ligands, CSF-1 and IL-34.[65] A phase I/II open-label study evaluating axatilimab in patients with active cGVHD after two lines of systemic therapy. Among the 22 evaluable patients in phase II, there were high response rates (ORR 50% at cycle 7, day 1, and 82% for the first six cycles) in the phase II cohort. In the entire study population, ORR was 67% (26 of 39), with responses seen in all organs with no differences in outcomes for moderate vs severe cGVHD. Responses were rapid, with a median response time of four weeks. Treatment-related grade ≥3 AEs were in 20% of patients. A phase 2 study evaluating axatilimab in cGVHD at three different dose levels is ongoing (AGAVE-201; NCT04710576).[66]

Conclusion

We anticipate continued improvement of prophylactic strategies for preventing GVHD; the identification of more accurate endpoints for determining the efficacy of treatment for cGVHD; and access to novel therapeutic agents to treat new and refractory cGVHD as well as established cGVHD. We further expect that cGVHD outcomes will continue to improve in allo-HCT recipients.

Authorship

*Monzr M. Al Malki and Amandeep Salhotra are joint senior authors.

Conflict-of-interest disclosure

Idoroenyi Amanam: no competing financial interests to declare.
Salman Otoukesh: no competing financial interests to declare.
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Off-label drug use

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EVIDENCE-BASED MINIREVIEW

Should posttransplant cyclophosphamide be considered standard of care for pediatric transplantation of acute leukemia?

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LEARNING OBJECTIVES

- Examine the adult experience for haploidentical stem cell transplant with PTCy and how it informs its application to pediatric patients
- Compare survival outcomes and incidence of GVHD in pediatric regimens using PTCy to the current standard or care for GVHD prophylaxis

CLINICAL CASE

A 7-year-old female diagnosed with CRLF2-positive pre-B acute lymphoblastic leukemia showed persistent disease at the end of consolidation. Complete remission followed treatment with blinatumomab, and the patient is referred for stem cell transplant. There are no siblings. Donor registry search identifies a single 10/10 human leukocyte antigen matched unrelated donor (MUD), but the donor is unavailable for 2 months. Both parents are healthy and in their thirties. Who is the preferred donor? Select the unrelated donor but risk disease recurrence while waiting, or select a haploidentical parent and proceed to transplant immediately using posttransplant cyclophosphamide (PTCy)?

Introduction

Hematopoietic stem cell transplantation (HSCT) can be a curative option for pediatric high-risk hematologic malignancies. Its use, however, still poses considerable logistical concerns and risks. Prompt identification and availability of a suitable donor can determine whether the patient undergoes transplantation free of minimal residual disease, a state recognized to impact posttransplant relapse. No less important, the HLA match between donor and patient influences the risk for severe acute and chronic graft-versus-host disease (GVHD). The need to address the dual mandate for timely donor access and prevention of GVHD has led to clinical trials utilizing haploidentical donors and incorporating PTCy for GVHD prophylaxis.

The Children's Oncology Group in clinical trials has favored a calcineurin inhibitor and methotrexate (CNI+MTX) for GVHD prevention. Now, with the emergence of haploidentical transplants using PTCy, the value of this treatment for children in need of transplantation must be determined. In this evidence-based mini-review, we assess the clinical experience and outcomes for PTCy and how this approach may relate to future transplantation in pediatric patients with hematologic malignancies.

What can we learn about PTCy from adult HSCT trials?

Reports suggest that the outcomes for adults with hematologic malignancies transplanted from haploidentical donors utilizing PTCy are comparable to MRD/MUD or mismatched donor transplants using CNI-based GVHD prophylaxis. The seminal randomized trial, BMT CTN 1101, compared outcomes for adult patients with lymphoma or acute leukemia following reduced intensity conditioning (RIC) and either a double umbilical cord blood with cyclosporine and mycophenolate mofetil (MMF) for GVHD prophylaxis or a haploidentical transplant with PTCy, tacrolimus, and MMF. GVHD outcomes were comparable, but overall survival (OS) was superior in the haplo-PTCy group (46% OS with double umbilical cord blood vs 57% OS with haplo-PTCy, $P = 0.037$).[1]

PTCy has not been limited to transplantation from haplo-donors. For example, in the BMT CTN 1703 trial, adults with high-risk hematologic malignancy with a matched related/unrelated or 7/8 mismatched unrelated donor underwent RIC and peripheral blood stem cell

(PBSC) infusion and were randomized to either CNI+MTX or PTCy, tacrolimus, and MMF. There was no difference in relapse or OS at 1 year, but the PTCy group experienced significantly superior GVHD-free, relapse-free survival (GRFS) (52.7% vs 34.5%), largely due to less acute and chronic GVHD.[2] Following a similar design, the HOVON-96 trial prospectively analyzed adult patients, receiving nonmyeloablative conditioning for hematologic malignancies with 8/8 MUDs or matched sibling donors (MSDs). One-year GRFS was 45% vs 21% in favor of PTCy[3] over cyclosporine and MMF as prophylaxis. Again, superior GRFS was attributed to the lower incidence of acute and chronic GVHD. The BMT CTN 1301 trial for adults with hematologic malignancies and MRD/MUD donors showed similar OS and chronic GVHD following myeloablative conditioning with either PTCy+tacrolimus+MMF or CNI+MTX, as GVHD prophylaxis.[4] When comparing outcomes of haplo-transplant to MUD transplant following myeloablative conditioning (MAC) with PTCy, CIBMTR registry data found no difference for OS, disease-free survival, or relapse rate, but a higher incidence of acute and chronic GVHD for patients transplanted from haploidentical donors.[5]

What are the strategies to decrease GVHD in pediatric HSCT?

The likelihood of identifying a matched related donor is less than 25%, and the focus for pediatric patients in emergent need of transplant has been on selecting the best alternative donor and proceeding immediately to transplant. Keating et al reported that for pediatric patients with acute myeloid leukemia the OS, leukemia free survival, and relapse rate (63%, 57%, and 22%, respectively) were similar whether selecting an matched related donor, an MUD, or a umbilical cord blood.[6] Chronic GRFS following umbilical cord blood and MRD transplantation was superior to MUD transplant, but the use of cord blood may be limited by other transplant considerations. Locatelli et al reported OS, LFS, and relapse rates (72% [68% for acute myeloid leukemia], 71% and 24%, respectively) for pediatric patients with acute leukemia receiving haploidentical transplants utilizing the GVHD prevention strategy of αβ T-cell and B-cell depleted grafts, outcomes that are comparable to those reported by Keating. When this cohort was compared to a cohort of similar patients transplanted from MRD or MUD, no difference was found for 5-year LFS and GRFS of 71%.[7] Despite these promising findings, αβ T-cell and B-cell depletion has limited application, lacking FDA approval and the need for institutional expertise to implement.

For children with high-risk malignancy and the immediate need of a donor, haploidentical transplant with PTCy may be the best alternative. To date, there are no randomized trials comparing haploidentical transplant with PTCy to standard of care, but results from nonrandomized trials are supportive. For example, two studies of pediatric and young adult patients with hematologic malignancies receiving nonmyeloablative conditioning followed by haplo-transplant and PTCy demonstrated low rates of nonrelapse mortality and acute and chronic GVHD, with OS and event-free survival (EFS) up to 56% and 46%, respectively.[8,9]

In a single-center study, children with hematologic malignancies received MAC followed by haploidentical HSCT with PTCy, calcineurin inhibitor, and MMF. OS was 70.5% and disease-free survival was 64.7%, with low incidence of acute GVHD and chronic GVHD.[10] A multicenter, prospective study evaluating outcomes for haploidentical transplant and PTCy with MAC enrolled children with high-risk hematologic malignancies and reported that no patient developed grade III-IV acute GVHD and the cumulative incidence of moderate to severe cGVHD at 1 year was 4%.[11] Additionally, 1-year OS and EFS were 77% and 68%, respectively, with a 0% treatment-related mortality (TRM). These promising outcomes and the practicality of using PTCy make it an option for centers that are unable to perform the aforementioned αβ T-cell depletion.

Further experience with PTCy for children in need of HSCT

The European Society for Bone Marrow Transplantation reported on 180 children with acute lymphoblastic leukemia receiving haploidentical transplant and PTCy. Patients received either MAC or RIC regimens and marrow or PBSC products. The reported incidence of grade III-IV aGVHD was 12.4%, and 2-year extensive cGVHD incidence was 9.5%,[12] but TRM was high at 19.6%. It is possible that PBSC, in contrast to marrow grafts, contributed to TRM. The use of bone marrow grafts may reduce TRM and improve EFS. Patients without active disease at the time of transplant also fared better.[12] In other reports, the incidence of grade III-IV GVHD for children receiving haplo-transplants with PTCy following MAC

Table 1. Comparison of haploidentical stem cell transplant outcomes using PTCy for pediatric hematologic malignancies

Reference	N	Donor type	Disease	Grade I-II aGVHD	Grade III-IV aGVHD	Mod-severe cGVHD	Graft failure	TRM	Relapse	EFS
Fierro et al (2023)[11]	32	Haplo (BM)	AL/MDS	13%	0%	4%	9%	0%	32%	68%
Symons et al (2020)[14]	29	Haplo	AL/MDS	17%	4%	14%	N/A	7%	28%	69%
Sharma et al (2021)[10]	17	Haplo (PBSC)	AL	12%	12%	18%	5.8%	5.8%	29%	64.7%
Srinivasan et al (2022)[15]	26	Haplo (PBSC)	Heme malignancy	N/A	11.5%	9.2%	3.8%	0%	19.3%	73.8%
Hong et al (2022)[16]	35	Haplo (PBSC)	AL	34.3%	2.9%	11.4%	0%	0%	25.6%	74.4%

AL, acute leukemia; BM, bone marrow; haplo, haploidentical; TRM, treatment related mortality.

regimens ranges from 0%-12% and the incidence of cGVHD from 4%-18%. The EFS, at 65%-74%, is reassuring and TRM is acceptable at 0%-7%[10,11,14-16] (Table 1). The overall experience for children undergoing PTCy haploidentical transplant compares favorably to outcomes for transplantation employing other donor options and other GVHD prophylaxis.

Just as for adults, this experience has extended the use of PTCy to alternative donor transplants in children and young adults. A recently published phase 2 study evaluated young adults and adults with 7/8 or 8/8 HLA-matched allogeneic transplants receiving MAC and PTCy, tacrolimus, and MMF for treatment of hematologic malignancy/myelodysplastic syndrome (MDS). There were no significant differences in survival outcomes between the 7/8 and 8/8 groups and overall low rates of severe cGVHD (5.5%) and grade III-IV aGVHD (5.5%).[13]

Although rates of relapse and acute and chronic GVHD have been the major focus, the use of PTCy has raised concern for delayed immune reconstitution and increased risk of infections, including cytomegalovirus and BK cystitis. These areas remain under active investigation, but no difference in infectious complications has been established.[14] Nor has it been shown that the incidence of cytomegalovirus reactivation is increased.[16]

Conclusions

HSCT with PTCy for adult patients receiving RIC regimens and PBSC products is safe and effective, and recent studies suggest that GVHD prophylaxis with PTCy is as effective as standard of care (CNI+MTX). While data are limited for children following transplantation from haploidentical donors with PTCy, results thus far show similar OS, EFS, and TRM compared to historical controls. Additionally, the occurrence of acute and chronic GVHD with PTCy compares favorably to that following MRD and MUD HSCT with CNI+MTX prophylaxis. Obviously, future efforts and time will define the true potential for using PTCy with close attention to the incidence of infectious complications, immune reconstitution, and late effects. Prospective studies comparing haploidentical PTCy outcomes to MUDs are an important area of investigation and will be addressed by the Children's Oncology Group ASCT2031 trial. Further studies are needed using PTCy with MUDs and MRDs and for transplantation of nonmalignant conditions. It must be acknowledged that should the FDA approve αβ T-cell depletion, this option warrants comparison to PTCy regimens. Finally, it is important to recognize that haplo-donors may not be interchangeable. Is there an advantage to selecting a haplo-sibling vs a haplo-parent? Is a younger haploidentical cousin a better donor than an aging parent? The results following haplo HSCT from second- and third-degree relatives suggest comparability to first-degree relatives.[17] For adults transplanted from haplo-donors after NMA conditioning, OS, PFS, and aGVHD statistically worsened with donor age.[18] Age has been recognized as a variable contributing to outcome. How age is integrated into donor selection for children and young adults undergoing HSCT will need further examination.

Recommendations for incorporation of PTCy in pediatric HSCT

1. Haploidentical transplant with PTCy should be considered for pediatric patients with high-risk malignancy when an MRD is not available to avoid unacceptable delays to treatment initiation and costs associated with procurement of unrelated donors (strong recommendation, moderate quality evidence).[11,14–17]
2. Post-transplant cyclophosphamide should replace the standard of care (CNI+MTX) for GVHD prophylaxis in pediatric patients with HLA-matched donors (weak recommendation, moderate quality evidence).[13]

Conflict-of-interest disclosure

Erin E. Doherty: no competing financial interests to declare.
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Off-label drug use

Erin E. Doherty: nothing to disclose.
Robert A. Krance: nothing to disclose.

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Improving long-term outcomes with intensive induction chemotherapy for patients with AML

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Intensive chemotherapy in combination with allogeneic hematopoietic cell transplantation and supportive care can induce long-term remissions in around 50% of acute myeloid leukemia patients eligible for intensive treatment. Several treatment optimization trials helped to refine schedule and dosing of the historic "7+3" combination. Together with the addition of novel agents, increased efficacy and tolerability led to improved long-term outcomes. Unsatisfactory outcomes in fit elderly patients and unfavorable genetic subgroups have raised the question of whether less-intensive venetoclax-based approaches may be beneficial as an alternative. Although tempting and worth exploring, this issue will remain controversial until the results of randomized comparisons appear. To date, intensive chemotherapy remains the only evident curative treatment option for long-term disease eradication in a fixed treatment time. With the advent of more novel agents and advances in minimal residual disease (MRD) detection and maintenance approaches, the face of intensive treatment could change in many ways. Several are being explored in clinical trials, such as (1) combinations of more than 1 novel agent with the intensive backbone, (2) head-to-head comparisons of novel agents, (3) replacement or dose reduction of cytotoxic components such as anthracyclines, and (4) MRD-guided escalation and de-escalation strategies. The combination of intensive treatment with individualized tailored innovative strategies will most certainly reduce treatment-related toxicities and increase the chances for long-term remission in the future.

LEARNING OBJECTIVES

- Outline the development and describe current standards of intensive chemotherapy in AML
- Compare strengths and shortcomings of intensive induction versus nonintensive approaches
- Explain novel approaches and sketch future scenarios for intensive AML treatment

CLINICAL CASE 1

After 2 weeks of progressive weakness, a 67-year-old woman notices recurring nose bleeds and consults her general practitioner. She has well-controlled arterial hypertension and is slightly obese. In addition to general paleness and petechiae around the ankles, the lab result reveals pancytopenia. After referral to a hematologist, bone marrow assessment shows 45% myeloid blasts, resulting in the diagnosis of acute myeloid leukemia (AML) according to both World Health Organization (WHO) and International Consensus Classification (ICC) criteria. What to do next?

CLINICAL CASE 2

A 70-year-old man seeks medical advice in the emergency department for fever and shortness of breath that did not get better despite over-the-counter self-medication. He has a slight leukocytosis with 25% atypical immature cells, anemia, and thrombocytopenia. Diagnostic workup shows an AML. Our patient has only one kidney due to congenital unilateral renal agenesis but is otherwise healthy. He is an active, well-informed pensioner who enjoys working in his garden and spending lots of time doing sports with his grandchildren. How shall we advise him?

Let's first look at the reasons why this woman and this man should be treated with intensive chemotherapy before we move on to the optimal regimen and potential study options.

Why should we use intensive chemo for fit patients?

Before the 1960s with no effective cytoreductive treatment options, our patients would have to face a life

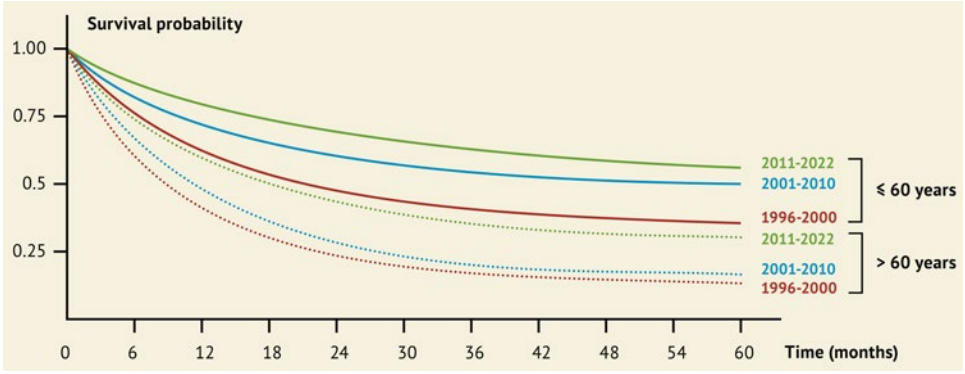

Figure 1. Overall survival in relation to age and period of treatment. Data based on clinical trials of the Study Alliance Leukemia Group (SAL) and the SAL AML registry.

expectancy of only around 2 to 4 months as shown from historic reports[1] or recent outcomes in elderly patients treated with best supportive care only.[2,3] A couple of decades ago, the advent of intensive chemotherapy led to a significant proportion of AML patients being cured. In the late 1960s, cytarabine and daunorubicin were identified as most effective to induce complete remissions even as single agents.[4,5] Since the establishment of their combination in the 7+3 schedule in the 1980s,[6] cure rates and life expectancy in patients fit for intensive treatment have continued to go up, mainly based on significant progress in supportive care and allogeneic hematopoietic cell transplant (HCT) technology, but also based on treatment optimization in the use of the cytostatic components (Figure 1). Recent data from clinical trials in younger fit AML patients across genetic subgroups show long-term remission rates around 60%, whereas 50% long-term remission can be achieved in combination with allogeneic HCT even in elderly fit patients with secondary AML.[7,8] Therefore, based on high evidence from thousands of treated patients, intensive chemotherapy is the most certain way to cure.

Whereas treatment is more manageable and delivers better results in younger patients, increased toxicity and fewer long-term remissions with intensive chemo in fit elderly patients led to an ongoing debate about its value. However, numerous arguments support the intensive approach also in this patient group.

1. There is no alternative treatment achieving better long-term remission results than intensive chemo in first-line treatment.
2. Depending on genetic risk, more than 50% of elderly patients can be cured with a combination of intensive induction and postremission treatment including allogeneic HCT.[9–11]
3. Disease eradication and prognostically relevant MRD reduction is quickly and profoundly achieved after 1 to 2 cycles of intensive induction with 60% to 80% of patients becoming MRD negative, providing a stronger disease control before postremission treatment or allogeneic HCT.[12–16]
4. Early mortality rates in intensively treated elderly AML patients have continually decreased throughout the past decades due to better supportive care.[17]
5. Intensive chemotherapy is generally a time-limited treatment, ie, patients will become treatment-free within a few months.

Based on these arguments, I would recommend intensive induction therapy to our case patients. Although there is no general consented definition of fitness, our two patients would be considered fit according to all existing sets of criteria, which generally incorporate performance status, adequate end-organ function, age, and geriatric assessment. Assuming patients or family had a medical background, we may anticipate several questions regarding clonal disease evolution under cytostatic drugs and the alternative use of venetoclax plus hypomethylating agents (HMA). However, despite preclinical data supporting clonal evolution and selection processes,[18] its clinical impact on long-term outcomes and the need for continuous treatment is less clear. And although the combination of venetoclax plus HMA means an outstanding leap forward for patients who cannot tolerate intensive chemotherapy, its capacity to achieve cure or even superiority over intensive induction has not been demonstrated in prospective randomized comparisons in fit patients. Retrospective analyses are prone to relevant issues around selection bias and statistical matching methodology, leading to mixed and partly contradictory results.

Therefore, while venetoclax will certainly play a role in the future of AML treatment, possibly also in fit patients in the context of combination approaches, for the time being, the value of HMA plus venetoclax compared to intensive treatment in fit patients has not been shown convincingly.

Several clinical trials explore the role of HMA plus venetoclax compared with standard intensive chemotherapy, as shown in Table 1. In addition, the alternative use of venetoclax not *instead* of but *with* intensive chemo is being explored in clinical trials (see section "The future of intensive chemotherapy in AML" and Table 2).

Since our two case patients are convinced by our arguments and agree with intensive induction, let us move on to see what we have learned about its use and what we should pay attention to during their induction.

How to use intensive induction

General aspects of induction

There are several ways to combine cytarabine and anthracyclines, and there are many variations of 7+3. They should all lead to similar outcomes when these combinations are used correctly. A few points are of general importance:

Table 1. Selection of RCTs comparing intensive chemotherapy with venetoclax-based nonintensive treatment in newly diagnosed fit patients

Target population/AML subgroup*	Age (years)	Experimental agent/intervention	Experimental arm	Control arm	Primary endpoint†	Planned patient number	Name PI, country (cooperative group)	Registry number
• All comers • No CBF • No *NPM1* mut in <60y • No *FLT3*-ITD or –TKD	≥18	Venetoclax + azacitidine	Venetoclax + azacitidine	7+3‡ or CPX-351	EFS	172	Fathi, USA	NCT04801797
• *NPM1* mut • No *FLT3*-ITD	≥60 or relevant comorbidity	Venetoclax + LDAC	Venetoclax + LDAC	DA+GO	Modified EFS	186	Dillon, UK (NCRI)	EudraCT 2020-000273-24, ISRCTN 15567173
• *NPM1* mut • No *FLT3*-ITD	18–70	Venetoclax + azacitidine	Venetoclax + azacitidine	DA + GO	Modified EFS	146	Röllig, Germany (SAL-AMLCG)	EudraCT 2021-00348-26, NCT05904106
• Adverse risk (ELN2017) • No *FLT3*-ITD or –TKD • No t(9;22)	18–59	7+3/CPX-351 + venetoclax or azacitidine + venetoclax	7+3/CPX-351 + venetoclax or azacitidine + venetoclax	7+3 or CPX-351	MRD after induction	268	Shami/NCI, USA, Canada (SWOG)	NCT05554406
• Intermediate risk • No *FLT3*-ITD or -TKD	18–59	7+3 + venetoclax or azacitidine + venetoclax	7+3 + venetoclax or azacitidine + venetoclax	7+3	MRD after induction	153	Savoie/NCI, Canada, USA (CCTG)	NCT05554393
• All comers	18–59	Venetoclax	Venetoclax + decitabine	7+3	ORR§	188	Suning, China	NCT05177731
• *TP53* mut	≥18	Magrolimab	Magrolimab + azacitidine	Venetoclax + azacitidine or 7+3	OS	356	Gilead, USA	NCT04778397
• Adverse risk, intermediate risk, and 50–70 y • No *FLT3*-ITD	18–70	Magrolimab	Magrolimab + venetoclax + azacitidine	DA, DA + GO, CPX-351, or FLAG-Ida	EFS	164	Craddock, UK (NCRI)	ISRCTN71474257

Trials for unfit patients, children, APL, relapsed or refractory disease and with purely maintenance or conditioning questions were excluded.

CBF, core binding factor; CRi, complete hematologic remission with incomplete hematologic recovery; DA, daunorubicin plus cytarabine (ara-c); EFS, event-free survival; GO, gemtuzumab ozogamicin; LDAC, low-dose cytarabine; MRD, measurable residual disease; mut, mutation; OS, overall survival; PI, principal investigator.

*sAML/tAML/HMA pretreatment not accounted for/mentioned in table. †Secondary end points for all trials include response rates, MRD, tolerability, rate of allogeneic HCT, patient-reported outcomes, and survival end points. ‡"7+3" stands for all variations of standard-dose cytarabine plus anthracycline/mitoxantrone and includes intensive consolidation for patients ineligible for allogeneic HCT. §ORR, overall response rate (CR+CRi+morphologic leukemia-free state MLFS).

Table 2. Selection of RCTs randomly evaluating modifications in intensive chemotherapy in newly diagnosed fit patients

Target population/AML subgroup*	Age (years)	Experimental agent/intervention	Experimental arm	Control arm	Primary endpoint†	Planned patient number	PI, country (cooperative group)	Registry number
• All comers	18–65	Venetoclax	Venetoclax +7+3	7+3	EFS	300	Wang, China	NCT05356169
• Intermediate or favorable cytogenetics • In CR/CRi after intensive induction	≥60	Venetoclax	Chemo consolidation with venetoclax + cytarabine	Chemo consolidation with idarubicin + cytarabine	RFS	134	Pigneux, France (FILO)	NCT04968015
• AML/MDS-EB2, • No *FLT3* mut	≥18	Venetoclax	Venetoclax +7+3	7+3	EFS	650	Döhner, Germany (AMLSG/HOVON)	NCT04628026
• Favorable/intermediate risk • No CBF-AML	18–60	Venetoclax	Venetoclax + IDAC as consolidation	IDAC as consolidation	RFS	200	Peterlin/Gastaud, France (FILO/ALFA)	NCT02416388
• All comers • No CBF-AML and *FLT3* mut	18–65	Venetoclax	Venetoclax + DAC	Placebo + DAC	EFS	311	Wierzbowska, Poland, (PALG)	EudraCT 2023-503394–37–00
• AML or MDS-EB2 with *FLT3*-ITD and/or *FLT3*-TKD	≥18	Gilteritinib	Gilteritinib +7+3	Midostaurin +7+3	EFS	768	Raajimakers, Netherlands (HOVON/AMLSG/SAKK, ALFA, FILO, ALLG, CETLAM)	NCT04027309
• *FLT3* mut • No CBF-AML	18–70	Gilteritinib	Gilteritinib +7+3	Midostaurin +7+3	CR/CRi *FLT3* negative	181	Luger, USA (PrECOG)	NCT03836209
• *FLT3*-ITD or *FLT3*-TKD AML	18–60	Crenolanib	Crenolanib +7+3	Midostaurin +7+3	EFS	510	AROG, USA	NCT03258931
• CBF-AML	18–65	Sorafenib	Sorafenib +7+3‡	7+3	CRmol	88	Shi, China	NCT05404516
• CBF-AML	18–70	Midostaurin	Midostaurin + GO +7+3	GO +7+3	EFS	66	Röllig, Germany (SAL-AMLCG)	NCT04385290
• AML or MDS-EB2 with *IDH1* or *IDH2* mut	≥18	Ivosidenib, enasidenib	Ivosidenib or enasidenib +7+3	Placebo +7+3	EFS	968	Wouters, Netherlands (HOVON/AMLSG/SAKK, ALFA, FILO, ALLG, CETLAM)	NCT03839771
• Favorable/intermediate risk (ELN2017) • No *FLT3*-ITD, -TKD	18–60	Glasdegib	7+3 + GO and postremission treatment, followed by glasdegib maintenance	7+3 + GO and postremission treatment	DFS	414	Venditti, Italy (GIMEMA)	NCT04168502
• *FLT3* mut AML	18–70	GO	GO + midostaurin +7+3	Midostaurin +7+3	EFS	130	Röllig, Germany (SAL-AMLCG)	NCT04385290
• All comers • No CBF-AML • No -5 or -7 • No *FLT3*-ITD or –TKD	≥18	Selinexor	Selinexor +7+3	7+3	OS	100	Pardee/NCI, USA	NCT02835222
• All comers • No *FLT3*-ITD or –TKD	18–75	Pembrolizumab	Pembrolizumab +7+3	7+3	MRDneg CR	124	Zeidan/NCI, USA	NCT04214249
• AML MR (WHO 2022) or AML with MR genetic changes (ICC 2022) or AML from MDS/MPN	18- 75	Pomalidomide	Pomalidomide + CPX-351	CPX-351	CR/CRi	78	Zeidner/NCI, USA	NCT04802161

Table 2. Selection of RCTs randomly evaluating modifications in intensive chemotherapy in newly diagnosed fit patients (*Continued*)

Target population/AML subgroup*	Age (years)	Experimental agent/intervention	Experimental arm	Control arm	Primary endpoint†	Planned patient number	PI, country (cooperative group)	Registry number
• MDS-IB2, MDS/AML, AML • Increased TRM score (less fit)	≥18	CPX-351	CPX-351	CLAG-M	OS	60	Walter, USA	NCT04195945
• HR-MDS, AML ≤30% blasts	18–75	CPX-351	CPX-351 before alloHCT	7+3 or Aza before alloHCT	EFS	150	Platzbecker, Germany (SAL)	NCT04061239
• All comers with • No *FLT3*-ITD or –TKD, no *NPM1* • No CBF or APL • No AML-MRC	≥50	CPX-351	CPX-351	7+3	MRDneg CR/CRi	210	Foussat, France (ALFA)	NCT05260528
• Intermediate/adverse risk (ELN2017) including AML-MRC and tAML	≥18	CPX-351	CPX-351	7+3	OS in *de novo* AML	882	Döhner, Germany (AMLSG)	NCT03897127
• All comers in CR after intensive induction	60–75	Idarubicin	Idarubicin + IDAC§ for consolidation	IDAC for consolidation	RFS	320	Hu, China	NCT04216771
• *FLT3*-ITD AML	18–65	Treatment intensification based on early blast clearance during 7+3 + midostaurin	HIDAC-based second induction and early alloHCT	Standard second induction and postremission treatment	EFS	172	Vannucchi, Italy (GIMEMA)	NCT04174612
• Intermediate risk in CR after induction	14–60	Decitabine	Decitabine + IDAC consolidation	IDAC consolidation	MRD	100	Jiang, China	NCT03417427
• CBF-AML in CR after intensive induction	18–60	Fludarabine	Fludarabine + IDAC for consolidation	HDAC for consolidation	Relapse rate	200	Song, China	NCT02926586

Selection of trials currently recruiting or planned at the time of writing. Trials for unfit patients, children, APL, relapsed or refractory disease and with purely maintenance or conditioning questions were excluded.

CBF, core binding factor; CRi, complete hematologic remission with incomplete hematologic recovery; CRmol, molecular CR; DA, daunorubicin plus cytarabine (ara-c); EFS, event-free survival; GO, gemtuzumab ozogamicin; HDAC, high-dose cytarabine; IDAC, intermediate-dose cytarabine; LDAC, low-dose cytarabine; MRD, measurable residual disease; MRDneg, MRD negativity; mut, mutation; OS, overall survival; PI, principal investigator; RFS, relapse-free survival; TRM, treatment-related mortality.

*sAML/tAML/HMA pretreatment not accounted for/mentioned in table.

‡"7+3" stands for all variations of standard-dose cytarabine plus anthracycline/mitoxantrone and includes intensive consolidation for patients ineligible for allogeneic HCT. †Secondary end points for all trials include response rates, MRD, tolerability, rate of allogeneic HCT, patient-reported outcomes, and survival end points. §IDAC=intermediate-dose cytarabine.

Cytarabine dose and schedule

Randomized comparisons suggest that 100 and 200 mg of continuous daily cytarabine are equally efficacious,[19,20] and there is no evidence on whether 7 or 10 or 5 days are better. However, the most extensive body of knowledge exists for 7 days of continuous infusion as it has been most widely used, also in establishing novel agent combinations. Higher doses of cytarabine have resulted in higher remission rates and superior relapse-free survival (RFS) than standard doses, but long-term beneficial effects on overall survival (OS) across all subgroups could not be shown.[21-24] Time-sequential splitting of induction shortens the critical leukopenia time, but its antileukemic efficacy is not significantly better than that of standard induction.[25] Based on this data, I use a dose of 100-200 mg/m^2 cytarabine as continuous infusion over 7 days in my clinical practice.

Anthracycline type, dose, and schedule

As the oldest anthracycline, experience and data are most robust for daunorubicin, but the anthracycline idarubicin and the anthracenedione mitoxantrone are equally effective according to randomized comparisons.[26,27] There is a lack of convincing randomized evidence for the acridine derivative m-amsacrine in induction treatment, not even in patients with impaired cardiac function.[28] To reduce the risk of cardiotoxicity, a cumulative dose threshold around 500 mg/m^2 daunorubicin equivalents or preexisting cardiac insufficiency should be considered as relative contraindication for anthracycline use. There are no comparisons indicating that application on days 1-3 versus 3-5 is more efficacious. The optimal dose of daunorubicin has been the subject of several randomized controlled trials (RCTs). Whereas 90 mg/m^2 is more efficacious than 45 mg/m^2 based on 3 randomized trials,[29-31] 60 mg/m^2 is equally effective as 90 mg/m^2, as shown in 2 randomized studies.[7,32] The NCRI-AML17 study showed a significant benefit of 90 mg in *FLT3*-ITD-mutated patients,[33] but this could not be reproduced in the DaunoDouble trial, possibly due to partial use of the FLT3 inhibitor midostaurin after its approval in the latter study.[7] Considering these results, I recommend 60 mg/m^2 daunorubicin over 3 days or, if not available, idarubicin 12 mg/m^2 as an alternative.

Additional nontargeted agents

Several attempts have been made to improve the efficacy of the cytarabine-anthracycline doublet by adding a third cytostatic drug. Thioguanine has historically been part of several induction regimens, but its potential additional benefit has never been assessed in randomized comparisons. The addition of fludarabine does not improve the outcome of newly diagnosed patients.[34] It is part of the high-dose cytarabine-based relapse combination FLAG-Ida, also used in first line in the MRC-AML15 and NCRI-AML19 trials, showing lower relapse rates with increased hematotoxicity,[35] and suggesting a higher survival efficacy than cytarabine plus daunorubicin in some forms of secondary AML and *NPM1-FLT3*-mutated AML without the use of midostaurin.[36,37] However, as fludarabine is a fixed component of FLAG-Ida, its additional cytoreductive effect cannot be clearly isolated from the other components. Cladribine was able to increase the complete remission (CR) rates from 56% to 68% in the Polish PALG study, leading to similar RFS but significantly longer OS in the cladribine arm.[34] A similar trial in elderly patients from 60 years could show a significant CR improvement in the age subgroup 60-65 years and improved OS in patients with favorable and intermediate karyotypes, but not in the entire study population.[38] When lomustin was combined with a chemotherapy backbone comprising induction, consolidation with low-dose cytarabine plus idarubicin and 6 reinduction cycles of low-dose cytarabine plus idarubicin, and maintenance with 6-MP and MTX, the OS was significantly longer than without lomustin in a French RCT on elderly patients without unfavorable cytogenetics.[39] The addition of clofarabine to standard induction significantly reduced the time to CR and the relapse risk, but due to similar CR rates and increased toxicity, OS was similar compared to standard induction alone, with the exception of European LeukemiaNet (ELN) 2010 intermediate-risk patients, who had a significant survival prolongation.[40] Trials testing all-trans retinoic acid (ATRA) in combination intensive chemotherapy have delivered heterogeneous results. A significant survival benefit was detected in 1 trial with elderly AML patients,[41] whereas a different RCT in younger patients showed improved OS in the per-protocol population but not in the whole intent-to-treat population.[42] However, 2 other randomized studies could not show a beneficial effect of ATRA.[43,44] Last but not least, attempts to improve leukemia outcomes by adding etoposide[35] or priming leukemic blasts by granulocyte colony-stimulating factor were not successful.[43-46]

Number of induction cycles

Double induction in younger AML patients was introduced in the 1980s mainly in Europe to establish a standardized dose intensity without treatment delay caused by response assessments. A recent randomized comparison of patients with a good early response to induction cycle 1 showed no relevant differences in CR rates or survival for single versus double induction.[47] Regimens containing higher doses of cytarabine should be considered for fit patients not responding to a first cycle of 7+3.[48,49]

Specific modification in patient subgroups

With the development and approval of novel targeted agents, comprehensive pretherapeutic diagnostics have become very relevant and should be available preferably within 5 to 7 days. In clinically stable patients such as our 2 case patients, waiting for diagnostic results for several days does not negatively affect the prognosis, whereas AML-associated complications, namely leukostasis, tumor lysis syndrome, or disseminated intravascular coagulation, must trigger an immediate start of specific treatment.[50,51]

We could offer our case patients an approved specific combination treatment of a 7+3 backbone with novel specific agents if initial diagnostics showed either CBF-AML, *NPM1*-mutated AML, or *FLT3*-mutated AML, whereas in the case of treatment-related or myelodysplasia-associated AML, the liposomal formulation of daunorubicin and cytarabine CPX-351 would be offered instead of 7+3. Treatment options are shown in Figure 2.

Most recent data and open questions around novel agents include the second-generation tyrosinkinase inhibitor (TKI) quizartinib, the use of gemtuzumab ozogamicin in *NPM1*-mutated and elderly AML patients, and the value of CPX-351 and FLAG-Ida in secondary-type mutations.

Quizartinib is a second-generation type-II TKI with a high specificity for mutated *KIT* and *FLT3*-ITD, while not active in *FLT3*-TKD-mutated cells. In the randomized-controlled QuANTUM-

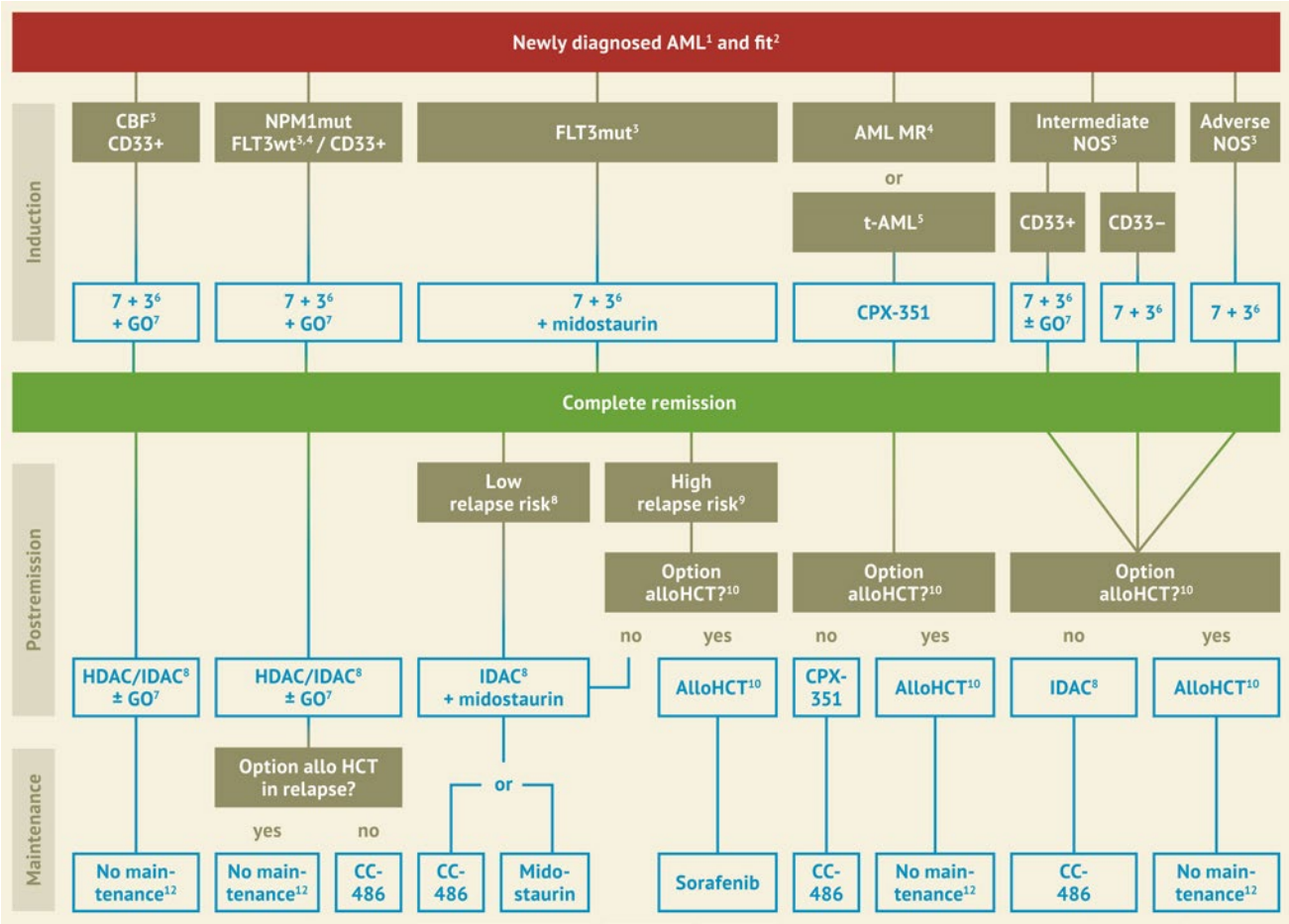

Figure 2. Treatment stratification for newly diagnosed patients fit for intensive treatment, modified after Onkopedia Guidelines for AML (www.onkopedia.com). [1]APL, acute promyelocytic leukemia excluded. [2]Fit for intensive therapy, based on ECOG status and comorbidity. [3]Genetically defined subgroups according to ELN 2022. [4]AML MR, AML with myelodysplasia-related changes (WHO) or entities "AML with myelodysplasia-related gene mutations", "AML with myelodysplasia-related cytogenetic abnormalities" and AML with the diagnostic qualifier "Progressed form MDS or MDS/MPN" (ICC). [5]t-AML, therapy-associated AML. [6]7+3, therapy regimen with cytarabine (Ara-C) on 7 days, daunorubicin on 3 days. [7]GO, gemtuzumab ozogamicin, recommended in patients up to 70 years. [8]HDAC, high-dose Ara-C; IDAC, intermediate-dose Ara-C. [9]Low risk of recurrence: *NPM1*-mut without relevant MRD. High risk of recurrence: *NPM1* wildtype or relevant MRD. [10]Allo HCT, allogeneic hematopoietic cell transplantation. [11]This recommendation includes *bZIP inframe CEBPA* mutated patients. [12]MRD monitoring recommended.

First trial, quizartinib or placebo were added to standard induction and chemoconsolidation treatment and as maintenance for up to 36 cycles after chemoconsolidation or allogeneic HCT in 539 fit newly diagnosed AML patients aged 18-75 years with an *FLT3*-ITD-mutation. While responses and event-free survival (EFS) were similar, quizartinib led to a significant prolongation of both RFS (median 39.3 versus 13.6 months, hazard ratio [HR] 0.61) and OS (median 31.9 versus 15.1 months, HR 0.78) with a similar toxicity profile as placebo.[52] In contrast to the pivotal trial for midostaurin (RATIFY),[53] the QuANTUM-First trial with quizartinib also enrolled patients ≥60 years, maintenance was 36 instead of 12 cycles and also allowed after allogeneic HCT. The results of the trial do not allow a direct comparison to midostaurin, but in the younger subgroup of patients 18-59 years, the HR for OS in *FLT3*-ITD patients was approximately 0.80 in RATIFY, whereas it was 0.68 in QuANTUM-First, indicating at least comparable or higher efficacy of quizartinib. Based on this data, the drug was approved by the FDA in July 2023 for combination with intensive chemotherapy and as maintenance in newly diagnosed *FLT3*-ITD-mutated AML. By the time of writing, trial data were still under review by the EMA.

Gemtuzumab ozogamicin (GO) is a well-established standard in patients with CBF-AML based on the results of the ALFA-0701 study and the meta-analysis of 5 randomized trials, showing a considerable survival prolongation in this subgroup, while the beneficial effect is smaller in intermediate-risk and absent in adverse-risk patients. The results of the AMLSG-0909 study have shown that patients with *NPM1* mutation also benefit from the addition of GO to intensive chemotherapy. A single dose of GO led to a deeper molecular remission and subsequently significantly prolonged RFS. Increased toxicity for the combination of GO with the quadruplet chemotherapy backbone (ICE plus ATRA) led to higher early mortality in patients 70 years and older and early crossing of EFS curves.[54] Subgroup analyses show a

significant EFS benefit in patients 18-60 and a trend for OS and EFS prolongation in patients 60-69 years. The results of the NCRI-AML18 study adding 1 versus 2 doses to induction with daunorubicin and cytarabine (DA) show higher rates of MRD negativity after 2 versus 1 cycles and an OS benefit in patients receiving allogeneic HCT as postremission treatment. The beneficial effect could not be observed in non-adverse-risk patients 70 years and older.[55] Although the study designs and data on GO are quite heterogeneous, these trial results confirm its potential to increase the depth of response in non-high-risk patients, prove a dose-response relationship, and indicate a caveat to use the drug in patients older than 70 years.

After the pivotal study for **CPX-351** enrolling older patients with secondary AML or MDS-like changes showed a significant improvement in OS with CPX-351 versus 7+3, the approved indication of CPX-351 was linked to the WHO category of AML-MRC. This category was revised both by the WHO and in a similar way by the ICC classifications in 2022.[56,57] Whereas the elimination of multilineage dysplasia at diagnosis as defining criterion for MDS relatedness from the definition and treatment indication of CPX-351 is evident due to the lack of prognostic significance and lack of study representation, the value of CPX-351 in patients with secondary-type mutations (STM: *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2*) is less clear. Retrospective analyses indicate that more than 40% of patients of the newly defined MDS relatedness categories are solely defined by STM mutations.[58] Post hoc analyses of the CPX-351 pivotal study show similar response rates and a trend for longer survival for CPX-351 versus 7+3 in STM patients, whereas no benefit was seen for *TP53*-mutated patients.[59] A small retrospective French analysis in CPX-351–treated patients shows superior OS in STM patients compared to other AMLs.[60] Finally, a post hoc analysis of the NCRI AML19 trial comparing FLAG-Ida induction with CPX-351 in younger, fit AML patients shows significantly longer survival after CPX-351 versus 7+3 in a small group of patients with STM and no adverse cytogenetics or *TP53* mutations.[36]

One day after the first bone marrow aspiration establishing the AML diagnosis in our 67-year-old woman, the RNA-based genetic result revealed the presence of a *RUNX1::RUNX1T1* fusion transcript. This established the WHO and ICC diagnosis of AML with the recurrent genetic abnormality and categorized the patient as favorable risk according to current ELN criteria. Additional genetic aberrations such as +8, which was later found in classic chromosome analysis, do not change the prognostic group. Based on the above-mentioned evidence, standard intensive induction treatment plus GO was recommended. Early marrow assessment on day 15 showed 3% myeloblasts, and on day 30, a CR was confirmed. Based on risk-benefit considerations, postremission chemotherapy with 3 cycles of intermediate-dose cytarabine was suggested to the patient. With this treatment, we can expect her long-term remission probability to be around 40%. If we bear in mind that this is the outcome for the best prognostic group in non-APL AML, it becomes clear that there is still considerable room for improvement, even in patients with "good" risk, but more so in all other risk groups.

The PCR-based mutational screening of our 70-year-old male patient showed *NPM1* and *FLT3*-ITD at an allelic ratio of 0.4, putting him in the intermediate-risk category of ELN 2022 and leading to the addition of midostaurin to 7+3 induction. Due to concerns about the renal function under immunosuppression and our patient's preference, chemoconsolidation plus midostaurin was chosen instead of allogeneic HCT as postremission treatment, followed by maintenance with CC-486 (oral azacitidine).

The future of intensive chemotherapy in AML

So far, we have looked at the evidence for intensive chemotherapy as the standard for curative treatment, and we have reviewed the approved novel agents used in addition to or instead of standard chemotherapy. However, since even favorable risk does not mean 90% to 100% cure, there is a clear need to develop this standard further. There are still several open questions in the context of intensive treatment ranging

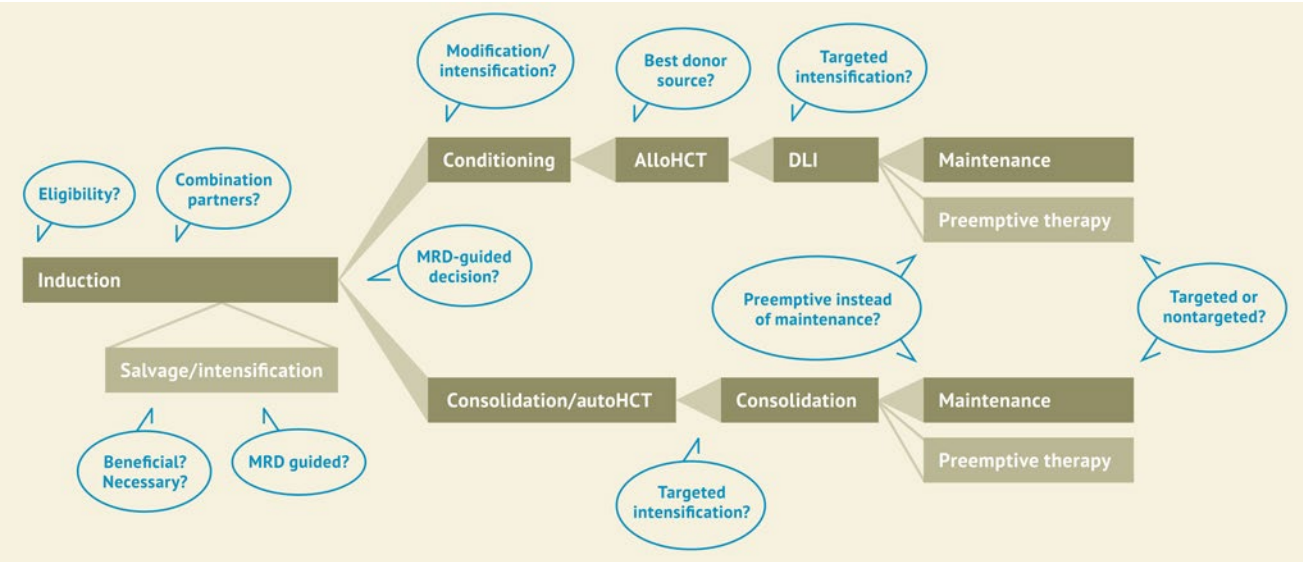

Figure 3. Open questions in intensive AML treatment.

from eligibility for intensive treatment based on fitness versus genomics, the value of MRD guidance, optimization options for allogeneic HCT, maintenance, and combination partners of standard chemotherapy (Figure 3). Based on 7+3 as a standard backbone, there are several ongoing studies that either (1) test the feasibility of single-agent treatment in combination with 7+3 or CPX-351 and (2) compare novel agent combinations head-to-head, but also (3) explore the many permutations of possible combinations of more than 1 novel agent with standard intensive therapy.

When we focus on intensive approaches in late clinical development currently evaluated in randomized trials, the intensive treatment standard 7+3 is combined either with subgroup-specific targeted agents or with drugs that work across different subgroups (Table 2).

General nontargeted approaches

Liposomal versus conventional 7+3: As the pivotal trial for CPX-351 focused on tAML and sAML in an elderly patient population, the AMLSG 30-18 trial is assessing the value of the liposomal agent in younger patients with intermediate and adverse genetic risk compared with standard 7+3 induction.

Venetoclax: Similarly, venetoclax or placebo is combined with standard intensive chemotherapy in newly diagnosed patients irrespective of their genetic profile in the AMLSG31-19/HOVON 501/AbbVie B18-982 study.

Targeted approaches

Several novel agents selectively inhibiting cellular pathways in genetically defined AML subgroups are tested in combination with intensive chemotherapy. The first step usually is to test the feasibility and efficacy of 1 novel agent versus placebo in combination with intensive chemotherapy. Novel agents currently evaluated are the IDH inhibitors ivosidenib and enasidenib, several menin inhibitors, the XPO1 inhibitor selinexor, the PD1-checkpoint inhibitor pembrolizumab, or the immune modulator pomalidomide.

Once established, longer approved novel agents can also be combined in addition to intensive chemotherapy. Apart from the question of additional beneficial effects, the excess hematologic toxicity remains the most burning open question to be answered here. One example for the latter development is the combination of midostaurin plus GO in addition to intensive standard treatment.

The third comparative study pattern is the head-to-head comparison of two novel agents such as the first- and second-generation FLT3 inhibitors midostaurin and gilteritinib in two RCTs and the second-generation FLT3 inhibitor crenolanib with midostaurin.

The long-term goal of treatment evolution in AML must be to increase the rates of long-term cure by intensifying and harnessing intensive chemotherapy to reduce toxicity and increase tolerability by dose reduction or substitution of particularly toxic components such as anthracyclines, and to further individualize treatment intensity using a standardized and refined MRD technology.

Although our 67-year-old female patient belonged to the "favorable" risk group with the rare CBF fusion transcript, long-term cure rates range only around 30% to 50%,[61-64] so that even she could benefit from novel options and should be offered a clinical trial. Currently, the combination of GO and midostaurin would be an option based on the finding that *KIT* is frequently overexpressed or mutated in CBF-AMLs and midostaurin is a KIT inhibitor. The *NPM1/FLT3*-ITD AML of our 70-year-old patient could be offered, eg, a trial comparing midostaurin with second-generation TKIs gilteritinib or crenolanib or combining midostaurin with GO.

Summary

With a chance for long-term remission of around 50% across all fit patients, treatment with intensive chemotherapy, followed by allogeneic stem cell transplant for appropriate patients, gives the highest potential for cure and forms the basis for further clinical development and treatment optimization in fit eligible patients.

The combination with more novel agents and advances in MRD detection and maintenance approaches will allow us to increase remission rates, deepen remission quality before postremission treatment, and prevent relapses to allow more patients to be cured in the future.

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Off-label drug use

Christoph Röllig: No off-label drug use outside the cited clinical trials.

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The approach of HMA plus VEN with or without BMT for all patients with AML

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Treatment options for acute myeloid leukemia (AML) have expanded over the last 5 years. New regimens are increasing the options for patients who previously may not have been offered any antineoplastic therapy. The use of the hypomethylating agent (HMA) decitabine or azacitidine combined with the BCL2 inhibitor venetoclax (HMA-VEN) has improved overall survival in an older and unfit population compared to HMA therapy alone. Delivering these regimens outside academic centers allows more patients with AML to be treated, though support and collaboration with allogeneic stem cell transplant (SCT) centers should still be considered to determine eligibility and promptly initiate a donor search for potential transplant candidates. Expanding the use of HMA-VEN to younger and fit patients who are also candidates for intensive chemotherapy (IC) is being studied prospectively and is not recommended at this time outside of a clinical trial. Retrospective studies suggest populations that may benefit from HMA-VEN over IC, but this is not yet confirmed prospectively. Utilizing HMA-VEN prior to allogeneic SCT is also under investigation, and some retrospective data show feasibility and the ability to achieve measurable residual disease negativity pretransplant. Upcoming prospective randomized clinical trials aim to answer the comparability or superiority of HMA-VEN vs IC in fit populations and its potential use as a standard pretransplant induction regimen.

LEARNING OBJECTIVES

- Describe the role of HMAs plus VEN in the treatment of older patients with AML
- Review data from the use of HMAs plus VEN in younger, fit patients with AML

CLINICAL CASE

A 61-year-old presents with a 4-week history of fatigue and dyspnea. Progressing symptoms over the last 3 days are unexplained bruising on the arms and legs and gingival tenderness. The patient's past medical history is pertinent for diffuse large B-cell lymphoma diagnosed 3 years ago and treated with six 21-day cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. The postchemotherapy treatment course was complicated by symptomatic lower-extremity edema and orthopnea 2 months after completion of chemotherapy, and subsequently the patient was found to have a decrease in cardiac ejection fraction from 65% prechemotherapy to 40%. The patient was evaluated and treated by oncocardiology with guideline-directed medical therapy. The patient's Eastern Cooperative Oncology Group (ECOG) performance status (PS) upon evaluation is 3, but history provided by the patient demonstrates an ECOG of 0 a few months ago. Mobility is severely limited, with dyspnea on exertion when arising from the chair or bed and significant fatigue, including sleeping 20 hours per day and being unable to perform some activities of daily living without assistance. The patient's complete blood count with differential 3 months ago was normal but now demonstrates a white blood cell count of 24 000 k/μL with 50% blasts, a hemoglobin level of 7.2 g/dL, and a platelet count of 65 000 k/μL. The physical examination is pertinent for normal jugular venous pressure, no peripheral edema, normal pulmonary function, mild splenomegaly, and ecchymoses. Peripheral blood flow cytometry demonstrates acute myeloid leukemia (AML). A repeat ejection fraction is 55%. Imaging demonstrates nodularity in the lung parenchyma and splenomegaly at 16 cm, both concerning for leukemic involvement. The patient is screened for available clinical trials but is excluded from open trials at the practice location due to an ECOG PS of 3. A bone marrow aspirate and biopsy confirm acute myelomonocytic leukemia without myelodysplastic changes. Rapid *TP53* and *FLT3* assays and fluorescence in

situ hybridization studies for −5,−7,−17p,inv16,t(8;21), and *MLL* rearrangement are all negative. Next-generation sequencing is pending. Therapeutic options are reviewed with the patient, who opts to pursue treatment. Following the American Society of Hematology (ASH) AML guidelines, the patient is started on azacitidine (AZA) at 75mg/m^2 on days 1 through 7 and venetoclax (VEN) on days 1 through 28 per a standard dose ramp-up for days 1 through 3. The dose is then adjusted for antifungal prophylaxis with a plan for disease assessment bone marrow biopsy between days 21 and 28.[1]

Introduction

AML treatment remains a challenge given its significant mortality despite the approval of new therapeutic options. In 2022 the American Cancer Society reported over 20 000 new index cases, with 11 310 deaths due to AML reported.[2] Of patients diagnosed with AML, only 28% of those aged 20 and older will be alive in 5 years.[3] Therapy for unfit or older adults presents a challenge as some will not tolerate intensive chemotherapy (IC).

Standard therapy for younger, fit patients has been IC since the 1970s, with anthracycline given for 3 days and cytarabine infusion for 7 days (commonly referred to as 7+3). Therapy for unfit patients was limited, with low-dose cytarabine (LDAC) data emerging in the 1980s. In the 2000s, drug approvals for myelodysplastic syndrome (MDS) included the hypomethylating agents (HMAs) AZA and decitabine. These agents began to be used off-label shortly thereafter for the treatment of AML in patients unfit for IC based on data from cooperative group trials in the 1980s that included patients with MDS and AML.

Fitness for IC is difficult to determine in some cases and is often subjective despite the increased use of geriatric assessment tools. In a disease such as AML, fitness can be rapidly affected by the disease itself, taking a patient who was "fit" a few weeks prior to presentation to a poor PS, as in our patient. Historically, many unfit patients older than 60 may have only been offered best supportive care. In our practice, when determining initial therapy, we consider both recent performance status and performance status at time of diagnosis. Consideration of prior anthracycline-induced heart failure and presenting performance status led against recommending treatment with liposomal daunorubicin and cytarabine (CPX-351) in this patient. However, CPX-351 is approved by the US Food and Drug Administration (FDA) and demonstrated superior overall survival (OS) compared to 7 plus 3 in a randomized phase 3 trial for newly diagnosed patients aged 60 to 75 years with therapy-related AML, AML with prior MDS or chronic myelomonocytic leukemia, or de novo AML with MDS-related cytogenetic abnormalities per 2008 World Health Organization criteria.[4]

Historical studies have shown that older patients given IC have inferior survival compared to younger patients. A study in 2020 reported that patients aged 60 and older had a 2-year OS of 15.8% compared to 78.6% in patients aged 40 or younger.[5] This location where patients receive their care also has an impact. In a 2016 retrospective study of a large community oncology practice analyzing newly diagnosed AML patients 60 years and older, only 5 of 922 patients received IC, and 43% received no antileukemic therapy.[6] The majority of patients treated received single-agent HMA therapy with decitabine or AZA.[6] Outcomes with single-agent HMAs were better than LDAC or best supportive care but lagged behind IC in rates of complete remission (CR), time to response, and OS, similar to reports in multiple other studies.[5,7-10] Community settings face challenges in providing intensive AML chemotherapy regimens, which often require weeks in the hospital and rigorous inpatient support and experience. A retrospective study evaluating outcomes in patients treated with IC in high-volume hospitals had lower mortality compared to low-volume hospitals (odds ratio, 0.8; 95% CI, 0.67-0.95; *P*=.01).[11]

Where HMA-VEN started

The inhibition of B cell lymphoma 2 (BCL2) protein has become an intriguing target in hematologic malignancies. Inhibiting BCL2 leads to the induction of apoptosis in blood cancer cells.[12] Multiple clinical trials were developed in the early 2010s utilizing VEN for treatment as a single agent or in combination therapy for a variety of hematologic malignancies. The first FDA approval came as a breakthrough designation in 2016 based on phase 2 data in chronic lymphocytic leukemia (NCT01889186) presented at the 2015 ASH annual meeting as a late breaking abstract.[13] Despite all AML cells not overexpressing BCL2, leukemia stem cells have dysregulated apopotosis.[14] VEN is a BCL homology domain (BH)-3 mimetic that is potent and highly selective for BCL2 (and not BCLXL, which is expressed on platelets) and able to inhibit BCL2, therefore increasing other proapoptotic factors to induce apoptosis in AML cells.[15] VEN was explored in a phase 2 study by Konopleva et al, where it was used as a single agent for patients unfit for IC and for relapsed/refractory patients.[16] This led to the exploration of combining it with other drugs, including HMAs. Preclinical work evaluating the synergism of VEN and HMAs ex vivo was described by Bogenberger et al in 2015.[17] Azacitidine reduces the antiapoptotic gene *MCL-1* and when combined with BCL2 inhibition was suggested to be moved into clinical studies. The phase 1b combination study of HMA-VEN in the frontline setting was published in 2018 by DiNardo et al and demonstrated safety, tolerability, and 60% CR and incomplete count recovery (CRi); therefore, it was the basis for future VIALE-A studies.[18] When combined with AZA, data have shown that leukemia stem cells can be targeted, leading to deeper remissions and achievement of measurable residual disease (MRD) negativity.[15,19]

How HMA-VEN is going

The treatment landscape for this older or unfit population changed in 2018 when the FDA granted accelerated approval to decitabine, AZA, or LDAC in combination with VEN. The pivotal phase 3 VIALE-A study of AZA plus or minus VEN and the VIALE-C study of LDAC plus or minus VEN led to regular FDA approval in 2020.[20]

VIALE-A included newly diagnosed AML in patients unfit for IC, defining unfit as aged 74 or over or aged 18 to 74 with comorbidities ineligible for IC.[21] Unfit for IC in this study was defined by a reduced cardiac ejection fraction of 50% or less, chronic angina, a pulmonary diffusion capacity of 65% or less or a forced expiratory volume of 65% or less, and a performance status of ECOG 2 or 3.[21] The majority of patients were over 75 (61% in the AZA-VEN group and 61% in the AZA-placebo group).[21] Patients were randomized 2:1 to receive AZA plus or minus VEN or placebo and met the primary end point of OS benefit at initial publication.[19] On long-term follow up reported in 2022, OS was 14.7

(95% CI, 12.1-18.7) months in the AZA-VEN arm vs 9.6 (95% CI, 7.4-12.7) months in the AZA-placebo arm.[21,22]

Updated data from community settings are not yet published, but with the rise of HMA-VEN combination therapy, AML treatment in a community setting has become more feasible. The availability of transfusion support, including weekend infusion and blood product availability, remains a challenge in many places. Partnership between community and academic settings is critical for optimal outcomes of this complex patient population, with benefits including increased clinical trial options, prompt allogeneic transplant eligibility determination, and shorter time to transplant.[23-25]

HMA-VEN use in relapsed or refractory AML has emerged within clinical practice and been explored within several retrospective studies and a few early phase prospective studies, but use in this setting remains an off-label indication. A 2022 article by Brancati S. et al highlighted the available prospective and retrospective data of use for HMA-VEN in relapsed/refractory patients with an ORR ranging from 38% to 62%, though it pointed to a much lower ORR in patients with prior HMA exposure.[26] Use as a salvage regimen requires further prospective studies.

CLINICAL CASE (continued)

Our patient, at age 61 with an ECOG PS of 3, would have met inclusion criteria in VIALE-A but would not have met inclusion criteria for liposomal CPX-351, and thus a less intensive regimen was chosen for the patient on the basis of performance status. Performance status impacted by disease burden is also a factor when determining treatment options, and IC could have been considered for this patient if the treating physician believed the symptoms to be reversible.

What is next for younger and fit patients?

The question of utilizing HMA-VEN combination for fit and/or younger patients or a direct comparator to IC has yet to be answered prospectively, but a large retrospective analysis by Cherry et al was performed on 143 patients who received AZA-VEN and 149 who received IC (Table 1).[27] The CR rate was 62.2% vs 64.4% in AZA-VEN vs IC, respectively.[27] Less than 25% of AZA-VEN patients went on to receive an allogeneic stem cell transplant (SCT) compared to 74.8% of those treated with IC.[27] After five years, OS favored IC over AZA-VEN (884 days vs 483 days).[27] Favorable survival in this case was likely largely impacted by who was offered and received allogeneic SCT, and when propensity matched controlling for age, European LeukemiaNet (ELN) risk group and transplant status, OS and progression-free survival were similar.[27] (Table 1).

CPX-351 was also retrospectively compared to AZA-VEN and included 217 patients who received CPX-351 and 439 who received AZA-VEN with a median OS of 13 months and 11 months, respectively (hazard ratio [HR] 0.88; 95% CI, 0.71-1.08; *P*=.22).[28] Of the 217 who received CPX-351, 61 (28%) went on to allogeneic SCT, and 44 (10%) underwent allogeneic SCT in the AZA-VEN group, with SCT in both groups improving OS (HR, 0.33; *P*≤.0005) regardless of the choice of therapy (HR, 0.97; *P*=.78).[28] The COVID-19 pandemic prompted the United Kingdom to expand the use of VEN to favorable-risk patients over 16 years of age with *NPM1* mutations (*FLT3-ITD* negative), patients under 50 with *NPM1* or *IDH1/2* mutations, and patients under 60 without favorable-risk cytogenetics.[29] Of the 301 patients registered, 85% received the AZA-VEN combination and 15% the LDAC and VEN, with a median age range that still skewed older at 72 (range, 34-90).[29] The composite CR rate was 70%, with higher rates seen in de novo, *NPM1*-mutated, *IDH1/2*-mutated, and ELN-favorable risk patients. Median OS in all patients was 12.8 months, with 71% of those who achieved CR with CRi alive at 1 year.[29]

IC has been the backbone of AML treatment for decades, with HMA-VEN thus far being the closest to having some equivalency to standard 7 plus 3 induction. The applicability of HMA-VEN to all patients is unlikely, but vigorous and thoughtful clinical trial design will hopefully lead to IC alternatives to reduce toxicity and hospitalization while retaining similar or superior efficacy.

Table 1. VEN and AZA compared with induction chemotherapy for newly diagnosed patients with acute myeloid leukemia

	Median OS (days)	*P*	ORR (%)	*P*	CR/CRi favored	OS favored
Entire cohort						
HMA-VEN (n=143)	483	.002	76.9	.2109	• Age >64 • *RUNXI* mutated • sAML	• Age >64 and *RUNX1* mutated
IC (n=149)	884		70.5		• Monocytic subtype	• Undifferentiated or minimal maturation subtypes • ELN intermediate risk • *FLT3-ITD* mutated • *RAS* mutated
Propensity matched for age, biological risk						
HMA-VEN	NR	.0667				
IC	705					

NR, not reached; ORR, overall response rate; sAML, secondary AML.
Reproduced from Cherry et al.[27]

Is HMA-VEN or IC better in certain populations?

In older populations, HMA-based induction has had some traction in favorable-risk AML patients excluded from the VIALE-A study population. A retrospective study presented at the 2021 ASH annual meeting reviewed ELN 2017 favorable-risk patients aged 70 and older. Ball et al found a 97% CR plus CRi rate, vs 66% in IC-treated patients (P=.0002).[30] Similar findings with *NPM1*-mutated patients have been seen retrospectively when compared with IC.[31] The CR rate was 89% in the HMA-VEN group and 85% in the IC group (P=.778; with median age significantly younger in the IC group, at 72 years vs 55 years, respectively).[31] Most notably, there was reduced risk of death in this older population compared to IC (HR, 0.31; 95% CI, 0.12-0.83; P=.038).[31]

Genomic subgroups are emerging that may also impact the choice of induction therapy between IC and HMA-VEN. Cherry et al identified *RUNX1* mutations favoring HMA-VEN (Table 1).[27] *IDH1/2* mutations were analyzed in the VIALE-A patient population and demonstrated composite CR rates of 79% and OS of 24.5 months.[32] When split out, *IDH2* mutations had a composite CR rate of 86% compared with 66.7% in the *IDH1*-mutant patients, and both maintained an advantage despite the cytogenetic risk group.[32] *TP53* mutations were also analyzed in the VIALE-A population, with OS of 5.2 months in the AZA-VEN group vs 4.9 months in the AZA monotherapy group, demonstrating no meaningful improvement in survival with the addition of VEN to AZA.[33] There was statistically significant improvement in the composite CR of AZA-VEN over AZA (41% vs 17%).[33] However, without improvement in OS the added toxicity of VEN calls its benefits into question in this population. A monocytic subtype was identified by Cherry et al (Table 1) as conferring an advantage to IC over HMA-VEN.[27] Pei et al also identified a loss of BCL2 target and heavy dependence of *MCL-1* in monocytic AML and suggested using flow cytometry to distinguish monocytic AML as a more mature entity and a potential future target to overcome resistance.[34]

The use of HMA-VEN instead of IC in younger, fit patients is the subject of current prospectively enrolling trials (Table 2). One is NCT03573024, a single-arm study enrolling fit patients aged 18 to 59, all receiving the AZA-VEN combination. Another phase 2 randomized, multicenter study comparing AZA-VEN to IC (7+3 or CPX-351) in fit, newly diagnosed patients is also currently enrolling (NCT04801797). The soon to launch National Cancer Institute–sponsored collaborative MyeloMATCH studies will enroll intermediate-risk (NCT05554393) and high-risk (NCT05554406) patients aged 18 to 59 to answer the question of IC plus or minus VEN vs HMA-VEN. Patients in the high-risk study will be randomized to 1 of 5 arms: cytarabine and daunorubicin (7+3); cytarabine, daunorubicin, and VEN (7+3 + VEN); AZA-VEN; CPX-351; CPX-351 and VEN. It is hoped that these trials will help determine outcomes for fit patients utilizing regimens other than the traditional 7 plus 3.

CLINICAL CASE (continued)

Returning to the clinical case, our patient clinically improved during treatment with VEN and AZA. By treatment day 5, the patient had improved dyspnea, and ECOG PS had improved to 2. Diagnostic testing demonstrated an *NRAS* mutation using next-generation sequencing and trisomy 8 using cytogenetics. The patient underwent evaluation by an allogeneic SCT physician, and a donor search was initiated. On day 21, a bone marrow biopsy was performed, which was 5% cellular with 1% blasts. VEN was held at this time, and upon absolute neutrophil count and platelet recovery 10 days later, the patient began cycle 2 of VEN-AZA, with an improved ECOG performance status of 1. Bone marrow aspiration/biopsy upon count recovery after cycle 2 demonstrated a normocellular marrow and 2% blasts (immunophenotypically normal). Multiparameter flow cytometry was negative along with normalization of cytogenetics, and *NRAS* mutation was not detected.

When does allogeneic SCT fit in with non-IC induction therapy?

One might think that if patient factors lead to the choice of a non-IC strategy such as HMA-VEN, then allogenic SCT is unlikely.

Table 2. Prospective studies evaluating younger, fit patients eligible for IC compared to AZA-VEN

Study	Phase	Type	Therapy	Key inclusion	Key exclusion
NCT04801797	II	Randomized	• IC • AZA-VEN	• Age 18+ • ECOG ≤2	• *FLT3* • Age <60 with *NPM1* mutated • Favorable risk
NCT03573024	II	Single arm	• AZA-VEN	• Age 18–59 • ECOG ≤2 • Adverse risk	• Willing to receive IC
NCT05554393 (MyeloMATCH)	II	Randomized	• 7+3 • 7+3 + VEN • AZA-VEN	• Age 18–59 • ECOG 0–3	• Favorable and adverse risk by ELN 2017 criteria • *FLT3-ITD/TKD* • Secondary or therapy-related AML
NCT05554406 (MyeloMATCH)	II	Randomized	• CPX-351 • 7+3 • AZA-VEN • 7+3+ VEN • CPX-351 + VEN	• Age 18–59 • ECOG 0–3 • Adverse risk per ELN 2017 criteria	• Favorable or intermediate risk • *FLT3- ITD/TKD*

Table 3. Currently reported studies utilizing HMA-VEN for remission induction prior to allogeneic SCT

Study	Type	Participating sites	Number of patients	12-month NRM (%)	12-month CIR	12-month RFS (%)	12-month OS (%)	Relapse (%)/median LFS (month)	OS (months)
Pasvolsky et al[35]	Retrospective	Multicenter	24	19.1		58	63		
Winters et al[36]	Retrospective	Single center	29			66.1	74.5		
Pollyea et al[37,38]	Retrospective	Single center	21	11		80			NR
Kennedy et al[39,40]	Retrospective	Multicenter	88	17	18		73		
Nizamuddin et al[41]	Retrospective	Single center	36					39/11.2	25.4
Rautenberg et al[42]	Retrospective	Single center	26			67	81		NR

CIR, cumulative index of relapse; NR, not reached; NRM, onrelapse mortality; RFS, relapse free survival; LFS, leukemia free survival.

Patients on HMA-VEN can show improvement in leukemia symptoms and therefore improved fitness and proceed to allogenic SCT. Several retrospective studies utilizing AZA-VEN as a path to allogeneic SCT demonstrated the feasibility of obtaining favorable outcomes and MRD negativity (Table 3). This is highlighted in a study showing that for patients who achieved a CR, CRi, or morphologic leukemia-free state, 70% of patients achieved MRD negativity by multiparameter flow cytometry in the AZA-VEN group compared to 64% in the IC arm (1 patient in the IC arm had refractory disease prior to allogeneic SCT).[35] Another study showed that those achieving a CR and MRD negativity with AZA-VEN, either in the frontline or relapsed settings, and then proceeding to SCT demonstrated a 1-year OS of 90% and 78%, respectively, and that those who proceeded to up-front allogeneic SCT lived longer than those who delayed allogeneic SCT.[36,37]

Prospective studies remain a need in this space. Gruppo Italiano Trapianto di Midollo Osseo recently completed a prospective multicenter study exploring HMA-VEN as a cytotoxic chemotherapy-free bridge to allogeneic SCT and enrolled newly diagnosed AML patients aged 60 to 75. Patients were treated with decitabine and VEN, and those who achieved CR, CRi, or morphologic leukemia-free state after 2 cycles proceeded to allogeneic SCT (NCT04476199). We eagerly await the results.

Conclusion

Our patient had a matched unrelated donor and proceeded to allogeneic SCT after achieving an MRD-negative CR after 2 cycles. The use of a nonintensive remission-induction strategy in this patient highlights the ability to improve initial performance status with disease control and shows that early evaluation for allogeneic SCT can improve outcomes for patients previously excluded from any antileukemic therapy. The use of HMA-VEN in a younger, fit population is best studied in the context of clinical trials, as its benefit over IC is yet to be demonstrated prospectively. HMA-VEN combinations expand options for antileukemia treatment beyond academic centers and large cities, and collaboration with leukemia centers is recommended for optimal long-term treatment planning for this complex patient population.

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The future paradigm of HMA + VEN or targeted inhibitor approaches: sequencing or triplet combinations in AML therapy

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The routine use of next-generation sequencing methods has underscored the genetic and clonal heterogeneity of acute myeloid leukemia (AML), subsequently ushering in an era of precision medicine–based targeted therapies exemplified by the small-molecule inhibitors of *FLT3*, *IDH1/IDH2*, and *BCL2*. This advent of targeted drugs in AML has broadened the spectrum of antileukemic therapies, and the approval of venetoclax in combination with a hypomethylating agent has been a welcome addition to our AML patients unable to tolerate intensive chemotherapy. Mounting evidence demonstrates that molecularly targeted agents combined with epigenetic therapies exhibit synergistic augmented leukemic cell kill compared to single-agent therapy. With such great power comes greater responsibility in determining the appropriate frontline AML treatment regimen in a molecularly defined subset and identifying safe and effective combination therapies with different mechanisms of action to outmaneuver primary and secondary resistance mechanisms in AML.

LEARNING OBJECTIVES

- Compare frontline treatment approaches in IC-ineligible *IDH1*-mutant AML and explain how to sequence targeted therapies upon relapse
- Evaluate frontline AZA-VEN therapy and targeted treatment options for IC-ineligible AML patients with *IDH2*, *FLT3*, *NPM1*, or *TP53* mutations

Introduction

The approval of venetoclax (VEN) and a hypomethylating agent (HMA; azacitidine [AZA] or decitabine) combination therapy for older adults (aged ≥75 years or not eligible to receive intensive chemotherapy [IC]) with newly diagnosed (ND) acute myeloid leukemia (AML) denotes a paradigm shift in the AML treatment landscape.[1] In the registrational VIALE-A trial for ND AML patients unable to receive IC (median age, 76 years), AZA-VEN combination therapy showed significantly improved composite complete remission (CRc; CR + CR with incomplete hematologic recovery [CRi], 66.4% vs 28.3%) and overall survival (OS) rates (14.7 vs 9.6 months) compared to AZA-placebo.[2] Long-term follow-up (43.2 months) of VIALE-A confirmed an ongoing survival benefit, albeit more pronounced in those who achieved measurable residual disease negativity.[3]

Nevertheless, this golden era of rationally targeted AML therapy is plagued by primary resistance and clonal evolution leading to adaptive resistance.[4] Specifically, in AML patients receiving frontline VEN-based therapy, kinase-activating mutations such as *FLT3*-ITD and *TP53* alterations appear to be the major drivers of adaptive drug resistance, while patients enriched for *NPM1* and/or *IDH1/IDH2* mutations appear particularly sensitive to VEN, with durable responses.[5] Sequential single-cell sequencing of diagnosis-remission-relapse samples of AML patients treated with HMA-VEN has illuminated confounding patterns of treatment resistance such as the expansion or acquisition of new *FLT3*-ITD clones or the parallel outgrowth of triple-mutant *FLT3*-ITD/*IDH1*/*NPM1* treatment-resistant subclones.[5] Furthermore, the emergence of *RAS* pathway mutations appears to be the leitmotif of AML treatment failure across the spectrum of HMA-VEN,[5] IDH inhibitors,[6,7] and FLT3 inhibitors (FLT3i).[8] In addition to the mutational spectrum and clonal hierarchy, other primary and acquired mechanisms of resistance, such as cell of origin/differentiation state (e.g., monocytic AML), pro- and antiapoptotic protein dynamics/*BAX* mutations, and mitochondrial metabolism alterations may also be at play.[9] This innate and treatment-induced selective pressure on AML clones has prompted

Table 1. Comparison of baseline demographics and efficacy outcomes in patients with ND *IDH1*^{mut} AML treated on the pivotal registrational trials of VIALE-A (pooled analysis) and AGILE

Efficacy-evaluable population	AZA with VEN N=33	IVO with AZA N=72
Age, median (range or IQR)	76 (64–90)	76 (58–84)
Response outcomes		
Composite CR (CRc*) rate	67%	53%
CR rate	27%	47%
Duration of response (mo)		
Median time to CRc (range)	1.2 (0.8–8.1)	4.0 (1.7–8.6)
Median duration of CRc (95% CI)	21.9 (7.8-NE)	NE (13.0-NE)
Survival outcomes (mo or percentage)		
Median OS (95% CI)	15.2 (7.0-NE)	24 (11.3–34.1)
Estimated 12-mo survival probability (95% CI)	57.6% (39.1–72.3)	~64%

IQR, interquartile range.
*Here defined as CR plus CRi in VIALE-A (pooled analysis) and CR/CRh in AGILE. CR plus CRi in AGILE was 54%.
Data derived from Pollyea et al[11] and Montesinos et al.[2]

unresolved questions regarding the categorical frontline treatment approach; namely, do we combine, add on, or sequence HMA-VEN and molecularly targeted therapies? Here we present case studies that illustrate the genomic complexity of AML and how we approach treatment in different settings.

Clinical scenario: older adult with ND *IDH1*-mutant AML

Mr. X, a 79-year-old man who dislikes hospitals, presented with a white blood cell (WBC) count of 1.0×10^9/L, hemoglobin level of 7.4 g/dL, and platelet count of 120×10^9/L during his periodic cardiac checkup. Bone marrow biopsy confirmed AML with 28% myeloblasts and trisomy 12, and molecular studies identified *NPM1* (variant allele fraction [VAF], 40%), *DNMT3A* (VAF, 35%), and *IDH1*-R132C (VAF, 48%) mutations. What is the appropriate frontline treatment option for this patient?

The National Comprehensive Cancer Network® version 3.2023 AML guidelines endorse either AZA-VEN or ivosidenib (IVO, an orally administered *IDH1*-targeted inhibitor) plus AZA as a category 1 recommendation (Table 1). Preclinical data have shown that *IDH1/2*-mutant cells exhibit BCL2 dependence, thus rendering them sensitive to VEN, a BCL2 inhibitor.[10] In the pooled analysis of AZA-VEN studies,[11] 44 patients with ND AML (n=498, 8%) harbored *IDH1*^{mut}. Among those who received AZA-VEN (n=33), the CRc and CR rate was 66.7% and 27%, respectively. Most patients achieved CR or CRi in the first cycle, the median duration of objective response (DoR) was 21.9 (95% CI, 7.8-NE [not estimable]) months, and median OS was 15.2 months (95% CI, 7.0-NE). In this pooled analysis of *IDH1/2*^{mut} AML patients treated with AZA-VEN (n=81), 87% had grade 3 or higher hematological adverse events (AEs); 42%, febrile neutropenia; and 46%, thrombocytopenia.[11]

In the phase 3 AGILE trial, IVO (500 mg daily) plus AZA (parenterally for 7 days) was compared to AZA-placebo in ND, IC-ineligible *IDH1*^{mut} AML patients.[2] In the intention-to-treat population (n=146), 72 patients received IVO-AZA: among those, the rate of CR or CR with partial hematological recovery (CRh) was 53%, including a CR rate of 47%. The median time to CR was 4.3 months (range, 1.7–9.2), and the median DoR was 22.1 months (95% CI, 13.0-NE). With a median follow-up of 12.4 months, median OS in the IVO-AZA arm was 24.0 months (95% CI, 11.3-34.1), compared to 7.9 months with AZA-placebo (*P*=.001). Among patients treated with IVO-AZA, 70% had grade ≥3 hematological AEs; 28%, febrile neutropenia; and 24%, thrombocytopenia. AEs of special interest (grade ≥3) included 10% QTc prolongation and 4% differentiation syndrome, which resolved with conservative management.[2]

In this case scenario, we will propose either AZA-VEN or IVO-AZA as a frontline treatment for IC-ineligible *IDH1*^{mut} AML after carefully considering the patient's comorbidities and the AE profile of the treatment regimen. Depending on test availability and institutional turnaround time for molecular studies, practical issues may arise with obtaining *IDH1* mutation status prior to initiating therapy, particularly in proliferative patients who may require more urgent treatment.

Relapsed/refractory *IDH1*-mutant AML

IVO or olutasidenib (OLU) is approved to treat relapsed/refractory (R/R) *IDH1*^{mut} AML (Table 2).[12] In the phase 1b study evaluating IVO in R/R *IDH1*^{mut} AML,[13] the rate of CR plus CRh was 30.4% (95% CI, 22.5-39.3), including a 21.6% CR rate in the primary efficacy population. With a median follow-up of 14.8 months (range, 0.2-30.3), the median duration of CR or CRh was 8.2 months (95% CI, 5.5-12.0), and the median OS was 8.8 months (95% CI, 6.7-10.2). AEs of special interest (grade ≥3) included QTc prolongation (7.8%) and IDH differentiation syndrome (3.9%). Of note, this study did not include patients exposed to VEN due to their contemporaneous approvals.[13]

OLU (FT-2102), an oral, selective *IDH1*^{mut} inhibitor, was approved in 2022 for R/R *IDH1*^{mut} AML. The phase 1 study evaluated OLU monotherapy and a combination with AZA (OLU-AZA) in both treatment-naive and R/R *IDH1*^{mut} AML/MDS, and a signal of efficacy was observed in all cohorts.[12] In the pivotal phase 2 cohort in R/R *IDH1*^{mut} AML, 153 *IDH1* inhibitor-naive patients received OLU at 150mg twice daily.[12] In this cohort the CR plus CRh rate was 35% (95% CI, 27.0-43.0), with a 32% CR rate. The

Table 2. Comparison of baseline demographics and efficacy outcomes in patients with R/R *IDH1*^{mut} AML treated on the pivotal registrational trials of IVO and OLU

Efficacy-evaluable population	IVO N=125	OLU N=147
Age, median (range or IQR)	67 (18–87)	71 (range, 32–87)
ECOG PS, n (%)		
0	27 (22)	45 (31)
1	64 (51)	76 (52)
2	32 (26)	23 (16)
3	2 (1)	0
AML type, n (%)		
De novo	83 (66)	97 (66)
Secondary	42 (34)	50 (34)
Cytogenetic risk, n (%)		
Favorable	–	6 (4)
Intermediate	66 (53)	107 (73)
Poor	38 (30)	25 (17)
Missing/unknown	21 (17)	9 (6)
Co-mutations, n (%)		
NPM1	24 (20)	31 (21)
FLT3	9 (8)	15 (10)
CEBPA	3 (3)	<10%
Prior regimens, median (range)	2 (1–6)	2 (1–7)
VEN, n (%)	0	12 (8)
HSCT, n (%)	36 (29)	17 (12)
Bone marrow blast percentage, median (range)	56 (0–98)	42 (4–98)
Response outcomes		
Composite CR (CR plus CRh) rate	30%	35%
CR rate	21%	32%
Duration of response (mo)		
Median time to CR/CRh (range)	2.7 (0.9–5.6)	1.9 (0.9–5.6)
Median duration of CR/CRh (95% CI)	8.2 (5.5–12.0)	25.9 (13.5-NE)
Survival outcomes (mo or percentage)		
Median OS (95% CI)	8.8 (6.7–10.2)	11.6 (8.9–15.5)*
Estimated 18-mo survival probability in patients with CR/CRh	50%	78%

ECOG, Eastern Cooperative Oncology Group; IQR, interquartile range; PS, performance status.
*In the 153-patient safety population (which included the 147-patient efficacy-evaluable population plus 6 patients with a lack of a centrally confirmed *IDH1* mutation).
Adapted from Venugopal and Watts.[41]

median duration of CR plus CRh was 25.9 months (95% CI, 13.5-NE), and median OS was 11.6 months (95% CI, 8.9-15.5). AEs of special interest (grade ≥3) included hepatic enzyme elevation (15%) and IDH differentiation syndrome (9%). In updated results from all phase 2 cohorts, 17 patients (15 receiving OLU monotherapy and 2 in combination with AZA) had prior exposure to VEN, of which 7 achieved a CR, CRh, or CRi (41%), and the median duration of CR/CRh (5 patients) was 18.5 plus months at the data cutoff date.[14,15] OLU-AZA was evaluated in multiple subcohorts on the phase 2 study and demonstrated durable clinical activity in treatment-naive AML and R/R patients without prior exposure to an HMA or *IDH1* inhibitor.[16] The activity of OLU after IVO treatment failure is unknown, although preclinical studies suggest it may have activity in second-site mutations.[17]

In R/R *IDH1*^{mut} AML, the management decision is dictated by the first-line treatment. Given available data, we would propose IVO or OLU monotherapy or an HMA-VEN regimen (prospective data are with decitabine) for patients with prior exposure to IC and consider OLU monotherapy for patients with prior exposure to AZA-VEN, given the limited prospective data.[18,19]

Clinical scenario: older adult with *IDH2*-mutant AML, treatment-naive and relapsed settings

For treatment-naive patients with *IDH2*^{mut} AML ineligible for IC, AZA-VEN is standard therapy. Enasidenib (ENA), an oral, selective *IDH2*^{mut} inhibitor, is approved in the R/R setting (Table 3.[20] In the pooled analysis of AZA-VEN studies,[11] 68 patients with ND AML (n=498, 14%) harbored *IDH2*^{mut}. Among those who received AZA-VEN (n=50), the CRc rate was 86% with a 56% CR rate. Most patients achieved their response (CR or CRi) in the first cycle, and the median DoR (95% CI, 16.7-NE) and median OS (95% CI, 17.6-NE) were not reached. The AG221-AML-005 trial evaluated ENA-AZA against AZA monotherapy in ND, IC-ineligible *IDH2*^{mut} AML (N=107). Among the efficacy-evaluable population (n=101), rates of CR plus CRh (57% vs 18%; *P*=.0002), CR (54% vs 12%; *P*<.0001), and median DoR (24.1 vs 9.9 months) were significantly higher in the ENA-AZA arm compared to AZA monotherapy. Nevertheless, there was no difference in median OS (15.9 vs 11.1 months; *P*=.11) between treatment arms, perhaps influenced by a lack of placebo control and the subsequent use of ENA in patients on the AZA monotherapy arm. Serious AEs included febrile neutropenia (13%) and differentiation syndrome (10%).[21] In a single institutional study of ENA-AZA in patients with *IDH2*^{mut} AML, outcomes were better in those with early vs late relapse, and a small group of R/R patients with prior exposure to ENA or AZA showed a signal of activity with ENA-AZA-VEN triplet therapy.[22]

IC-ineligible adults with treatment-naive *IDH2*^{mut} AML are highly sensitive to AZA-VEN, and we would strongly recommend this approach as first-line therapy. In the relapsed setting post AZA-VEN, ENA monotherapy is the standard of care, and in R/R patients without prior exposure to HMA or VEN, ENA or an HMA-VEN regimen may be considered.

Future directions in *IDH1/2*^{mut} AML: triplet (AZA-VEN-IDH1/2 inhibitor) vs. sequencing with *IDH1/2* inhibitors

In a phase 1b study evaluating the safety and efficacy of IVO-VEN plus or minus AZA (IVO-VEN-AZA) in *IDH1*^{mut} myeloid malignancies (n=31),[23] the AE profile was similar to that of IVO or AZA monotherapy, and the maximum tolerated dose was not reached. Of note, VEN was given for 14 days starting with cycle 1 and was studied at both 400 mg and 800 mg daily given potential pharmacokinetic interactions with IVO. Clinical activity was observed with IVO-VEN and IVO-VEN-AZA (CRc, 83% and 90%, respectively) with no difference in survival between IVO-VEN and IVO-VEN-AZA (42.1 months vs not reached [NR]; *P*=.13), and enrollment continues on the IVO-VEN-AZA cohort.[23] In another nonrandomized study evaluating total oral therapy with cedazuridine-decitabine (days 1-5), VEN (days 1-14), and IVO or ENA, robust treatment response was observed in treatment-naive patients. In the R/R setting, responses were more pronounced in those without prior VEN exposure.[24] Triplet regimens may increase the likelihood of receiving a curative-intent hematopoietic stem cell transplant in a selected subset of older adults. The I-DATA study (NCT05401097) comparing the order of treatment with IVO-ENA monotherapy and AZA-VEN in treatment-naive, IC-ineligible patients should offer some clarity on the sequencing of these agents. Ultimately, randomized studies of AZA-VEN-IDH inhibitor vs AZA-VEN (possibly followed by an IDH inhibitor) are needed, and differences in sensitivity to AZA-VEN by the *IDH*^{mut} subtype and the unique clinical profiles of available IDH inhibitors should be considered. Switch maintenance studies (AZA-VEN followed by *IDH1* inhibitor-AZA once remission is achieved) are also under consideration.

Clinical scenario: older adult with relapsed *NPM1*^{mut} AML

Mr. X, now 81 years of age, presents with prolonged pancytopenia prior to starting his 14th cycle of IVO-AZA. The bone marrow biopsy evaluation confirmed relapsed AML, and molecular studies identified *NPM1* (VAF, 50%), *DNMT3A* (VAF-40%), and *BCOR* (VAF, 25%) mutations. What is the appropriate treatment regimen for this patient?

Approximately 10% to 40% of patients with *NPM1*^{mut} AML relapse, depending on various factors, and a single-center report suggests that adding VEN to either high- or low-intensity salvage

Table 3. Comparison of baseline demographics and efficacy outcomes in patients with *IDH2*^{mut} AML treated on the VIALE-A (pooled analysis), AG221-AML-005, and ENA (registrational trial)

Efficacy-evaluable population	AZA with VEN N=50	ENA with AZA N=68	ENA N=109
Age, median (range or IQR)	76 (64–90)	75 (70–79)	67 (19–100)
Trial design and setting	Phase 3, ND	Phase 1b/2, ND	Phase 1/2, relapsed/refractory
Response outcomes			
Composite CR (CRc*) rate	86%	57%	26.8%
CR rate	56%	54%	20.2%
Duration of response (mo)			
Median time to CRc (range or IQR)	1.1 (0.7–8.8)	4.6 (2.3–6.7)	3.7 (0.7–11.2)
Median duration of CRc (95% CI)	NE (16.7-NE)	NE (10.2-NE)	8.8 (0.7–11.2)
Survival outcomes (mo or percentage)			
Median OS (95% CI)	NE (17.6-NE)	22.0 (14.6-NE)	9.3 (8.2–10.9)
Estimated 12-mo survival probability (95% CI)	75.6% (61–85.3)	72% (60–82)	39%

IQR, interquartile range.
*Here defined as CR plus CRi in VIALE-A (pooled analysis) and enasidenib and CR plus CRh in AG221-AML-005. CR plus CRi in AG221-AML-005 was 63%.
Data derived from Pollyea et al[11]; DiNardo et al[21]; and Stein et al.[20]

regimens may improve outcomes in these patients.[25] In addition, preclinical studies have shown that deregulation of the *HOXA9/MEIS1* axis drives leukemogenesis in *NPM1*^{mut} AML and that menin-*MLL* inhibition abrogates this phenotype.[26] In a phase 1 study evaluating patients with R/R AML including *NPM1*^{mut}, revumenib (SNDX-5613), a selective oral menin inhibitor, demonstrated tolerability and clinical activity (CRc, 36% in *NPM1*^{mut} AML) in a heavily pretreated population. AEs of special interest included QTc prolongation (53%) and differentiation syndrome (16%).[27] In the absence of a clinical trial evaluating targeted therapies in *NPM1*^{mut} AML, we would recommend a VEN-based regimen for this patient with relapsed *NPM1*^{mut} AML after prior treatment with IVO-AZA.

Clinical scenario: older adult with *FLT3*^{mut} AML

Mr. Z, a 78-year-old man, presents with a WBC of 40×10^9/L, a hemoglobin level of 7.4 g/dL, and a platelet count of 70×10^9/L. Bone marrow biopsy confirms AML with 67% myeloblasts and a normal karyotype, and the molecular studies identify *NPM1* (VAF, 40%), *FLT3*-ITD (allele ratio, 0.83), and *IDH2* (VAF, 22%) mutations. What is the appropriate frontline treatment option for this patient?

Currently, there are no approved targeted options for treatment-naive patients with *FLT3*^{mut} AML who are IC ineligible. In the pooled analysis of AZA-VEN studies,[28] 64 patients with ND AML (n=498, 13%) harbored *FLT3*^{mut}. Among those who received AZA-VEN (n=42), 30 had *FLT3*-ITD and 12, *FLT3*-TKD mutations. The CRc rates in patients with *FLT3*-ITD and *FLT3*-TKD were 63.3% and 77%, respectively. Among these patients, median OS was longer in those with *FLT3*-TKD (19.2 months; 95% CI, 1.8-NE) compared to *FLT3*-ITD (9.9 months; 95% CI, 5.3-17.6).[28] In a proof-of-concept study allowing the addition of an FLT3i to a decitabine-VEN backbone (n=25), CRc rates were 92% (polymerase chain reaction/next-generation sequencing negativity, 91%) and 62% (polymerase chain reaction/next-generation sequencing negativity, 100%) in ND (IC-ineligible) and R/R *FLT3*^{mut} AML (including prior FLT3i exposed), respectively.[29] Triplet therapy combining the FLT3i gilteritinib (GILT) with AZA-VEN, with modified dosing for myelosuppression, is also being evaluated in this population,[30] and a multicenter study is planned (VICEROY).

In the R/R setting, the VEN-GILT doublet has shown promising clinical activity in a phase 1b study (n=61 patients with R/R *FLT3*^{mut} AML). The primary end point was a modified CRc rate (mCRc, CR minus CRi plus CRp plus morphologic leukemia-free state), and prior VEN or FLT3i (other than GILT) was allowed (64% had prior FLT3i exposure). VEN at 400 mg plus GILT at 120 mg/day was chosen as the recommended phase 2 dose based on clinical activity (75% mCRc rate) and tolerability. Cytopenias of grade 3 or above were observed in 80% of patients, and approximately 50% required dose interruptions secondary to VEN or GILT-related AEs. While VEN-GILT demonstrated activity even in patients with prior FLT3i exposure (67% mCRc rate), myelosuppression can be prolonged, and dose modifications for tolerability were common, highlighting the need for robust clinical trials to further examine the safety and efficacy of VEN-based combinations.[31]

In IC-ineligible patients with *FLT3*^{mut}AML, we recommend AZA-VEN in the frontline setting and GILT monotherapy in the R/R setting, outside of a clinical trial.

Clinical scenario: older adult with *TP53*^{mut} AML

Ms. Y, a 76-year-old woman with a previous history of large-cell lymphoma treated with chemotherapy 5 years ago, presents with a WBC of 2.5×10^9/L, a hemoglobin level of 7.9 g/dL, and a platelet count of 45×10^9/L. Bone marrow biopsy confirms AML with 67% myeloblasts and a complex karyotype with 17p deletion, and molecular studies identified a *TP53* R282W (VAF, 73%) mutation. What is the appropriate frontline treatment option for this patient?

Improving the short-lived remissions and inferior survival in *TP53*^{mut} AML remains the Sisyphean endeavor of the leukemia research community. In particular, *TP53* multihit status is notoriously refractory to treatment.[32] Retrospective and prospective studies have shown that adding VEN to HMA or chemotherapy does not improve survival in *TP53*^{mut} AML.[33,34] In the pooled analysis of AZA-VEN studies, there was no difference in OS between those who received AZA-VEN and AZA (5.2 vs 4.9 months).[35] Magrolimab (MAGRO) is a first-in-class humanized anti-CD47 antibody that blocks CD47 binding to signal-regulatory protein-α and promotes phagocytosis of leukemic cells.[36] MAGRO-AZA has been evaluated in patients with untreated higher-risk myelodysplastic syndrome (n=95),[37] of which 26.3% harbored a *TP53* mutation (n=25). Anemia was the most common MAGRO-related AE (37.9%), which resolved with subsequent cycles. Among those with *TP53*^{mut}, 10 (40.0%) achieved CR. At the median follow-up of 12.5 months, DoR was 7.6 months (95% CI, 3.1-13.4), and median OS was 16.3 months (95% CI, 10.8-NR) in *TP53*^{mut} patients.[37] Given this encouraging activity, the phase 3 ENHANCE-2 study is evaluating MAGRO-AZA vs AZA-VEN or IC in treatment-naive patients with *TP53*^{mut} AML (NCT04778397). Triplet therapy with MAGRO-AZA-VEN is underway and has demonstrated preliminary activity in frontline and R/R AML, *TP53*^{mut} and wild type,[38] and the phase 3 ENHANCE-3 study is evaluating triplet MAGRO-AZA-VEN vs AZA-VEN in all-comers with ND, IC-ineligible AML (NCT05079230). Immunotherapy approaches such as anti-CD3/CD123 bispecific antibodies, with or without an AZA-VEN backbone, may also have a role,[39,40] and other therapies exploiting CD47/signal-regulatory protein-α are under investigation.

Conclusions

Increased biological understanding of AML has translated into an expansion of the AML treatment landscape with rational combinations incorporating molecularly targeted therapies. In the era of precision medicine, tolerable regimens combining targeted agents with an HMA-VEN backbone may improve outcomes in IC-ineligible patients across various molecular subsets (*IDH1/2*, *NPM1*, *FLT3*, *TP53*). Ongoing and future clinical trial evaluations should guide us in the choice of treatment regimens, answering critical questions about topics such as the use of frontline triplet combinations, compared with the sequencing of targeted therapies. Triplet regimens may be associated with increased toxicity, and equipoise is needed to optimize treatment approaches and delineate survival advantages in multicenter trials. Despite unresolved questions, having these tailored treatment options available for our patients represents a major advance compared to less than a decade ago.

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For all our patients, who teach us so much every day.
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Conflict-of-interest disclosure

Sangeetha Venugopal: no competing financial interests to declare.

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Recognizing, defining, and managing CAR-T hematologic toxicities

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Autologous CAR-T cell therapy (CAR-T) has improved outcomes for patients with B-cell malignancies. It is associated with the well-described canonical toxicities cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), which may be abrogated by corticosteroids and the anti-IL6 receptor antagonist tocilizumab. Practitioners and researchers should be aware of additional toxicities. Here we review current understanding and management of hematologic toxicities after CAR-T, including cytopenias, coagulopathies, bleeding and clotting events, hemophagocytic-lymphohistiocytosis, and tumor lysis syndrome. We pay particular attention to cytopenias, recently termed immune effector cell-associated hematological toxicity (ICAHT). While the "H" is silent, hematotoxicity is not: ICAHT has the highest cumulative incidence of all immune adverse events following CAR-T. Early cytopenia (day 0–30) is closely linked to lymphodepleting chemotherapy and CRS-related inflammatory stressors. Late ICAHT (after day 30) can present either with or without antecedent count recovery (e.g., "intermittent" vs "aplastic" phenotype), and requires careful evaluation and management strategies. Growth factor support is the mainstay of treatment, with recent evidence demonstrating safety and feasibility of early granulocyte colony-stimulating factor (G-CSF) (e.g., within week 1). In G-CSF refractory cases, autologous stem cell boosts represent a promising treatment avenue, if available. The CAR-HEMATOTOX scoring system, validated for use across lymphoid malignancies (B-NHL, multiple myeloma), enables pretherapeutic risk assessment and presents the potential for risk-adapted management. Recent expert panels have led to diagnostic scoring criteria, severity grading systems, and management strategies for both ICAHT and the recently termed immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS), now clarified and defined as a distinct entity from CRS.

LEARNING OBJECTIVES

- Review the classifications, categories, incidence, and management of ICAHT
- Evaluate baseline and postinfusional risk factors for prolonged cytopenia and infectious complications
- Characterize further hematologic complications of CAR-T, including coagulopathies, bleeding and thrombosis, IEC-HS, and tumor lysis syndrome

Introduction

Chimeric antigen receptor (CAR) T-cell therapy has altered the treatment landscape for an ever-increasing number of relapsed and refractory B-cell malignancies,[1-7] but it requires specialized attention to recognize and manage a unique toxicity profile. Next to CRS and ICANS as prototypical CAR-T side effects, additional hematologic toxicities are both frequent and clinically relevant.[8] Cytopenia following CAR-T, recently termed immune effector cell-associated hematological toxicity (abbreviated as ICAHT and pronounced "eye-cat") is perhaps the most common noncanonical CAR-T toxicity.[9] A less frequently observed hematologic complication outside of CRS is a distinct hemophagocytic syndrome, recently termed immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS), which often manifests following resolution of CRS. Coagulopathies are common after CAR-T with both bleeding and thrombotic events experienced in some patients. Tumor lysis syndrome can manifest, although clinical impact is rare.

Here, we present a summary of our current understanding of the manifestations and management of these complications following CAR-T. This information is intended to allow practitioners to recognize and manage these complications promptly, to inform translational investigators trying to elucidate their mechanisms and optimal management approaches, and to prompt experts to action in further standardization of definitions and management algorithms.

CLINICAL CASE

A 62-year-old man with relapsed/refractory mantle cell lymphoma (MCL) was referred for CD19-directed CAR-T therapy with brexucabtagene autoleucel (brexu-cel). His prior disease course included chemoimmunotherapy, autologous transplantation, and ibrutinib. Chemotherapy bridging was employed during manufacturing. At lymphodepletion, his laboratory studies were notable for an elevated serum LDH, increased serum inflammatory markers (CRP 4.1 mg/dL, ferritin 881 ng/mL), and bilineage cytopenia (hemoglobin 8.8 g/dL, platelets 122 G/L). Baseline risk assessment was performed using the CAR-HEMATOTOX score, a risk stratification tool comprised of markers of hematopoietic function and baseline inflammation, with this patient presenting with a high-risk score of 4. Bone marrow studies revealed ~70% cellular involvement by blastoid MCL. On day 2 following CAR-T, the patient developed fever and progressive hemodynamic and respiratory insufficiency (maximal CRS grade 3), which resolved over the course of several days to grade 1 with administration of tocilizumab and corticosteroids. He also developed mild neurocognitive impairment on day 5 with a minimal ICE score of 8 (ICANS grade 1), which resolved on day 7. Following a rapid taper of steroids, on day 10, he presents with pronounced pancytopenia (ANC 0.1 G/L, Hb 7.1 g/dL, platelets 8 G/L).

Cytopenia after CAR-T: immune effector cell-associated hematological toxicity

Profound and/or prolonged cytopenias can predispose patients to significant infectious complications,[10] result in extended hospital stays,[11] and prevent subsequent salvage therapies at relapse.[12] However, the underlying pathophysiology remains enigmatic. While early cytopenia is expected after lymphodepleting chemotherapy, low counts can last weeks, months, or even years after CAR-T.[13] Neutrophil recovery typically follows either a biphasic or an aplastic trajectory (Figure 1).[8,14,15] Understanding underlying risk factors for hematotoxicity is critical for the application of risk-adapted management strategies.[9]

Incidence of ICAHT from pivotal trials to real-world evidence

Direct comparison of the incidence of post-CAR-T hematotoxicity, including cytopenias, across trials, and disease entities, is difficult due to differences in trial design, CAR construct, cohort size, and patient population. However, the observed degree and duration of hematotoxicity varies depending on the disease subtype (B-cell precursor acute lymphoblastic leukemia, B-NHL, multiple myeloma) and target antigen (CD19, BCMA) (Table 1). A recent meta-analysis demonstrated higher incidence of post-CAR-T cytopenia in BCP-ALL, likely related to extensive bone marrow infiltration or more intensive prior therapy.[16] High rates of cytopenia have also been noted for MCL, in line with the generally high toxicity burden in these

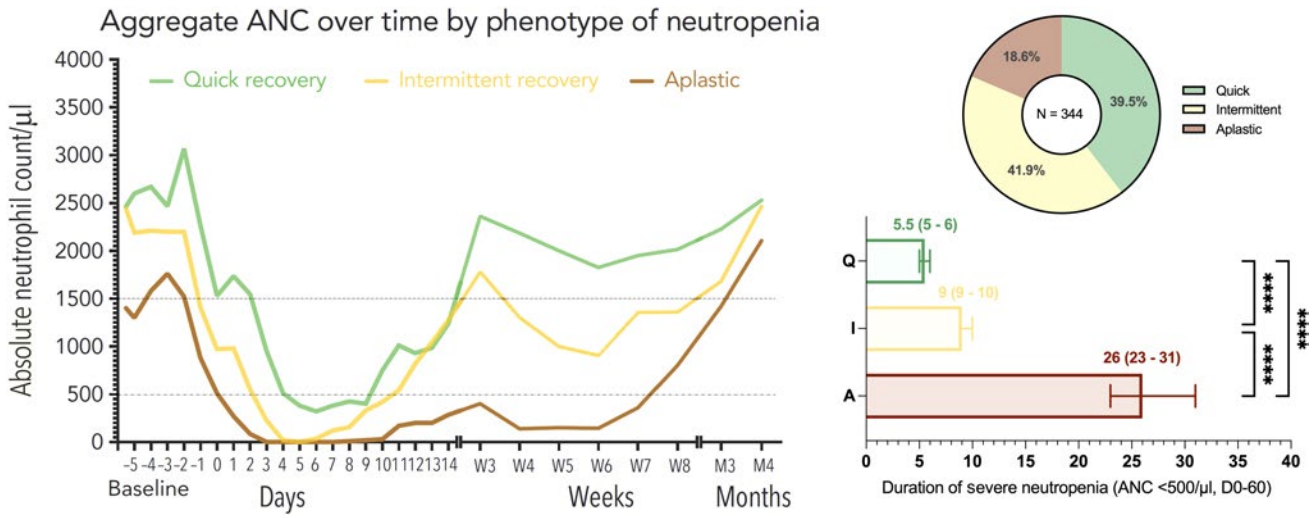

Figure 1. Phenotypes of neutrophil recovery following CAR T-cell therapy. Left panel: Quick recovery is defined as sustained neutrophil recovery without a second dip below an ANC <1000/µL. Intermittent neutrophil recovery (ANC >1500/µl) is followed by a second dip with an ANC <1000/µL after day 21. Aplastic is continuous severe neutropenia (ANC <500/µL) ≥14 days. (Adapted with permission from Rejeski et al., Blood. 2021.[8]) Right panel: Pie chart shows the relative distribution of neutrophil recovery phenotypes in a cohort of 344 relapsed/refractory LBCL patients treated with axi-cel or tisa-cel in a real-world setting. The duration of severe neutropenia (ANC <500/µL) during the first 60 days following CAR-T infusion is shown on the bottom. (Adapted with permission from Rejeski et al., ASH annual meeting 2022, abstract number 1987[21]). ANC, absolute neutrophil count.

Table 1. Incidence of post CAR-T cytopenias in clinical trials

Trial/product	Disease	Target/endodomain/vector	Lymphodepletion	Grade ≥3 neutropenia, %	Grade ≥3 thrombocytopenia, %	Grade ≥3 anemia, %	Reference
ZUMA-3 Brexu-cel	BCP-ALL	CD19/CD28z/RV	Flu 25 mg/m^2 × 3 Cy 900 mg/m^2 × 1	27%	30%	49%	Shah et al. Lancet 2021[3]
ZUMA-1 Axi-cel	LBCL	CD19/CD28z/RV	Flu 30 mg/m^2 × 3 d Cy 500 mg/m^2 × 3 d	78%	38%	43%	Locke et al. Lancet Oncol 2019[73]
JULIET Tisa-cel	LBCL	CD19/4–1BB/LV	Flu 25 mg/m^2× 3 d Cy 250 mg/m^2× 3 d	33%	28%	39%	Schuster et al. NEJM 2019[74]
TRANSCEND Liso-cel	LBCL	CD19/4–1BB/LV	Flu 30 mg/m^2× 3 d Cy 300 mg/m^2× 3 d	60%	27%	37%	Abramson et al. Lancet 2020[75]
ZUMA-7 Axi-cel	LBCL	CD19/CD28z/RV	Flu 30 mg/m^2× 3 d Cy 500 mg/m^2× 3 d	69%	15%	30%	Locke et al. NEJM 2022[1]
TRANSFORM Liso-cel	LBCL	CD19/4–1BB/LV	Flu 30 mg/m^2× 3 d Cy 300 mg/m^2× 3 d	82%	50%	52%	Abramson et al. Blood 2023[2]
ZUMA-2 Brexu-cel	MCL	CD19/CD28z/RV	Flu 30 mg/m^2× 3 d Cy 500 mg/m^2× 3 d	85%	51%	50%	Wang et al. NEJM 2020[4]
ELARA Tisa-cel	FL	CD19/4–1BB/LV	Flu 25 mg/m^2× 3 d Cy 250 mg/m^2× 3 d	32%	9%	13%	Fowler et al. Nat Med 2022[76]
ZUMA-5 Axi-cel	FL	CD19/CD28z/RV	Flu 30 mg/m^2× 3 d Cy 500 mg/m^2× 3 d	33%	9%	25%	Jacobson et al. Lancet Oncol 2022[77]
KarMMa-1 Ide-cel	MM	BCMA/4–1BB/LV	Flu 30 mg/m^2× 3 d Cy 300 mg/m^2× 3 d	89%	52%	60%	Munshi et al. NEJM 2021[78]
KarMMa-3 Ide-cel	MM	BCMA/4–1BB/LV	Flu 30 mg/m^2× 3 d Cy 300 mg/m^2× 3 d	76%	42%	51%	Rodriguez-Otero et al. NEJM 2023[79]
CARTITUDE-1 Cilta-cel	MM	BCMA/4–1BB/LV	Flu 30 mg/m^2× 3 d Cy 300 mg/m^2× 3 d	95%	60%	68%	Berdeja et al. Lancet 2021[80]
CARTITUDE-4 Cilta-cel	MM	BCMA/4–1BB/LV	Flu 30 mg/m^2× 3 d Cy 300 mg/m^2× 3 d	90%	41%	36%	San-Miguel et al. NEJM 2023[81]

Cytopenias are graded according to clinical trial reporting (common terminology of adverse events—CTCAE).
Axi-cel, axicabtagene ciloleucel; BCMA, B-cell maturation antigen; BCP-ALL, B-cell precursor acute lymphoblastic leukemia; brexu-cel, brexucabtagene autoleucel; CD, cluster of differentiation; cilta-cel, ciltacabtagene autoleucel; cy, cyclophosphamide; d, day; FL, follicular lymphoma; flu, fludarabine; ide-cel, idecabtagene vicleucel; G, grade; MCL, mantle cell lymphoma; MM, multiple myeloma; LBCL, large B-cell lymphoma; LV, lentiviral vector; liso-cel, lisocabtagene maraleucel; RV, γ-retroviral vector; tisa-cel, tisagenlecleucel.

patients.[17-19] Conversely, CAR-T trials for follicular lymphoma demonstrated low rates of hematotoxicity (Table 1), although real-world evidence is needed to confirm. When considering the co-stimulatory domain, matched comparison has revealed increased cytopenias in patients receiving CAR products harboring a CD28z as opposed to 4-1BB co-stimulatory domain.[16,20]

Real-world studies have confirmed the high rate of grade 3 or higher hematological toxicity and especially prolonged cytopenias following both CD19- and BCMA-directed CAR T-cell therapy (Table 2). Detailed studies on the nature of CAR-T-related cytopenias have shed light on the biphasic pattern of neutropenia, with second or even multiple decreases.[14] There are three distinct *phenotypes* of post-CAR-T neutrophil recovery (Figure 1).[8] These range from transient lymphodepletion-associated cytopenia ("quick") to the aforementioned biphasic course ("intermittent") and the clinically challenging "aplastic" phenotype associated with high morbidity and mortality.[15,21] The relative distribution of these phenotypes after CAR-T therapy is approximately 40%, 40%, and 20% (quick vs intermittent vs aplastic). Patients with the aplastic phenotype are often refractory to G-CSF and can develop prolonged neutropenia (Figure 1 bottom right shows median duration of severe neutropenia is 26 days). Interestingly, biphasic neutrophil recovery is linked to favorable treatment outcomes and higher levels of CAR T-cell expansion and persistence.[21] Of note, the thrombocytopenic nadir is commonly observed in the second month following CAR-T infusion.[8]

Cytopenias can persist long after lymphodepletion and resolution of CRS and ICANS. However, there is marked heterogeneity in the reporting and definitions of these long-term hematological side effects across studies. To address this, an expert panel recently developed a consensus grading system for early (day 0-30) and prolonged/late (after day +30 and day +90 respectively) ICAHT (Table 3). These clear definitions will ease reporting in trials, enable comparative studies of ICAHT severity across disease entities and CAR products, and provide a basis for severity-based management recommendations.

Short-term management of ICAHT using granulocyte colony-stimulating factor (G-CSF)

The case highlights a key decision point in managing early ICAHT: namely, when to initiate G-CSF and whether to defer G-CSF in case of coincident immunotoxicity (e.g., severe CRS

Table 2. Definition and incidence of prolonged cytopenias in the real-world setting

Reference	Disease	Sample size	Product	Definition of prolonged, day	Neutropenia	Thrombocytopenia	Comments
Nahas et al. Leuk Lymph 2020[82]	LBCL	21	Axi-cel	42	38%	-	2 cases of MDS
Strati et al. Haematologica 2021[83]	LBCL	31	Axi-cel	30	29%	42%	MDS (*n*=4)**
Fried et al. BMT 2019[14]	ALL, B-NHL	35	Local product/ CD19/CD28	42	62%	44%	Biphasic neutropenia and thrombocytopenia; proposed mechanism for late CART cytopenia: SDF-1 alterations
Cordeiro et al. BBMT 2020[13]	ALL, B-NHL, CLL	86	Local product/ CD19/4-1BB	90	16%	-	4 cases of MDS, long-lasting nature of cytopenia after CAR-T infusion
Jain et al. Blood Adv 2020[28]	BCL, ALL MM	83	Axi-cel, tisa-cel, local product	90	20%	10%	Delayed hematopoietic recovery associated with high-grade CRS/ICANS, MDS (*n*=1)*
Rejeski et al. Blood 2021[8]	LBCL	235	Axi-cel, tisa-cel	21	64%	-	Description of 3 typical neutrophil recovery phenotypes; thrombo-cytopenic nadir in month 2; development of CAR-HEMATOTOX score with independent validation
Wang et al. Front Oncol 2021[84]	ALL	76	Local product/ CD19; CD22/ 4-1BB	80	70%	48%	
Logue et al. Haematologica 2021[41]	LBCL	85	Axi-cel	30	30%	26%	Description of long-term immune reconstitution in LBCL patients treated with axi-cel
Logue et al. Blood Adv 2022[42]	MM	52	Ide-cel	30	39%	51%	Real-world data on hematotoxicity in multiple myeloma
Juluri et al. Blood Adv 2022[53]	ALL, B-NHL, CLL	173	Local product/ CD19/4-1BB	28	9%	14%	F/U (40M): persistent neutropenia (9%) and thrombocytopenia (14%); association of hematotoxicity with high-grade CRS and CRS-related inflammatory patterns
Bethge et al. Blood 2022[85]	LBCL	319	Axi-cel, tisa-cel	28/100	26%/10%	67%/32%	Defined as absence of count recovery; delayed hematopoietic recovery associated with NRM
Bachy et al. Nat Med 2022[20]	DLBCL	418	Axi-cel, tisa-cel	30/90	17%/6%	16%/5%	Matched-paired comparison of CAR products; increased hematotoxicity incidence with axi-cel > tisa-cel
Penack et al. JITC 2023[86]	LBCL	398	Axi-cel, tisa-cel	30/100	severe (CTC grade ≥3) cytopenia: 9%/12%		Association between number of prior treatment lines and incidence of cytopenia

Axi-cel, axicabtagene ciloleucel; ALL, acute lymphoblastic leukemia; BCL, B-cell lymphoma; BCMA, B-cell maturation antigen; B-NHL, B-cell non-Hodgkin lymphoma; brexu-cel, brexucabtagene autoleucel; CD, cluster of differentiation; CLL, chronic lymphocytic leukemia; CRS, cytokine release syndrome; d, day; DLBCL, diffuse large B-cell lymphoma; F/U, follow-up; G, grade; ide-cel, idecabtagene vicleucel; ICANS, immune effector cell-associated neurotoxicity syndrome; M, month; M1, neutropenia/thrombocytopenia at 1 month; MDS, myelodysplastic syndrome; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NRM, non-relapse mortality; LBCL, large B-cell lymphoma; SDF-1, stromal cell-derived factor 1; tisa-cel, tisagenlecleucel.

*MDS diagnosed in relapse after CAR-T-cell treatment.

**No difference in MDS incidence between patients with and without >G3 cytopenia at M1.

Adapted with permission from Rejeski, Subklewe et al, Blood. 2023.[9]

Table 3. Novel ICAHT grading

Grading	I	II	III	IV
Early ICAHT (day 0–30)				
ANC ≤ 500/μL	<7 days	7–13 days	≥14 days	Never above 500/μL
ANC ≤ 100/μL	-	-	≥7 days	≥14 days
*Late ICAHT (after day +30)**				
ANC ≤ 1500/μL				
ANC ≤ 1000/μL				
ANC ≤ 500/μL				
ANC ≤ 100/μL				

*Measured ≥2 time points or non-transient neutropenia.

Adapted from Rejeski et al, Blood 2023 with permission.[9]

or ICANS, typically during the first 2 weeks). Hesitation to administer G-CSF stems from preclinical data suggesting that GM-CSF may exacerbate toxicities.[22] However, retrospective analyses from real-word data sets have demonstrated an acceptable safety profile with early G-CSF without increases in the rate of high-grade (e.g., ASTCT grade 3 or higher) CRS or ICANS.[23-27] For example, Miller et al showed that patients receiving prophylactic G-CSF prior to CAR-T infusion (mostly pegylated G-CSF) displayed faster neutrophil recovery, comparable treatment outcomes, and similar rates of severe ICANS.[26] Importantly, patients presenting with low-grade toxicity did not exhibit worsening CRS severity with G-CSF. A separate study by Lievin et al found that early G-CSF administration (day +2) reduced febrile neutropenia without increased high-grade CRS or ICANS.[25] Notably, G-CSF did not impact CAR-T expansion or efficacy.[24,25] Taken together, these data support early G-CSF in high-risk patients to shorten severe neutropenia and prevent infections. Nonetheless, the optimal day of initiation and G-CSF protocol (prophylactic vs early; pegylated vs non-pegylated) in the context of CAR-T remains unclear. It must be noted that most CAR-T patients (>80%) will adequately respond to growth factor support with count recovery.[12,28]

CLINICAL CASE (continued)

On day 21, the patient had continued pancytopenia despite daily G-CSF and was transfusion-dependent for platelets and red cells. He developed hospital-acquired pneumonia and received broad anti-infective treatment. Bone marrow studies demonstrated aplasia and no MCL. Viral causes and substrate deficiency were ruled out. Myelotoxic co-medications were paused.

Clinical management of G-CSF refractory ICAHT cases

Diagnostic evaluation

Cases of ICAHT in which patients do not respond to G-CSF can be challenging, and this typifies the aplastic neutrophil recovery phenotype.[8,12] For diagnostic workup, a judicious incremental approach is warranted (Table 4). A first diagnostic tier should rule out other causes of BM insufficiency, including medications, viral infections, substrate deficiency, and severe IEC-HS (further described later). In patients without prior prophylactic G-CSF, we advocate initiation of G-CSF support on day 5-7 in neutropenic patients, particularly those with a high baseline CAR-HEMATOTOX score and in case the first diagnostic tests are inconclusive. If counts have not recovered despite G-CSF support, BM aspiration and biopsy should be employed no later than day 28 to rule out persistent BM infiltration (e.g. progression), perform IEC-HS diagnostics, and evaluate for dysplasia indicative of underlying myeloid dyscrasias which can evolve rapidly following CAR-T infusion.[29] The longer cytopenia persists beyond CAR-T infusion, the greater the impetus to perform in-depth cytogenetic studies and/or next-generation sequencing (myeloid panel). This is particularly important for workup of prolonged (day 30-90) and late (beyond day 90) cases of marrow aplasia or new-onset cytopenias long after CAR-T infusion.

Therapeutic interventions for severe and/or persistent ICAHT

In G-CSF refractory cases with an available cryopreserved autologous or allogeneic stem cell product from a prior treatment line (Figure 2), hematopoietic cell boosts should be strongly considered given their favorable safety profile and encouraging engraftment rates.[30-34] Unfortunately, these are available in only a minority of cases, with myeloma patients sometimes having extra stem cells for a potential second consolidative transplant.[35] Other options include thrombopoietin receptor agonists (TPO-RA) such as eltrombopag or romiplostim, though the data are restricted to a few small case series.[36-38] The potential improvement of hematopoietic function would mirror the efficacy of TPO-RA in other cases of acquired BM failure.[40] If the underlying etiology is deemed associated with inflammation or is HLH-like in nature, anti-inflammatory measures such as pulse-dose steroids or anticytokine therapy with anakinra or tocilizumab can be pursued. If the options described above do not facilitate count recovery and grade 4 ICAHT persists, allogeneic hematopoietic cell transplantation (allo-HCT) represents the last resort. However, gradual count recovery can occur over weeks to months, and allo-HCT will inevitably eradicate CAR T-cells. Before commencing with allograft, it is imperative to carefully consider all factors including time from CAR-T, the possibility of spontaneous count recovery, the risk for fatal infections, the likelihood of disease progression, donor suitability/availability, and the patient's goals of care.[28,41,42] Regardless of the treatment

Table 4. Diagnostic workup

Diagnostic category	Included diagnostic tests	When to initiate	Additional comments
Basis workup (tier 1)	• Check medication list for myelotoxic co-medications • Rule out active infections: blood cultures, procalcitonin • Vitamin deficiency: B12, folic acid • Consider secondary HLH/MAS: serum ferritin	In case of severe neutropenia (ANC <500/µL) beyond day +7 after CAR-T infusion	Low threshold to perform (minimal workup)
Advanced workup in case of severe ICAHT (tier 2)	• Bone marrow aspiration and biopsy • Advanced viral studies (parvovirus B19, CMV)	Grade 3 or higher ICAHT beyond day +14	Especially in patients with underlying marrow infiltration
Clinical suspicion for therapy-related myeloid neoplasm	Immunohistochemistry, flow cytometry, cytogenetics; next-generation sequencing (myeloid panel)	In case of persistent bone marrow aplasia beyond 1 month; unclear and/or new-onset cytopenia; cytopenia refractory to therapeutic measures	t-MN after CAR-T therapy is an emerging field of study*

ANC, absolute neutrophil count; CMV, cytomegaly virus; HLH/MAS, hemophagocytic lymphohistiocytosis/macrophage activation syndrome; ICAHT, immune effector cell-associated hematotoxicity; t-MN, therapy-related myeloid neoplasm.

*Incidence rate as high as 6% of t-MN after CAR T-cell infusion (see Gurney et al., EHA 2023; abstract number S263[87]).

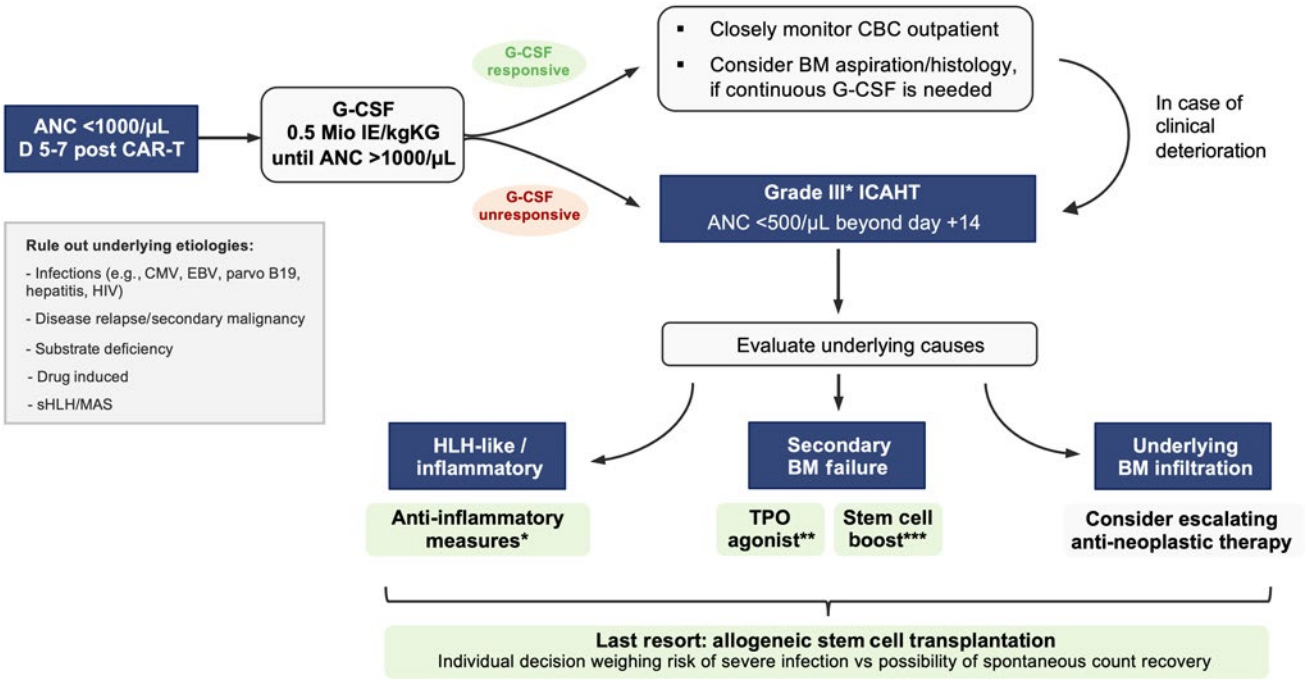

Figure 2. Treatment algorithm for immune effector cell associated hematotoxicity. *Consider dexamethasone-pulse (20 mg over 4 days) or anticytokine-therapy (e.g., anakinra or tocilizumab). **Consider eltrombopag (e.g., 50 mg×7 days). ***If available, contact apheresis unit.

strategy, mitigating the risk of severe infections with adequate and broad anti-infective therapy is critical. Fatal infections are possible and represent the main driver of non-relapse mortality.[11,15,43,44] Due to the broad spectrum of opportunistic pathogens, infectious disease consultation is recommended.

Moving toward risk-adapted management of ICAHT using the CAR-HEMATOTOX score

The management of CAR-T-related toxicities ideally should be tailored to the patient's individual risk profile. To this end, the CAR-HEMATOTOX (HT) score was developed and externally validated to predict severe hematotoxicity in relapsed and/or refractory (r/r) LBCL.[8] It was then subsequently extended to patients receiving CAR-T therapy for r/r MCL and multiple myeloma.[17,45] Calculated prior to lymphodepletion (day −5), the score incorporates both factors that relate to the baseline inflammatory state (e.g., C-reactive protein [CRP], ferritin) and the patient's hematopoietic reserve (e.g., hemoglobin, absolute neutrophil count [ANC], platelet count) (Figure 3). High-risk patients (score ≥2) exhibited increased rates of severe

infection (especially bacterial infections), higher non-relapse mortality, and inferior treatment outcomes compared to their low-risk counterparts (score 0-1).[11,45,46] We posit that the score may be used to restrict antibacterial prophylaxis and prophylactic G-CSF support to patients with a high-risk profile, as HT^{high} patients are more likely to benefit from these supportive measures because of their significantly higher rate of febrile neutropenia and infections.[11,26,45,47] On the other hand, HT^{low} patients may be suitable for avoidance of anti-infectives (i.e., fluoroquinolones, anti-mold agents), which may be beneficial due to the important role of an intact gut microbiome in the context of CAR-T therapy.[48,49] In addition, longitudinal measurements of serum procalcitonin (PCT) may be used to help rule out infections in the context of CRS (e.g., HT^{low} patients with non-elevated PCT at time of first fever).[46,50] Finally, the score may be useful to identify patients who require more extensive baseline diagnostic evaluations (e.g., pre-CAR-T BM biopsy). Prospective or retrospective data supporting or refuting these strategies should be generated. Limitations of the score relate to the lower specificity and positive predictive value and the fact that it has not been validated for use in BCP-ALL, follicular lymphoma, and pediatric patients (especially following relapse after hematopoietic stem cell transplantation).

Pathophysiology of ICAHT

The CAR-HEMATOTOX score underlines the importance of baseline hematopoietic function and systemic inflammation as risks for hematological toxicity. Baseline cytopenias likely reflect underlying impairment of the hematopoietic stem and progenitor cell (HSPC) compartment as a result of prior cytotoxic therapies.[51] They are also more frequently observed in cases of disease infiltration of the bone marrow, which represents an independent risk factor for hematotoxicity due to local inflammatory stressors and effects on HSPCs. In line with this observation, patients with prolonged cytopenias following BCMA-targeting CAR-T were more likely to display increased concentrations of CAR T-cells in the marrow post-infusion.[52] The high degree of systemic inflammation and secondary inflammatory insults that are stimulated by CAR-T infusion play a relevant pathophysiologic role, with several reports linking high-grade CRS and the associated inflammatory markers to prolonged cytopenias.[28,53] This may in part explain the more extensive hematotoxicity observed with the CD28z-endodomain harboring CAR-T products, although lymphodepletion dosing may also contribute.[5] In what manner the presence of preexisting clonal hematopoiesis of indeterminate potential (CHiP) may contribute to an underlying inflammatory state and subsequent development of prolonged cytopenias remains to be explored.[54,55]

Oligoclonal CAR-T-cell expansion and T-cell receptor restriction have been observed on the single-cell level in patients with protracted BM aplasia, together with an inflammatory micromilieu reminiscent of acquired aplastic anemia.[56] Strati et al employed single-cell RNA sequencing of bone marrow from patients with prolonged cytopenia to demonstrate that clonally expanded *CXCR1*^{hi}, *IFN-γ* expressing cytotoxic T cells were associated with hematopoietic stem cells (HSCs) that express IFN-γ response signatures.[57] This is consistent with data that IFN-γ can impair self-renewal and skew HSC differentiation.[58,59] Most importantly, these results expose potential therapeutic vulnerabilities, as IFN signaling can be targeted using IFN-neutralizing antibodies (e.g., emapalumab) or TPO-RA like eltrombopag.

Coagulopathy and hypofibrinogenemia

Patients undergoing CAR-T cell therapy are at risk for a spectrum of coagulopathies, from asymptomatic laboratory abnormalities to disseminated intravascular coagulation (DIC). Petechiae and ecchymosis are the most common manifestations of these coagulopathies. We discuss hemorrhage and bleeding complications, which are infrequent but potentially life-threatening, in a later section. Although coagulation abnormalities have been associated with poor progression-free survival (PFS), these reports

Prior to lymphodepleting chemotherapy (day -5)

→ Determine patient-individual risk of heme-tox and infections using the **CAR-HEMATOTOX score**

- Leniency time period for lab values: 3 days

Features	0 Point	1 Point	2 Points
Platelet count	> 175.000/µl	75.000 - 175.000/µl	< 75.000/µl
Absolute neutrophil count (ANC)	> 1200/µl	≤ 1200/µl	-
Hemoglobin	> 9.0 g/dl	≤ 9.0 g/dl	-
C-reactive protein (CRP)	< 3.0 mg/dl	≥ 3.0 mg/dl	-
Ferritin	< 650 ng/ml	650-2000 ng/ml	> 2000 ng/ml
Low: 0-1 High: ≥2			

Low risk (HT 0-1) | High risk (HT 2-7)

Risk profile

	LBCL (n = 235)	MCL (n = 103)	MM (n = 113)
Median duration of severe neutropenia (ANC<500/µL, D0-60)	5.5 days (95% CI 5-8 days)	6 days (95% CI 5-7 days)	3 days (95% CI 2-5 days)
Aplastic phenotype	2.6%	0%	3%
Severe infection rate	8%	5%	5%
Severe bacterial infection rate	0.9%	5%	3%

	LBCL (n = 235)	MCL (n = 103)	MM (n = 113)
Duration of severe neutropenia (ANC<500/µL, day 0-60)	12 days (95% CI 10-16 days)	14 days (95% CI 9-18 days)	9 days (95% CI 7-13 days)
Aplastic phenotype	36%	47%	32%
Severe infection rate	40%	30%	40%
Severe bacterial infection rate	27%	28%	34%

Figure 3. Using the CAR-HEMATOTOX score for risk-adapted toxicity management. Reproduced with permission from Rejeski et al., *Blood* 2023.[9]

are severely limited in their interpretability given their correlations to CRS and ICANS and other inflammatory markers such as CRP and ferritin, which are known to be higher in the face of an immunosuppressive tumor microenvironment and elevation of suppressive myeloid cells in the tumor and periphery.[60,61]

The common toxicities of CAR-T, CRS and ICANS, are associated with a systemic inflammatory response and endothelial breakdown, respectively, both of which are likely contributors to the pathogenesis of consumptive coagulopathy and DIC. In a trial of primarily adult ALL patients treated with CAR-T, increased IL-6 and severe CRS corresponded to laboratory-defined coagulopathies, including D-dimer elevation, increased fibrin degradation products, and activated partial thromboplastin time (aPTT) elongation, while treatment of CRS aligned with their resolution.[62] Elevations of platelet endothelial cell adhesion molecule-1 (PECAM-1) and tissue factor (TF) were also seen, consistent with a disruption of endothelial integrity in the setting of CRS and ICANS.[62] The incidence of severe CRS has decreased with intervention using tocilizumab and corticosteroids at earlier grades, and the impact of these interventions on the incidence of coagulation abnormalities remains unclear.[1,63]

The frequency and degree of coagulation testing is not standardized across CAR-T trials or in real-world settings. With frequent evaluation, over 50% of patients may be discovered to have coagulation abnormalities within the first month after receiving CAR-T. Prothrombin and thrombin time prolongation, aPTT, and D-dimer elevation typically peak within the first 6-9 days, while fibrinogen nadir may be slightly later at 12-14 days.[60,64] Several attempts have been made to apply standardized DIC criteria scores to laboratory results in CAR-T patients; however, an association with bleeding has not been seen.[62,64] For example, Johnsrud et al applied the International Society on Thrombosis and Hemostasis DIC criteria to LBCL CAR-T-treated patients and found high rates of DIC yet no correlation with bleeding events. They postulated that thrombocytopenia after CAR-T is more likely related to lymphodepleting chemotherapy or immune-mediated suppression rather than platelet consumption seen in classical DIC.[65] While evaluation of laboratory tests of coagulation may help aid decision-making and management in the CAR-T patient, the diagnostic utility of DIC-scoring algorithms remains uncertain, and more evaluation is needed. There is wide variation across practitioners and institutions on which tests, including D-dimer, fibrin degradation products, thrombin time, prothrombin time, aPTT, and fibrinogen, are checked and when. Furthermore, there is little data to indicate that utilization of these parameters to guide interventions improves outcomes. Our general practice in adult patients is to evaluate these parameters at baseline and again after CAR-T only when a patient experiences severe or refractory CRS or bleeding events.

Fibrinogen concentrate or cryoprecipitate can effectively correct hypofibrinogenemia in the setting of CAR-T therapy. In pediatric and adolescent/young adult ALL patients, severe CRS and consumptive coagulopathies are more commonly observed than in the adult population. Tisagenlecleucel investigators summarized coagulopathies seen in BCP-ALL patients and developed practical guidelines for monitoring and managing coagulopathies, particularly fibrinogen replacement. Despite the relatively common occurrence of hypofinrinogenemia, severe bleeding events were relatively rare, occurring in 1.4% of cases. Buechner and colleagues recommend intervening with cryoprecipitate or fibrinogen only when grade 3-4 CRS occurs and fibrinogen levels are very low at <1 g/L, replacing to >1.5 g/L until CRS resolves to <G3.[66] In a series of adult LBCL patients undergoing CAR-T, severe hypofibrinogenemia was detected in 6% of patients between day 0 and 100, and it was not associated with bleeding events. However, the fibrinogen level was only checked in 20% of patients and was at the discretion of the treating physician, thus preventing extrapolation of incidence or bleeding risk.[64] Although grade 3-4 CRS in adult patients is rare, and the incidence of concomitant hypofibrinogenemia is uncertain, practitioner awareness of these guidelines is important when the situation may arise.[66]

Bleeding and thrombosis after CAR-T

While severe bleeding events are rare, the risk of hemorrhage after CAR-T may be underappreciated. In pivotal trials of CAR-T cell therapy, severe bleeding events were rare. Bleeding events are most likely to occur within the first 30 days after CAR-T.[65] In one retrospective series, bleeding events were seen in 11% of LBCL CAR-T patients, a minority of which were severe, and they were seen more frequently in the elderly or those with prior bleeding events, those with baseline and concomitant thrombocytopenia, and patients with ICANS consistent with its known association with endothelial dysfunction.[65] Reports of cerebral bleeding events, particularly in the setting of CRS and ICANS, may be attributed to this endothelial dysfunction.[29]

The incidence of thrombotic events after CAR-T are variably seen in 2-11% of cases, having associations with D-dimer elevation and ICANS.[67] In contrast to bleeding events, thrombosis events are more likely to occur up to day +90. Importantly, anticoagulation medications, either as prophylactic or treatment, have not been associated with bleeding events. In a series of 148 LBCL CAR-T patients, 11% of patients experienced a thrombotic event, and these were safely managed with anticoagulation. In this series, routine prophylaxis was not employed. However, therapeutic anticoagulation after thrombotic events was not associated with bleeding.[64] A more recent series, also in LBCL patients, reported only a 2% rate of thrombotic events when prophylactic anticoagulation was used in most patients.[68] Taken together, these data suggest that patients at moderate to high risk of thrombosis or with a prior history of thrombosis requiring anticoagulation can safely receive prophylactic or therapeutic anticoagulation, respectively, after CAR-T with cessation at platelet counts <50,000/μL, and such intervention may reduce the incidence of thrombotic events after CAR-T.

Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS)

Secondary hemophagocytic lymphohistiocytosis can occur after CAR-T, typically with hemaphaogocytosis and other HLH-like features occurring after the resolution of acute CRS and, more commonly, with CD22-directed CAR-T.[69,70] Classical definitions of secondary HLH have diagnostic criteria that overlap with the common features of CRS, which itself is associated with the HLH adjacent macrophage activation syndrome. A need to further delineate CRS from this discrete CAR-T-related HLH entity led one panel of experts on HLH and cell therapy to define and name

it IEC-HS, as well as create a severity grading algorithm and management recommendations.[71]

According to the American Society for Transplantation and Cellular Therapy (ASTCT) expert panel, IEC-HS refers to "the development of a pathological and biochemical hyperinflammatory syndrome independent from CRS and ICANS that (1) manifests with features of macrophage activation/HLH, (2) is attributable to immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome therapy, and (3) is associated with progression or new onset of cytopenias, hyperferritinemia, coagulopathy with hypofibrinogenemia, and/or transaminitis."[71] A requirement for diagnosing the syndrome is an elevated and/or rapidly rising serum ferritin. As biphasic CRS can occur, classical recurrence of CRS as an alternative diagnosis should be considered.

Early recognition is critical, and alternative etiologies such as malignancy progression or infection should be ruled out. Initial therapy with the IL-1 receptor antagonist anakinra, with or without corticosteroids, is suggested. Following 48 hours for response assessment, additional agents such as the JAK1/2 inhibitor ruxolitinib, the anti-IFN-γ antibody emapalumab, or low-dose etoposide chemotherapy could be considered depending on the patient's trajectory.[71]

Tumor lysis syndrome

Although tumor lysis syndrome (TLS) following CAR-T cell therapy can occur, clinically significant manifestations of the syndrome are unusual in ALL, lymphoma, or myeloma patients and potentially more common in chronic lymphocytic leukemia. As with coagulopathies, evaluation of electrolyte abnormalities may suggest tumor lysis; however, clinical manifestations such as creatinine elevation may have multifactorial etiologies, including hypotension, in the setting of CRS or nephrotoxicity from concomitant co-medications.[72] In patients with a history of TLS or those with high circulating tumor burden, allopurinol prophylaxis can be considered.

Conclusion

Recognition of noncanonical hematologic toxicities after CAR-T cell therapy is critical for the practitioner. The development of uniform diagnostic criteria and severity grading systems will inform reporting on their incidence and help investigators elucidate their mechanisms. Significant strides have been made to define ICAHT and IEC-HS as distinct toxicity categories of CAR T-cell therapy. This now sets the stage for evaluating severity-based management recommendations and studying the applicability of these grading systems across disease entities and indications, including for solid tumors, pediatric patients, bispecific antibody therapies, and novel CAR constructs. Future studies will need to examine the potential of prophylactic stem cell collection in patients at ultra high risk for ICAHT, although this may incur an additional logistic burden and increase costs. More work must be done to quantify and define monitoring and management strategies for coagulopathies, bleeding and thrombosis events, and tumor lysis after CAR-T. Emerging areas of interest relate to the association between CHiP and the development of prolonged cytopenias, CRS, ICANS, and therapy-related myeloid neoplasms. Innovative single-cell and multi-omic approaches and modern imaging techniques may help to uncover the still vexing pathophysiology of ICAHT and IEC-HS. Finally, these efforts must ideally leverage the power of multicenter collaborations that span multiple CAR-T centers and countries to optimize resources and leverage diverse patient populations.

Authorship

*Kai Rejeski and Marion Subklewe contributed equally to this article.

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Off-label drug use

Kai Rejeski: nothing to disclose.
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Checkpoint inhibitors

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Immune checkpoint inhibitors are a class of antineoplastic therapies that unleash immune cells to kill malignant cells. These medications commonly cause immune-related adverse effects due to activated adaptive and innate immune cells, autoantibody production, and/or cytokine dysregulation. Hematologic toxicities are rare and of uncertain mechanism, and therefore management is often based on experiences with familiar conditions involving these perturbed immune responses. Management is challenging because one must attend to the hematologic toxicity while simultaneously attending to the malignancy, with the imperative that therapeutic effects be maintained or minimally interrupted when possible.

LEARNING OBJECTIVES

- Recognize ICI toxicity within a complex clinical milieu
- Manage hematologic toxicity without threatening the management of malignancy

CLINICAL CASE

A 39-year-old woman was diagnosed with stage IV M1d melanoma metastatic to the liver, bone, soft tissues, lymph nodes, and brain. She was started on dual checkpoint inhibitor therapy with ipilimumab + nivolumab and completed 4 cycles followed by maintenance nivolumab. Four months into maintenance therapy, PET-CT revealed a good overall response but a single new metastasis in the left ilium. She continued nivolumab and received radiation therapy to the bone lesion. Two months after completing radiation therapy, PET-CT showed a new fluorodeoxyglucose-avid left common iliac node. Single-agent nivolumab was stopped and dual checkpoint inhibitor therapy reinitiated. She received ipilimumab + nivolumab for 3 cycles, at which time it was stopped because of continued disease progression. While red cell and platelet counts remained near normal during the second round of dual checkpoint therapy, the absolute neutrophil count (ANC) fell from 2990/μL before it was resumed to 1250/μL after 2 cycles to 10/μL after 3 cycles. At no time were there fevers, mouth sores, or any other symptoms or signs of infection.

What are immune checkpoint inhibitors?

Ipilimumab is a human IgG4 monoclonal antibody that binds and inhibits cytotoxic T lymphocyte associated protein-4 (CTLA-4). CTLA-4 is a protein receptor expressed on activated CD4+ and CD8+ lymphocytes that inhibits these immune cells. It does this by directly transducing an inhibitory signal and by subverting a stimulatory pathway mediated by lymphocyte CD28 binding to CD80/86 present on antigen-presenting dendritic cells (APCs) and macrophages. It subverts CD28-mediated lymphocyte activation because its higher affinity for CD80/86 results in competitive inhibition of CD28 binding to CD80/86 (Figure 1).

Nivolumab is a human IgG4 monoclonal antibody that binds to and inhibits programmed cell death protein-1 (PD-1). PD-1 is a protein receptor found on activated CD4+ and CD8+ T lymphocytes, as well as on B lymphocytes, macrophages, natural killer cells, and myeloid-derived suppressor cells. It binds to programmed death-ligand 1 (PD-L1) expressed on antigen-presenting dendritic cells and macrophages and tumor cells, and this binding transduces inhibitory signals (Figure 2).

Most FDA-approved immune checkpoint inhibitors (ICIs) are directed against PD-1 or PD-L1; ipilimumab is the only available CTLA-4 inhibitor.[1] Many clinical trials are underway evaluating checkpoint inhibitors of B lymphocytes,[2] as well as checkpoint inhibitors of innate immunity mediated by natural killer cells, neutrophils, macrophages, and myeloid-derived suppressor cells.[3-5]

Is this patient's severe neutropenia caused by ipilimumab + nivolumab?

The parsimonious explanation is that this is ICI toxicity. While rare, ICI-associated neutropenia and agranulocytosis

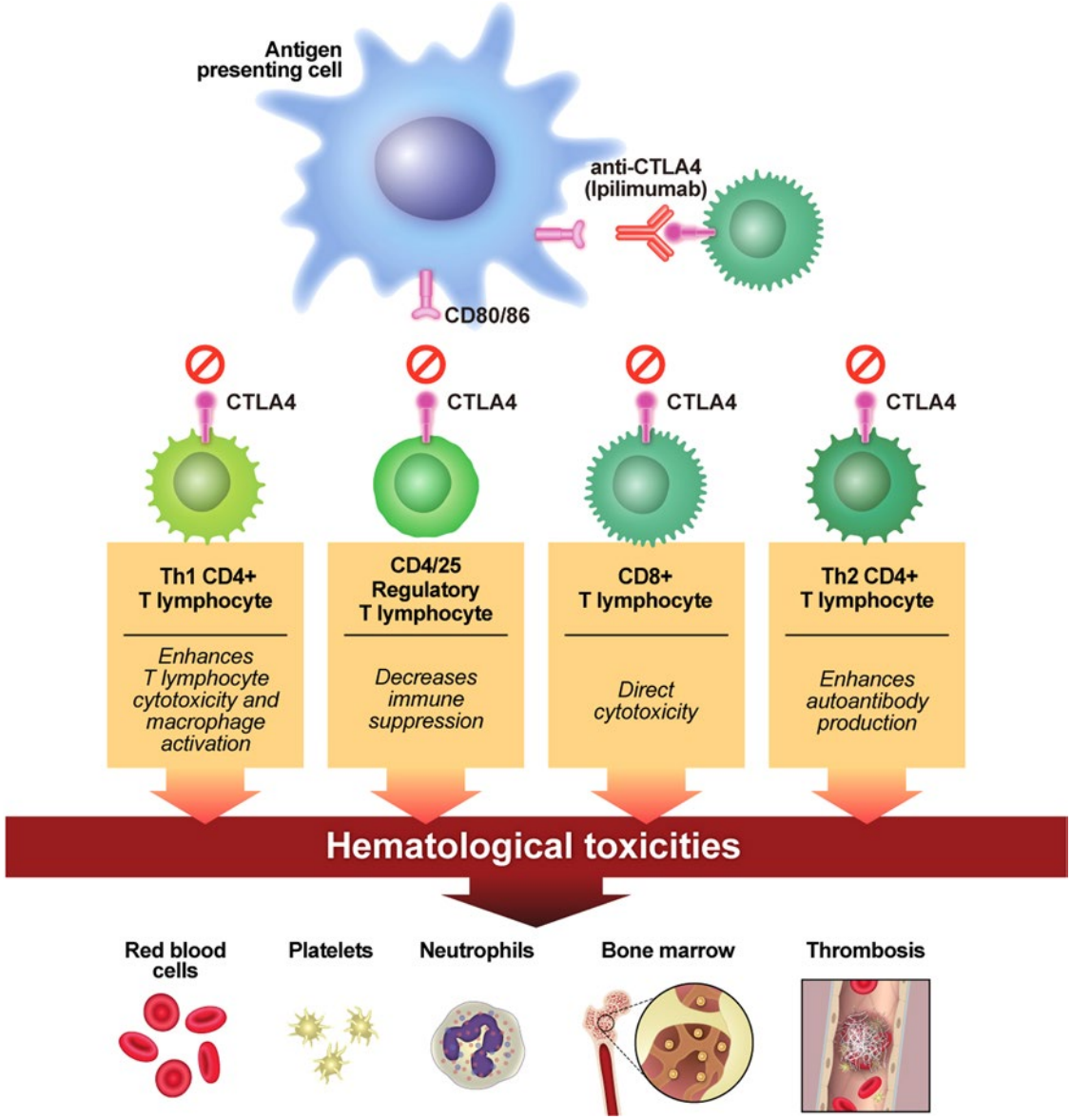

Figure 1. How the CTLA-4 inhibitor works. Anti-CTLA-4 therapy blocks inhibitory signals to cytotoxic (CD8+) and helper (Th1 and Th2) T lymphocytes and suppresses the activation of regulatory T cells (CD4/25+). (Reprinted from Kroll et al[1] with permission.)

have been catalogued.[6] One analysis of 47 clinical trials encompassing over 9000 patients calculated the incidence of common terminology criteria for adverse events grade 3 (<1000/μL), 4 (<500/μL), and 5 (lethal) neutropenia as ranging from 0.4 to 1.7%.[7] Another catalogue of 5923 patients from 19 clinical trials identified grade 2 (<1500/μL) or worse neutropenia in 0.61%.[8] Dual checkpoint blockade, as was used in this case, may double the risk of immune-mediated toxicity.[9] Radiotherapy, as was also used in this case, appears to not increase the rate of ICI toxicities, but the combination of ipilimumab + radiotherapy is associated with higher-grade toxicities.[10] Further evidence that this is an ICI effect is that the time of onset of our patient's grade 4 (also referred to as *severe*) neutropenia occurred sometime between week 8 and 12 after reinitiating dual ICI therapy, similar to the typical median onset of severe neutropenia as described in several reports.[11-14] Finally, the absence of fever in our patient is not unexpected, as fewer than half of those with severe neutropenia suffer febrile episodes.[13]

How can one be sure that there isn't another cause of this patient's neutropenia?

The first step is to review medications to determine if a recently added nonchemotherapy drug caused the severe neutropenia.[15] In this case no drug culprit emerged. The next step, here and in all cases of ICI-associated cytopenia, is to rule out myelophthisis from metastatic cancer. This can often be identified on the blood smear, but in the absence of leukoerythroblastic features and uncertainty about metastatic marrow involvement, it is reasonable to examine the bone marrow (Figure 3). It was not done in this case because other blood counts were preserved and the blood smear was unremarkable other than the absence of myeloid elements. In one case series of ICI-induced neutropenia,

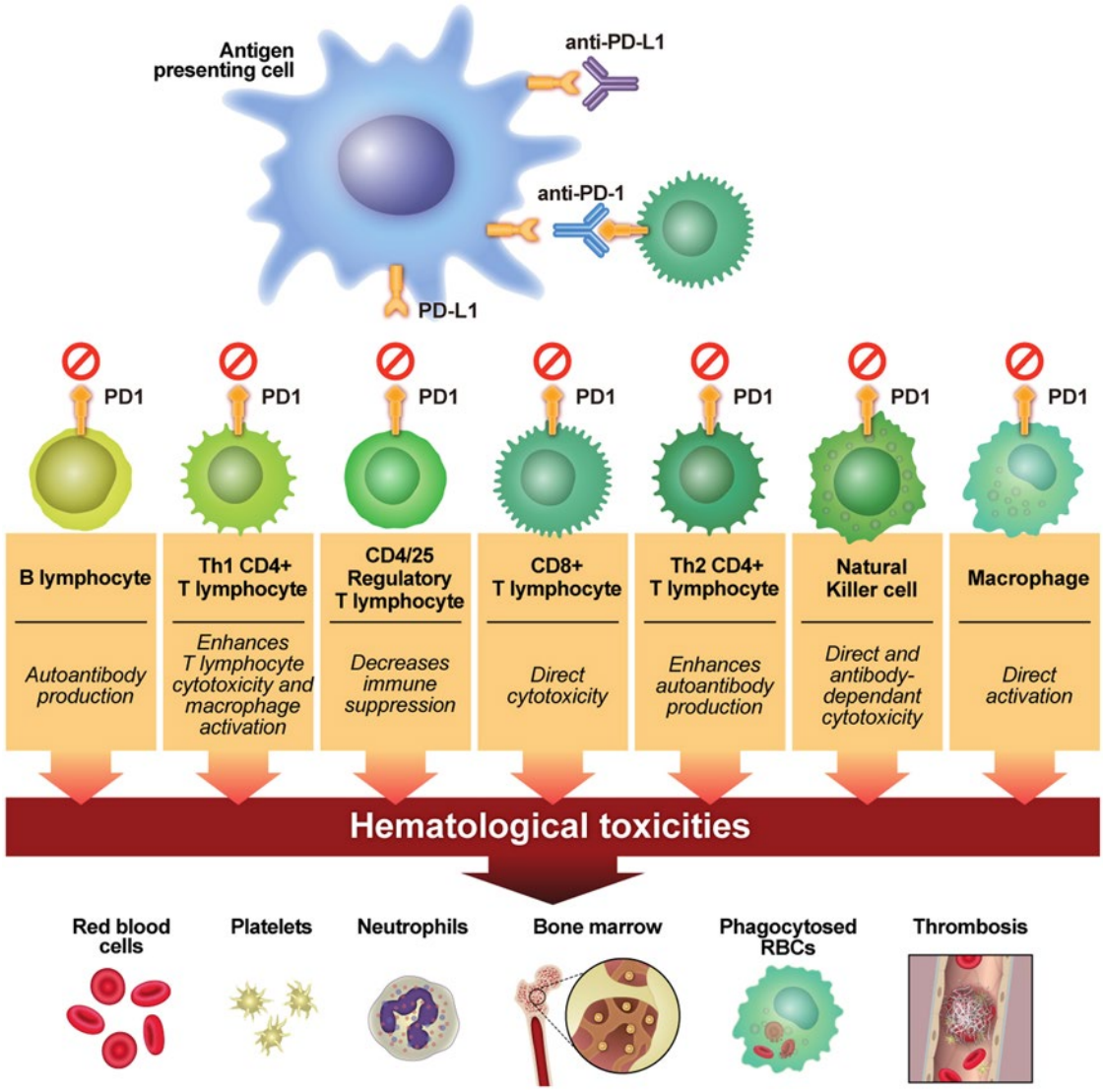

Figure 2. How PD-1 and PD-L1 inhibitors work. PD-1/PD-L1 therapies have block inhibitory signals to cytotoxic and helper T lymphocytes, B lymphocytes, natural killer cells, and macrophages and suppress the activation of regulatory T cells. RBCs, red blood cells. (Reprinted from Kroll et al[1] with permission.)

bone marrow examination done on 24 patients suggested pleiotropic pathologic mechanisms: ~45% were normal, ~10% were hyperplastic, and ~45% demonstrated either myeloid hypoplasia or maturation arrest.[13] These results provide insight into the mechanism and may direct therapy (see below). Antineutrophil antibodies were not measured in this case, as this same case series showed that they are unlikely to be useful: only 4 of 32 patients were tested, 2 of whom tested positive and none of whom were managed differently.[13]

CLINICAL CASE (continued)

Nivolumab and pembrolizumab were stopped because of progressive melanoma coincident with the development of severe neutropenia (ANC 10/μL). She was started on prednisone. After 2 weeks of prednisone 40 mg twice daily her ANC rose to 2900/μL. Prednisone was continued at 30 mg twice daily for 2 weeks followed by 20 mg twice daily for 2 weeks, at which time the ANC was 2300/μL. Prednisone tapering continued.

Was this patient treated appropriately?

It is recommended that ICIs be held when neutrophils fall below 1500/μL (Figure 3). Our case supports this recommendation, as the additional cycle of dual checkpoint inhibitors given at the time her ANC was below 1500/μL culminated in severe neutropenia, permitting speculation that this could have been mitigated had the ICIs been stopped immediately after neutropenia was identified. As done in this case, active therapy begins when the ANC falls below 1000/μL using corticosteroids without or with granulocyte colony-stimulating factor (G-CSF). G-CSF was not needed in this case because the patient was asymptomatic

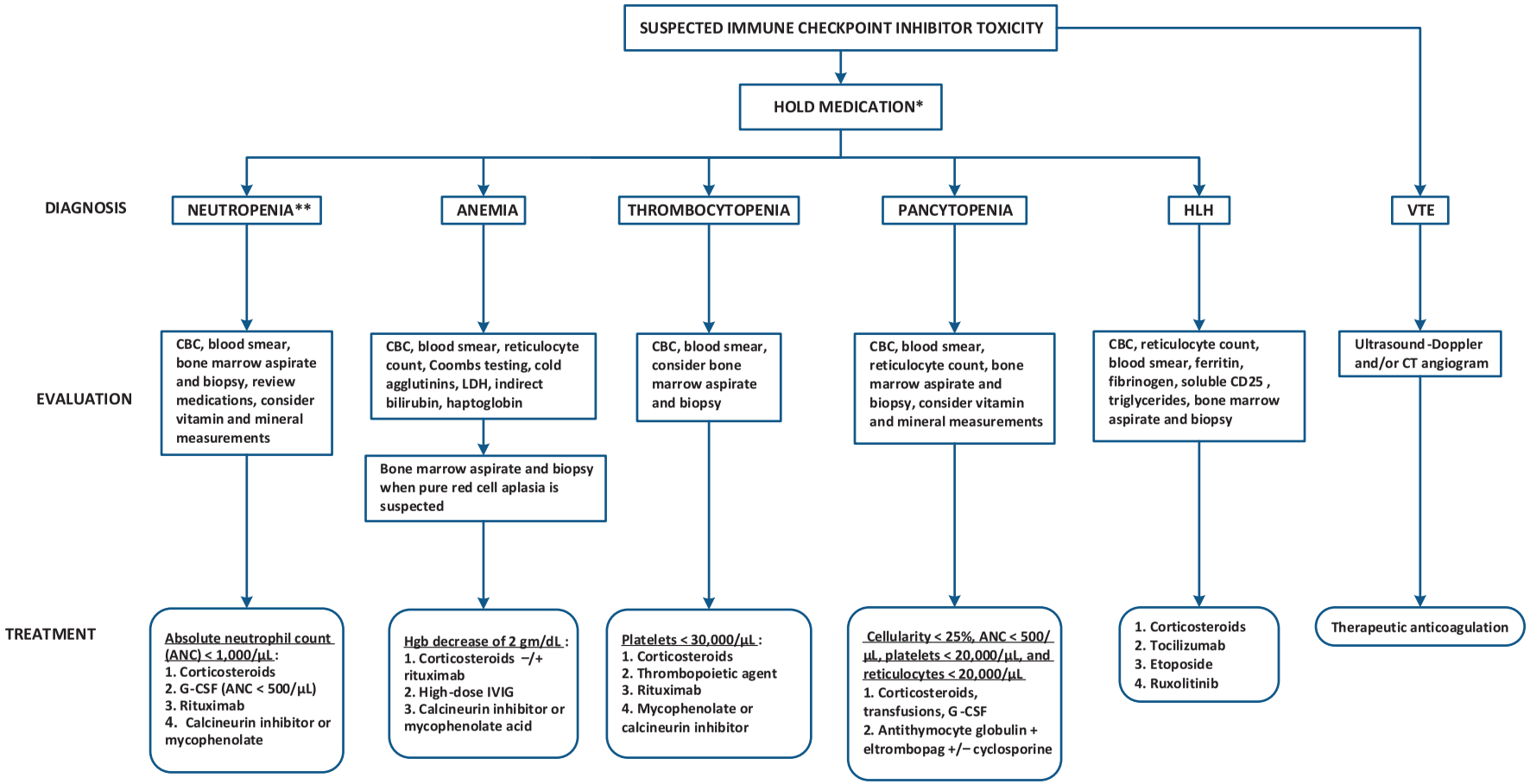

Figure 3. Management of immune checkpoint inhibitor hematologic toxicity. *Recommended thresholds for holding therapy are 1500/µL neutrophils, 75 000/µL platelets, and 8 g/dL serum hemoglobin concentration. **Please be aware of the possibility of Duffy null associated neutrophil count (previously designated "benign ethnic neutropenia") among patients of African or Middle Eastern ancestry. CBC, complete blood cell count.

and the neutrophil count began rising within days of beginning prednisone 40mg twice daily. Rituximab is sometimes used; it has been given during induction, during maintenance (to support corticosteroid taper), or following relapse when steroids are resumed.

When there is no early response, high-dose intravenous immunoglobulin (IVIG) can be administered to those with normal or hypercellular bone marrow (considered to suffer splenic autoantibody-mediated immune destruction) and a calcineurin inhibitor (cyclosporine or tacrolimus) or mycophenolate for those with hypoplastic or maturation-arrested bone marrow (considered to suffer CD8+ T lymphocyte–mediated neutrophil precursor destruction or suppression). When there are no bone marrow biopsy results, a calcineurin inhibitor or mycophenolate is preferred as second-line therapy, as clinical responses to IVIG are infrequent.[6,13] Treatment generally works: in 1 systematic review of 27 patients, 70% responded to immune suppression, with the median time to response of less than 1 month.[12] Two patients died, but death was attributed to cancer progression rather than intractable neutropenia.

Are there other hematologic complications from checkpoint inhibitors?

The previously acknowledged systematic review identified hematologic toxicities in 3.6% of all patients with cancer treated with ICIs.[7] In 2023 over 1 900 000 Americans will be diagnosed with cancer (excluding nonmelanoma skin cancer), and it is estimated that 12% or ~228 000 will receive and benefit from ICIs, leading to ~8000 new cases of ICI-associated hematologic toxicity.[16,17] In addition to neutropenia, which develops in at least 0.4% of patients (perhaps 50 patients this year), the systematic review identified immune thrombocytopenia (ITP) (1%), aplastic anemia/pancytopenia (0.6%), hemolytic anemia (0.6%), hemophagocytic lymphohistiocytosis (HLH, 0.4%), and pure red cell aplasia (0.3%).[7] Venous thromboembolism may also be associated with ICIs, although risk data are ambiguous due to complex comorbid prothrombotic factors affecting these individuals.[18,19] Finally, the American Society of Clinical Oncology management guidelines list thrombotic thrombocytopenic purpura, atypical hemolytic uremic syndrome, lymphopenia, and acquired hemophilia A as ICI toxicities.[20] Except for HLH (see below), PD-1, PD-L1, and CTLA-4 inhibitors appear to be associated with hematologic toxicities of similar rates, types, magnitudes, and clinical courses.

Immune thrombocytopenia

The Registre des Effets Indésirables Sévères des Anticorps Monoclonaux Immunomodulateurs en Cancérologie (REISAMIC) is a prospective multicenter registry of patients treated with anti-PD-1 or anti-PD-L1 therapy. Nine patients in the REISAMIC registry presented with thrombocytopenia.[11] Seven of nine patients had laboratory evaluations and bone marrow biopsies consistent with ITP. Thrombocytopenia was severe with a median nadir platelet count of 5000/μL. An antibody to platelet glycoprotein IIb/IIIa was identified in one patient's serum. Severe bleeding developed in 2 of 9 patients, but there was no fatal bleeding.

Management derives from routine therapies for de novo ITP (Figure 3). One would hold the ICI when the platelet count falls below 75 000/μL and begin immune suppressive therapy when the platelet count is below 30 000/μL. Treatment begins with corticosteroids for all—either dexamethasone 40 mg per day for 4 days or prednisone 1-2 mg/kg per day. High-dose IVIG should be added for patients who are bleeding, and we recommend rapidly introducing a thrombopoietic agent when corticosteroids and/or IVIG do not work. We prefer a thrombopoietic agent to rituximab because it decreases immunosuppression, which may have an adverse effect on tumor progression.[21] In one survey, recovery occurred in 21 out of 36 patients (58%).[12] Drugs that target activated cytotoxic CD8+ T lymphocytes, such as a calcineurin inhibitor or mycophenolate, may be particularly useful for refractory ITP, as cytotoxic T lymphocytes frequently mediate steroid-refractory ITP, and drugs targeting them can lead to platelet recovery.[22,23]

Cytopenias and bone marrow failure

The REISAMIC registry reported 4 of 5 patients with pancytopenia whose bone marrow showed severe trilineage hypoplasia; 1 patient's bone marrow was "near-normal"; 1 of 5 died of neutropenic sepsis; and 1 of 5 recovered over 8 months.[11] A summary of reported cases describes a broad range in the time of onset of bone marrow failure, but the majority occurred within 2–3 months of beginning the ICI.[12]

One should hold the ICI while providing transfusion and G-CSF support for patients with nonsevere aplasia and begin immunosuppression when aplasia is severe (marrow cellularity <25% with ANC <500/μL, platelets <20 000/μL and reticulocytes <20 000/μL) (Figure 3). Treatment includes corticosteroids + antithymocyte globulin + eltrombopag, with cyclosporine added for those with severe aplasia and active cancer demonstrating a poor or uncertain response to the ICI.[24] Similar parameters for treatment, based on the bone marrow cellularity and the myeloid:erythroid ratio, have been used for patients with pure red cell aplasia or bicytopenia. One case of amegakaryocytic thrombocytopenia was treated effectively with prednisone and eltrombopag.[25] In contrast to other hematologic toxicities, responses are infrequent (~30%) and mortality is high (~30%).[12]

Hemolytic anemia

REISAMIC included 9 cases of hemolytic anemia, identified by any CTCAE grade 2 (Hgb <10 g/dL) or worse, a US multicenter retrospective cohort analysis included 14 cases, and the FDA Adverse Events Reporting System identified 68 cases.[11,26,27] In REISAMIC all 9 cases had a positive direct antiglobulin test: 3 for IgG and 6 for complement factor 3d; 3 of the latter 6 patients also had IgM autoantibodies (cold agglutinins) in their serum. Among 13 US patients tested in the US multicenter analysis, a positive direct antiglobulin test was identified in 8 (62%); the median number of ICI cycles was 3 (range of 1-12), all events were CTCAE grade 3 or 4 (Hgb <8 g/dL), the median nadir hemoglobin was 6.3 g/dL, and red blood cell transfusion support was required in 11 of 14, including the transfusion of 4 or more units of packed red blood cells in 7 of 14 patients.[26] No serologic testing was available in the FDA database. It therefore appears that many cases are due to autoantibody production, possibly due to decreased regulatory T lymphocyte–mediated immune suppression and/or B-cell activation (Figures 1 and 2).

Treatment aims at autoantibody-mediated hemolysis: corticosteroids and rituximab, given simultaneously or sequentially,

possibly along with IVIG (Figure 3). Failing that, a calcineurin inhibitor or mycophenolate acid is recommended with the caveat that little data are available to support an approach targeting cytotoxic T lymphocytes.[28]

Hemophagocytic lymphohistiocytosis

Over 200 cases of hemophagocytic lymphohistiocytosis have been reported.[11,29,30] Most are associated with anti-PD1, anti-PD-L1, and combination therapies; only 7/190 (5.7%) were associated with single-agent ipilimumab.[30] Their onset was anytime between 1 week and over a year after the ICI was begun. The most common presenting symptoms were fever and organomegaly, the mean ferritin level was 27 000µg/L, and 16 of 18 patients had demonstrable hemophagocytosis in the bone marrow aspirate or blood smear. In over half of the cases alternative potential predispositions or triggers for HLH were identified, such as progressive malignancy, infection, and 1 deleterious perforin mutation.[31] Guidelines for managing ICI-related HLH are derived from HLH Society guidelines, which recommends starting corticosteroids and tocilizumab, and adding etoposide if there is no response after 48 hours (Figure 3).[32] In 1 case series of 20 patients with ICI-associated HLH, all were treated with ICI withdrawal and corticosteroids, 6 were treated with etoposide, 1 with tocilizumab, and 1 with anakinra: 15 of 20 patients recovered and 3 of 20 patients died.[29]

Thrombosis

Venous thromboembolism risk from ICIs is uncertain and there appears to be no risk of arterial thrombosis.[18,19,33,34] Pharmacologic thromboprophylaxis is not recommended among ambulatory patients with cancer receiving an ICI, and ICI therapy should not be stopped when acute venous thromboembolism is diagnosed.

CLINICAL CASE (continued)

Within 2 weeks of completing an 8-week course of prednisone, the ANC fell to 550/µL. Prednisone was resumed and rituximab started. About 1 month later the ANC was 2800/µL.

Is relapse unusual, and how much do we know about long-term outcomes?

Relapse after medication discontinuation and steroid taper is not uncommon: 1 systematic review reported a 9% relapse rate.[6] General conclusions about long-term clinical outcomes are confounded by heterogeneous clinical factors and varying therapies. Nonetheless, except for patients with pancytopenia (who have poorer outcomes) and HLH (who, surprisingly, have better outcomes), about two-thirds of patients with hematologic toxicity will recover within 1 month following the initiation of immune suppression. For patients who recover and whose malignancy was controlled by the ICI, the ICI can sometimes be safely resumed (along with ongoing but minimized corticosteroid dosing).[1,6,12,21] For those who don't respond, second-line immune suppression is begun with either rituximab—as was used in this case—or medications that target cytotoxic T lymphocytes such as cyclosporine, tacrolimus, or mycophenolate, the latter of which may be preferred for isolated thrombocytopenia.[35] For those whose toxicity resolves and require another treatment with targeted therapy, there appears to be no risk of recurrent or worsening ICI toxicity.[36] While persistent organ-specific ICI toxicities are being increasingly recognized, to date persistent hematologic toxicities are not among them; and, while up to 15% of patients suffering from ICI-associated hematologic toxicity die, death is only rarely related directly to the hematologic problem.[6,37]

CLINICAL CASE (continued)

Following neutrophil recovery and identification of an activating mutation in tumor N-Ras, she was considered for enrollment in the phase 2 NAUTILUS trial of the histone deacetylase inhibitor OKI-179 + the mitogen-extracellular activated protein kinase inhibitor binimetinib. Unfortunately, the ANC fell to 1760/µL during prednisone taper, making her ineligible for OKI-179 therapy. She is currently exploring phase 1 studies targeting mutant N-Ras.

Conclusion

ICI-associated hematologic toxicities are rare but easily recognizable. Except for thromboses, management begins with pausing the offending agent. Treatments follow standards developed for patients with related hematologic disorders. ICI discontinuation and therapeutic immunosuppression must be balanced against the need to maintain an effective antitumor immune response.

Acknowledgments

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Conflict-of-interest disclosure

Michael H. Kroll: no competing financial interests to declare.

Off-label drug use

Michael H. Kroll: The use of cyclosporine, tacrolimus, mycophenolate, rituximab, tociluzimab, or anakinra to treat ICI-associated hematological toxicities is off-label.

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Bispecific antibody therapies

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Management of hematological malignancies is rapidly evolving from chemotherapy-based regimens toward targeted agents and immunotherapies, including bispecific antibodies (BsAbs). These novel and highly active treatments come with new side effect profiles. The hematological toxicities are common and potentially harmful, and the side effects have hitherto not been reviewed. With many BsAbs recently approved and entering routine clinical use, we have reviewed the rather limited published data and propose recommendations on the management of these toxicities. Our review of the available data confirms that hematological toxicities are among the most common toxicities, with potentially harmful consequences for the patients. Fortunately, hemophagocytic lymphohystiocytosis and disseminated intravascular coagulation are rare. Severe neutropenia and hypogammaglobulinemia are manageable, and their timely treatment and prevention may reduce morbidity and mortality.

LEARNING OBJECTIVES

- Review the incidence and severity of hematological toxicities associated with bispecific antibody therapies in hematological malignancies
- Discuss the background for such hematological toxicities and their potential complications
- Propose management strategies to prevent and/or treat the hematologic toxicities

CLINICAL CASE

A 55-year-old female was referred for experimental treatment of her third relapse of non-germinal center B-cell diffuse large B-cell lymphoma. She was first diagnosed in 2013 and had three times relapsed after initial response to rituximab-containing polychemotherapy, including high-dose chemotherapy with autologous stem cell support. In 2019, a CT scan showed a third relapse in the mediastinum. She was in a good general condition and was enrolled in the phase 1 study of the CD20xCD3 BsAb glofitamab (NP30179) in January 2020. She tolerated the step-up regimen well, with cytokine release syndrome (CRS) grade 1 after the 2.5mg priming dose and after the 10 mg intermediate dose but with no signs of CRS on subsequent doses, and she achieved a complete response after 2 treatment cycles. After 3 cycles, she encountered recurrent respiratory infections that led to dose delays in cycle 4 and cycle 5 and that were preceded by glofitamab-induced lymphopenia. After cycle 6, the lymphopenia was accompanied by neutropenia, and she was admitted to an intensive care unit with neutropenic sepsis and bilateral pneumonia. She improved on broad-spectrum antibiotics and vasopressor support. The neutropenia resolved after more than one week of granulocyte colony stimulating factor (G-CSF) treatment. After an additional (and again delayed) cycle 7 of glofitamab, she again developed profound neutropenia, this time with a very slow response to G-CSF, and she was taken off protocol per physician's decision. Lymphocyte and neutrophil counts normalized within the following months, and more than 3 years after the end of glofitamab treatment, she remains in complete response. She receives subcutaneous immunoglobulin injections in to reduce the risk of infections.

Introduction

The management of hematologic malignancies is evolving, and immunotherapies are rapidly becoming part of the treatment paradigm. Along with the introduction of these new strategies such as bispecific antibodies, CAR-T cell therapy, and checkpoint inhibiting antibodies,[1,2] new

toxicities are also emerging. We address adverse events to BsAbs, while the latter modalities will be covered in accompanying articles.

Bispecific antibodies (BsAb) are monoclonal antibodies binding to an effector cell surface antigen (typically CD3 on T-cells) and to a surface antigen on the tumor cell, leading to effector-cell mediated tumor cell killing. These antibodies are so far only recommended for use as single agents, but they are also under study in combination with chemotherapy, other immunotherapies (NCT05849610), checkpoint inhibitors (NCT03533283), other bispecifics (NCT04586426), or costimulatory immune agonists (NCT05219513, NCT04077723).

There is a paucity of data on common hematological toxicities observed in the context of BsAbs. While some of these toxicities are well characterized as "on-target, off-tumor" effects of the T-cell bispecifics, particularly the profound lymphopenia and hypogammaglobulinemia seen in most B-cell non-Hodgkin lymphoma (B-NHL) patients, the biological mechanism behind the observed neutropenia, thrombocytopenia, and the rare cases of hemophagocytic lymphohystiocytosis (HLH) are poorly understood.

The scope of this article is to review hematological complications to bispecific antibody and bispecific-T-cell-engager (BiTE) treatment in hematologic malignancies, based on published trials and abstracts, and to propose some recommendations for their management.

We reviewed the main articles and abstracts published about BsAbs in the treatment of B-NHL, B-cell acute lymphoblastic leukemia (B-ALL), multiple myeloma (MM), with a focus on hematological side effects. Data are summarized and presented in a tabular format, derived from articles with information about anemia, neutropenia, febrile neutropenia, lymphopenia, thrombocytopenia, hypogammaglobulinemia, HLH, and disseminated intravascular coagulation (DIC), by group of disease.

Hematological toxicities are common in the context of BsAbs, regardless of indications and tumor targets. The reasons for these phenomena are multifactorial and associated with the disease (which is often bone marrow based), previous therapies (most patients are heavily pretreated), the patient's immune repertoire, and the effect of the individual bispecific molecule in inflammation, B-cell/plasma cell depletion and T-cells exhaustion.[3-5] Production of cytokines by the bone marrow environment may also affect hematopoiesis during BiTE and BsAb therapy.

Acute lymphoblastic leukemia

Although the prognosis of ALL has improved over past decades, a substantial proportion of adults relapse or are refractory (R/R) to first-line treatment. For such patients, the 1- and 5-year overall survival (OS) are 22% and 7%, respectively.[6] Blinatumomab, targeting CD19 on B-cells, was the first FDA-approved BiTE used in clinical practice.[7]

In the phase 2 study of blinatumomab in adult patients with R/R B-ALL, hematological toxicities were the most frequent ≥ grade 3 adverse events (AE). In this trial, 4 of 188 patients (2%) developed DIC, a rare but feared complication of T-cell hyperactivation, while 23/188 (12%) suffered a fatal AE, mainly related to infectious complications.[8] In the phase 3 TOWER trial, the hematological adverse events of ≥ grade 3 were more common in the control arm (chemotherapy) than in the blinatumomab arm. The exceptions were hypogammaglobulinemia (6% vs 0.9%) and HLH (1.5% vs 0%), which almost exclusively occurred in the blinatumomab arm.[9] The RIALTO study evaluated blinatumomab in a pediatric population with R/R B-ALL. In this study, there was one event of DIC (0.9%) and febrile neutropenia ≥ grade 3 occurred in 9.1% of patients. There was no blinatumomab-related fatal AE, and no reports of HLH.[10]

Data on the hematological toxicities of the BiTEs in ALL are summarized in Table 1.

B-cell non-Hodgkin lymphoma

B-NHL is a heterogeneous group of diseases, ranging from the aggressive subtypes such as diffuse large B-cell lymphoma (DLBCL) to the more indolent subtypes, including follicular lymphoma.[11]

DLBCL accounts for approximately 28% of NHL cases.[12] DLBCL generally responds well to first-line chemoimmunotherapy, but 40% of patients eventually experience R/R disease and require subsequent treatment.[13] For patients who are refractory to second-line chemotherapy and/or unfit for high-dose chemotherapy and autologous stem cell transplant (ASCT), as well as for patients who relapse after ASCT, the prognosis with conventional chemotherapy-based treatment is dismal. CAR-T cell therapy represents a very important improvement for patients, both in first relapse where it replaces ASCT as standard of care as well as in later treatment lines. However, the majority of chemo-refractory patients eventually fail the treatment for their relapsed disease, even with CAR-T cell therapy. The indolent lymphomas are also difficult to control with conventional chemoimmunotherapy in patients with refractory or early relapsing.[14] A number of BsAbs have been developed to meet this need that target B-cell surface antigens (CD19, CD20, CD22, CD79) and the T-cell antigen CD3.

Glofitamab is a bivalent CD20-targeting and T-cell-engaging full-length antibody. In the first-in-human phase 1 trial of glofitamab in B-NHL, 2.9% of patients withdrew from treatment because of an AE. At the recommended phase 2 dose (RP2D), the occurrence of hematological toxicity ≥ grade 3 was as follows: neutropenia in 25.7%, thrombocytopenia in 8.6%, and febrile neutropenia in 5.7%, with G-CSF used in 21.6% of patients. Infections were seen in 42.9% at the RP2D, without fatal AEs in this group.[15] Also, in the expansion cohort, the most common grade ≥3 AE was neutropenia (27% of patients). Infections ≥ grade 3 occurred in 15% of subjects.[16]

Epcoritamab also targets CD20 on the malignant B cells and CD3 on the T-cells. The phase 1/2 trial of epcoritamab in R/R B-NHL reported no cases of febrile neutropenia ($N = 68$).[17] In the dose expansion cohort, however, neutropenia occurred in 21.7% of subjects, with febrile neutropenia in 4/157 (2.5%). G-CSF support was used in 16 patients (10.2%).[18]

Mosunetuzumab is another full-length IgG1-based humanized BsAb targeting CD20 and CD3. In group B of the phase 1 trial, for R/R B-NHL (dose escalation with step-up dosing during cycle 1), the most common ≥ grade 3 AE was neutropenia (25%), with a median duration of neutropenia of 9 days. Febrile neutropenia occurred in only 3.6% of patients, 22.3% received G-CSF, and anemia was reported in 18.8% of group B patients. There were four AE-related deaths in the trial, and one patient developed HLH secondary to Epstein-Barr virus infection.[19] In the phase 2 expansion study in R/R follicular lymphoma (grade 1-3a), at

Table 1. Incidence of hematological toxicities on bispecifics for acute lymphoblastic leukemia published trials

Reference	Population	Drug/target	N*	Anemia**	Neutropenia**	Thrombocytopenia**	Febrile neutropenia**	Lymphopenia**	Low IgG**	HLH**	DIC**
Kantarjian et al. (2017)[9]	Adult R/R ALL	Blinatumomab CD19×CD3	267	69 (25.8%)/NA	NA/101(37.8%)	47(17.6%)/NA	NA/57(21.3%)	NA/4(1.5%)	16(6%)/ 7(2.6%)	4(1.5%)/ 4(1.5%)	NA/NA
Topp et al. (2015)[8]	Adult R/R ALL	Blinatumomab CD19×CD3	188	38(20%)/ 27(14%)	33(17%)/ 30(16%)	21(11%)/16(8%)	53(28%)/ 48(25%)	NA/NA	NA/NA	NA/NA	4(2%)/NA
Locatelli et al. (2022)[10]	Pediatric R/R ALL	Blinatumomab CD19×CD3	110	20(18.2%)/ 5(4.5%)	11(10%)/ 10(9.1%)	22(20%)/16(14.5%)	NA/ 10(9.1%)	NA/NA	NA/NA	NA/NA	1(0.9%)/ 1(0.9%)

ALL, acute lymphoblastic leukemia; DIC, disseminated intravascular coagulation; HLH, hemophagocytic lymphohystiocytosis; NA, not available; R/R, relapsed/refractory.

*N is the population considered for that extracted data, which may be the whole cohort or a subpopulation.

**Adverse event, any grade(%)/grade ≥3(%).

baseline the patients had peripheral B-cell counts below normal limits. As expected, mosunetuzumab induced deep and durable B-cell depletion, while NK- and T-cell levels in general remained within normal limits. Most common ≥ grade 3 AE was neutropenia (27% of subjects), with a median duration of 8 days. Of the neutropenic patients, 18 of 26 (69%) received G-CSF support. There were no infection-related deaths.[20] In the cohort of R/R DLBCL and transformed follicular lymphoma, data on lymphocytopenia were consistent with those of the previous cohort. The most common hematological AE was neutropenia (27.3%) and anemia (17%). Nineteen patients received G-CSF. There was one case of fatal sepsis.[21]

Odronextamab is a CD20×CD3 completely human IgG4-based drug. In the first-in-human study of odronextamab in B-NHL (ELM-1), the distribution of AEs was consistent with other BsAbs. Most common grade ≥3 AEs were anemia (25%), lymphocytopenia (19%), neutropenia (19%) and thrombocytopenia (14%).[22]

Data on the hematological toxicities of the BsAbs in B-NHL are summarized in Table 2.

Multiple myeloma

Incorporation of immunomodulatory drugs, proteasome inhibitors, and monoclonal antibodies, in triplet or quadruplet combinations, has dramatically improved the response rates and survival in MM.[23,24] However, virtually all patients eventually relapse and ultimately develop disease resistant to conventional therapies. Like in the B-cell lymphomas, T-cell engaging therapies have the potential to improve the landscape of R/R MM.[25,26]

Teclistamab is a bispecific IgG4 antibody targeting BCMA on plasma cells and CD3 on the T-cells. In the Majestec-1 trial study, among patients treated at the RP2D ($N = 40$), hematological toxicities were the most frequent grade ≥3 AEs, with neutropenia, anemia, and thrombocytopenia occurring in 40%, 28%, and 20%, respectively. Infectious complications occurred in 45%, including 23% of grade ≥3. In the overall cohort ($N = 157$), 11% of patients received prophylactic immunoglobulin (Ig) therapy and 6% received Ig therapy for treatment of a hypogammaglobulinemia-related adverse event.[27] In the pivotal phase 1-2 study with the RP2D of 1.5mg/Kg, AEs followed the same pattern, with most common toxicities being hematological, including neutropenia in 70.9%, anemia in 52.1%, thrombocytopenia in 40%, and four cases (2.4%) of febrile neutropenia. Of 117 neutropenic patients, 91 received G-CSF at the discretion of physicians. Hypogammaglobulinemia occurred in 123 patients (74.5%), and immunoglobulin substitution was given in 65 of those 123 patients.[28]

Elranatamab also targets BCMA and CD3. In the first-in-human trial, for 58 safety-evaluable patients, hematological toxicities followed a similar pattern, with neutropenia ≥ grade 3 in 60% of patients and lymphopenia ≥ grade 3 in 64% of patients.[29] In the phase 2 MagnetisMM-3 trial, with 123 patients, neutropenia ≥ grade 3 occurred in 43.1% and lymphopenia ≥ grade 3 in 23.6%.[30]

ABBV-383 is a fully human monoclonal IgG4 targeting BCMA and CD3. In its phase 1 trial, neutropenia occurred in 37% of patients (≥3 in 34%) and hypogammaglobulinemia in 14%. It is described that 29 of 124 (23%) of patients required Ig substitution.[31]

Alnuctamab, targeting BCMA×CD3, showed neutropenia ≥ grade 3 in 30% and anemia ≥ grade 3 in 17%.[32]

Table 2. Incidence of hematological toxicities on bispecifics for non-Hodgkin lymphoma published trials

Reference	Population	Drug/target	N*	Anemia[&]	Neutropenia[&]	Thrombocytopenia[&]	Febrile neutropenia[&]	Lymphopenia[&]	Low IgG[&]	HLH[&]	DIC[&]
Hutchings et al. (2021)[15]	Adult CD20+ NHL	Glofitamab CD20×CD3	35	NA/0(0%)	NA/9(25.7%)	NA/3(8.3%)	NA/2(5.7%)	NA/NA	NA/NA	NA/NA	NA/NA
Dickinson et al. (2022)[16]	Adult CD20+ NHL	Glofitamab CD20×CD3	154	47(30.5%)/ 10(6.5%)	58(37.7%)/ 41(26.6%)	38(24.7%)/12(7.7%)	NA/4(3%)	NA/5(3.2%)	NA/NA	NA/NA	NA/NA
Hutchings et al. (2021)[17]	Adult CD20+ NHL	Epcoritamab CD20×CD3	68	16(23%)/9(13%)	NA/17(25%)	NA/8(12%)	0/0	NA/4(6%)	NA/NA	NA/NA	NA/NA
Thieblemont et al. (2023)[18]	Adult CD20+ NHL	Epcoritamab CD20×CD3	157	28(17.8%)/ 16(10.2%)	34(21.7%)/ 23(14.6%)	21(13.4%)/9(5.7%)	4(2.5%)/NA	NA/NA	NA/NA	NA/NA	NA/NA
Budde et al. (2022)[20]	Adult CD20+ NHL	Mosunetuzumab CD20×CD3	197	37(18.8%)/ 18(9.1%)	56(28.4%)/ 50(25%)	5(2.5%)/4(2%)	7(3.6%)/ 7(3.6%)	9(4.5%)/8(4%)	NA/NA	NA/NA	NA/NA
Budde et al. (2022)[19]	Adult CD20+ NHL	Mosunetuzumab CD20×CD3	90	12(14%)/7(8%)	26(28%)/ 24(27%)	9(10%)/4(4%)	0/0	NA/NA	NA/NA	NA/NA	NA/NA
Bartlett et al. (2023)[21]	Adult CD20+ NHL	Mosunetuzumab CD20×CD3	88	15(17%)/8(9.1%)	24(27.3%)/ 19(21.6%)	NA/3(3.4%)	NA/5(5.7%)	NA/NA	NA/NA	NA/NA	NA/NA
Bannerji et al. (2022)[22]	Adult CD20+ NHL	Odronextamab CD20×CD3	145	55(38%)/36(25%)	35(25%)/ 27(19%)	40(28%)/20(14%)	NA/NA	32(22%)/ 28(19%)	NA/NA	NA/NA	NA/NA

DIC, disseminated intravascular coagulation; HLH, hemophagocytic lymphohystiocytosis; NA, not available; NHL, non-Hodgkin lymphoma.

*N is the population considered for that extracted data, which may be the whole cohort or a subpopulation.

[&]Adverse event, any grade(%)/grade ≥3(/%).

Talquetamab is a bispecific directed to GPRC5D on plasma cells and CD3 on T-cells. MonumenTAL-1 was the first-in-human, phase 1 study with 232 R/R MM subjects. As other BiTEs, most common grade ≥3 AEs were hematological. For RP2D SC cohorts ($N = 130$), grade ≥3 hematological events were neutropenia (45.4%), anemia (27.7%), lymphopenia (32.3%), and thrombocytopenia (20%). Low IgG serum levels (<500 mg/dL) were observed in 71% to 87% of patients, depending on the cohort.[33]

In the ongoing phase 1 study of the GPRC5D×CD3 targeting BsAb forimtamig ($N = 105$), hematological toxicities are infrequent, with anemia ≥ grade 3 ranging from 5.2% to 13.7% and neutropenia ≥ grade 3 from 11.8% to 16.7% for different routes of administration.[34]

Cevostamab targets FcRH5 and CD3. FcRH5 is expressed on B-cells and is highly expressed on MM plasma cells. In the phase I data (NCT 03275103) of cevostamab in R/R MM anemia, ≥ grade 3 was seen in 21.9% of patients, neutropenia ≥3 in 30.1% of patients, with one reported case of fatal HLH.[35]

Data on the hematological toxicities of the BsAbs in MM are summarized in Table 3. The higher rates of cytopenias compared to data from the B-NHL studies perhaps is no surprise, as MM is a disease of the bone marrow, more so than B-NHL. Nevertheless, given that MM patients are generally more prone to infections than B-NHL patients, the frequent cytopenias are clinically important.

How to manage hematological toxicities to biscpecific antibodies

Hematological toxicities are among the most common adverse events and the most common grade ≥3 AEs related to bispecific antibody therapy. Although we do not have prospective trials guiding the management of these toxicities, data from the early-phase trials provide some guidance. Different authors have proposed guidelines on these toxicities[3,4] that are mainly extrapolated from similar situations in the context of CAR-T cell therapy and hematopoietic stem cell transplant. The following points represent a proposal that should take into account institutional standards:

A) Anemia and thrombocytopenia: No clear recommendations can be made regarding eryhtropoietin and thrombopoitin agonists because we only have anecdotal reports of beneficial effects in this context.[36] Transfusions should be administered according to clinical indication.

B) Lymphopenia and hypogammaglobulinemia: They may be present before the initiation of BsAbs. We propose monthly monitoring and replenishing IgG to keep levels at ≥400 mg/dL. In the case of repetitive infections, replenishing to ≥600 mg/dL should be considered, in keeping with most recommendations for patients with hypogammaglobulinemia after CART.[37] Both lymphopenia and hypogammaglobulinemia can last many months after discontinuation of BsAb therapy (as discussed in the clinical case).

C) Neutropenia: We advise using G-CSF in case of neutropenia <1000/mm^3 while avoiding dosing delays. The pathogenesis behind neutropenia remains elusive, if it is not due to infection or dysplasia. Cytokine-mediated damage to hemopoietic precursors or an effect similar to what is seen in delayed-onset neutropenia post-rituximab could be speculated.[37,38]

Table 3. Incidence of hematological toxicities on bispecifics for multiple myeloma published trials

Reference	Population	Drug/target	N*	Anemia&	Neutropenia&	Thrombocytopenia&	Febrile Neutropenia&	Lymphopenia&	Low IgG&	HLH&	DIC&
Usmani et al. (2021)[27]	Adult R/R MM	Teclistamab BCMA×CD3	40	20(50%)/ 11(28%)	26(65%)/ 16(40%)	18(45%)/8(20%)	NA/NA	7(9.6%)/ 7(9.6%)	14(9%)/NA	NA/NA	NA/NA
Moreau et al. (2022)[28¥]	Adult R/R MM	Teclistamab BCMA×CD3	165	86(52.1%)/ 61(37%)	117(70.9%)/ 106(64%)	66(40%)/35(21%)	4(2.4%)/NA	57(34.5%)/ 54(32.7%)	123(74.5%)/NA	NA/NA	NA/NA
D'Souza et al. (2022)[31]	Adult R/R MM	ABBV-383 BCMA×CD3	124	36(29%)/ 20(16%)	46(37%)/ 42(34%)	29(23%)/15(12%)	NA/NA	19(15%)/16(13%)	17(14%)/NA	NA/NA	NA/NA
Chari et al. (2022)[33#]	Adult R/R MM	Talquetamab GPRC5D×CD3	130	63(48.5%)/ 36(27.7%)	67(51.5%)/ 59(45.4%)	39(30%)/26(20%)	NA/3(2.3%)	42(32.3%)/ 42(32.3%)	NA/NA	NA/NA	NA/NA

DIC, disseminated intravascular coagulation; HLH, hemophagocytic lymphohystiocytosis; MM, multiple myeloma; NA, not available; R/R, relapsed/refractory; RP2P, recommended phase 2 dose.

*N is the population considered for that extracted data, which may be the whole cohort or a subpopulation.

&Adverse event, any grade(%)/grade ≥3(/%).

¥Data from cohort of phase 1 and cohort of phase 2 that used the RP2D; *N* = 165.

#Data from all subcutaneous cohorts (supplement material); *N* = 130.

D) Broad-spectrum antibiotics for primary antibacterial prophylaxis: They may be considered in individual cases but cannot be generally recommended. Antifungal prophylaxis should be considered in cases of neutropenia <500/mm^3 for more than 7–14 days in patients with recent invasive fungal infections or long-term high-dose corticosteroid use.

E) We recommend prophylaxis for herpes simplex virus and/or varicella-zoster virus and prophylaxis against *Pneumocystis jirovecii* during treatment and until total lymphocyte and CD4 counts approach normal levels.

F) Hemophagocytic lymphohystiocytosis (HLH): This hyperinflammatory state is most often driven by infection, malignancy, autoimmune diseases, or drugs. It is the extreme end of a hyperinflammatory continuum and should be regarded as a potential complication of BsAb, along with macrophage activation syndrome. HLH can occur immediately post-treatment in the direct context of CRS, but more classic HLH is typically delayed from treatment onset. Diagnosis can be supported by HLH-2004 criteria[38,39] or the calculation of the HScore.[40] In a scenario of BsAbs-induced HLH, the use of tocilizumab and corticosteroids is recommended (extrapolated from the experience in CAR T cell-treatment). HLH also occurs in rare instances of checkpoint inhibiting therapies and often responds to glucocorticoid monotherapy.[4,41] Other anti-inflammatory agents (e.g., anti-IL1ra, JAK-inhibition, anti-IFNγ) should be considered in refractory cases. Etoposide and cytostatic agents probably play a limited role in this context.[4,42] It is mandatory to search for causal pathogens (i.e., Epstein-Barr virus, cytomegalovirus, COVID-19, *Aspergillus fumigatus*), including local triggers like leishmaniasis and rickettsial disease or histoplasmosis in the US. We use microbiome evaluation of bone marrow examinations in HLH patients because some infections may be latent for many years,[43] and some infections (e.g., the newly diagnosed HLH-trigger Neoehrlichia mikurensis) are undetectable by serology and blood cultures).[4,44]

G) Disseminated intravascular coagulation: This is a very rare but potentially fatal complication, especially in the context of CRS or sepsis. There is no data to suggest that management of BsAb-associated DIC should differ from that of conventional DIC.

H) In all cases of severe hematological toxicities, discontinuation of BsAb therapy should be carefully considered.

BsAbs have significant potential for adverse events. It is important to acknowledge that the agents they replace may have worse infectious complications and less efficacy (e.g., blinatumomab vs conventional chemotherapy).[9] Prevention of infectious complications remains important. This includes immunizations, immunoglobulin substitution, and G-CSF support. Physicians should be aware of the risk of hyperinflammation leading to HLH in the context of CRS.

Conflict-of-interest disclosure

Luiz Henrique de Assis: no competing financial interests to declare.
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Off-label drug use

Luiz Henrique de Assis: This paper discusses use of drugs which are still under early-phase investigation. Blinatumumab is approved for the treatment of r/r B-ALL and for the treatment of B-ALL n first or second complete remission with minimal residual disease. Glofitamab and Epcoritamab are approved for the treatment of r/r DLBCL with at least two prior lines of treatment. Mosunetuzumab is approved for the treatment of r/r FL with at least two prior lines of treatment. Teclistamab, Talquetamab, and Elranatamab are approved for the treatment of r/r MM with at least four prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent and an anti-CD38 monoclonal antibody. All other drugs discussed are purely investigational and their use regarded as investigational or off-label, including the use of tocilizumab in the treatment of HLH.

Daniel El Fassi: This paper discusses use of drugs which are still under early-phase investigation. Blinatumumab is approved for the treatment of r/r B-ALL and for the treatment of B-ALL n first or second complete remission with minimal residual disease. Glofitamab and Epcoritamab are approved for the treatment of r/r DLBCL with at least two prior lines of treatment. Mosunetuzumab is approved for the treatment of r/r FL with at least two prior lines of treatment. Teclistamab, Talquetamab, and Elranatamab are approved for the treatment of r/r MM with at least four prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent and an anti-CD38 monoclonal antibody. All other drugs discussed are purely investigational and their use regarded as investigational or off-label, including the use of tocilizumab in the treatment of HLH.

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Identifying and treating iron deficiency anemia in pregnancy

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Anemia is common during pregnancy, and while most anemia is physiologic, the most common pathologic cause is iron deficiency. The American College of Obstetricians and Gynecologists (ACOG) recommends confirmation of iron deficiency anemia with iron studies when anemia is diagnosed during pregnancy but acknowledges that presumptive treatment for suspected iron deficiency anemia is common in practice. Currently ACOG does not recommend treating iron deficiency without anemia during pregnancy. Though the benefits of treating iron deficiency anemia during pregnancy are clear, the optimal route of iron repletion remains uncertain. Results of ongoing large, randomized trials will help define the optimal route of iron treatment for pregnant patients diagnosed with iron deficiency anemia.

LEARNING OBJECTIVES

- Overview physiologic versus pathologic anemia during pregnancy
- Discuss screening and treatment guidelines for iron deficiency anemia in pregnancy
- Review current data on optimal iron treatment method (oral versus intravenous) for iron deficiency anemia in pregnancy

CLINICAL CASE

A patient has had an uncomplicated pregnancy and undergoes routine third-trimester prenatal laboratory screening for gestational diabetes and anemia. The hemoglobin results at 10.2 g/dL. The obstetrician prescribes oral iron with instructions to take 1 tablet once daily in the evenings with orange juice. The patient initially follows these instructions but stops taking the iron tablet within a few weeks due to feeling noticeably more bloated and constipated. On routine laboratory evaluation upon admission for delivery, the hemoglobin is 9.0 g/dL. The labor course is notable for fetal distress requiring cesarean delivery, which was complicated by postpartum hemorrhage due to uterine atony. Total estimated blood loss was 1500 mL. The hemoglobin on postoperative day 1 is 7.2 g/dL. The patient receives a blood transfusion for symptomatic anemia.

Anemia in pregnancy

According to the World Health Organization (WHO), nearly 40% of pregnancies are complicated by anemia.[1] Despite its high prevalence, there is no standard definition of anemia during pregnancy: the WHO defines anemia as a hemoglobin <11 g/dL or hematocrit <33% at any time during pregnancy,[2] whereas the Centers for Disease Control and Prevention (CDC) as well as the American College of Obstetricians and Gynecologists (ACOG) propose a trimester-based definition. Specifically, ACOG and the CDC define anemia in pregnancy as hemoglobin <11 g/dL (hematocrit <33%) during the first or third trimesters or hemoglobin <10.5 g/dL (hematocrit <32%) during the second trimester (Table 1).[3] Historically, there were different diagnostic thresholds for anemia during pregnancy that were based on maternal race.[4] To reduce racial inequities in screening and treating anemia in perinatal populations, the same standard is now applied universally to diagnose anemia during pregnancy.

Differentiating between physiologic and pathologic causes of anemia in pregnancy

The high prevalence of perinatal anemia is driven by physiological changes that occur during pregnancy. Because plasma volume expands by 40% to 50% but erythrocyte mass expansion is only 15% to 25%, a physiological dilutional anemia commonly develops as pregnancy progresses.[3] Though physiologic dilutional anemia typically

Table 1. Contrasting definitions of anemia and iron deficiency during pregnancy

	World Health Organization[2,11]	American College of Obstetricians & Gynecologists[3]
Ferritin	<15 ng/L*	<30 ng/L**
Hemoglobin		
First trimester	<11 g/dL	<11 g/dL
Second trimester	<11 g/dL	<10.5 g/dL
Third trimester	<11 g/dL	<11 g/dL

*WHO definition for iron deficiency during first trimester of pregnancy;
**ACOG definition for iron deficiency during any trimester of pregnancy.

results in mild anemia (hemoglobin of 10 to 11 g/dL), it is impossible to differentiate between physiologic dilutional anemia and pathological causes of anemia during pregnancy without a laboratory workup. This workup targets the more common pathologic causes of anemia, which include, but are not limited to, iron deficiency anemia, anemia of chronic disease, folic acid deficiency anemia, anemia associated with vitamin B12 deficiency, or inherited hemoglobinopathies such as thalassemia or sickle cell anemia.[3] Specifically, ACOG guidelines recommend screening all pregnant people for anemia with a complete blood count twice during routine prenatal care: once in the first trimester and again between 24 weeks and 0 days gestation and 28 weeks and 6 days gestation.[3] Though ACOG guidelines do not recommend routine repeat anemia screening in the third trimester or at term, this is commonly done in the United States.

When anemia is identified on the screening complete blood count, ACOG recommends additional evaluation, which "may include a medical history, physical examination, and measurements of the complete blood count, red blood cell indices, serum iron levels, and ferritin levels" with consideration for peripheral smear, hemoglobin electrophoresis, or genetic testing based on personal or family history.[3] However, ACOG also endorses empirical treatment with iron and additional investigations if there is no response.[3] Perhaps due to ACOG's inconsistency, there is a wide variation in practice patterns concerning the diagnosis of anemia during pregnancy. For example, some obstetricians screen for hemoglobinopathies universally during the first trimester, while others obtain hemoglobin electrophoresis only in the setting of anemia and family history or geographic location. Moreover, serum iron or ferritin are not routinely performed for anemic pregnant patients. Rather, the presumptive diagnosis for anemia during pregnancy is iron deficiency anemia, and iron repletion therapy is often initiated without confirming the diagnosis for most patients.

Prevalence of iron deficiency and iron deficiency anemia during pregnancy

Iron deficiency anemia is the most common pathologic cause of anemia, affecting nearly 1 in every 5 pregnant persons in the United States.[5] Each pregnancy requires approximately 1000 milligrams (mg) of total iron to support increased erythrocyte production, normal placental and fetal development, and anticipated blood loss at delivery.[3] Many pregnant people are unable to ingest or absorb sufficient dietary iron,[6] leaving them vulnerable to developing iron deficiency or iron deficiency anemia. Indeed, according to data from the United States National Health and Nutrition Examination Survey from 1999 to 2006, 25% of all pregnant people in the United States have iron deficiency, with rates of 7%, 24%, and 39% in the first, second, and third trimester, respectively.[5] Rates of iron deficiency during pregnancy differ by demographic factors: compared with those who are non-Hispanic White or have fewer than 3 children, the prevalence of iron deficiency is markedly higher among non-Hispanic Black and Mexican-American pregnant people and those who have 3 or more children.[5]

To reduce iron deficiency and the risk of subsequently developing iron deficiency anemia, the CDC recommends that all pregnant patients begin low-dose iron supplementation (27mg of elemental iron daily) at the first prenatal visit.[7] Most nonchewable prenatal vitamins include 27 mg of elemental iron. The United States Preventive Task Force does not recommend for or against routine iron supplementation during pregnancy (beyond low-dose supplementation), as it is unclear whether iron supple-

Table 2. Ongoing trials comparing IV to oral iron for iron deficiency anemia during pregnancy[36–40]

Trial	Location/ funding	Inclusion	IV iron (1)	IV iron (2)	Oral iron	Masking	Primary outcome	N
EDIVA[39]	Bangladesh Gates	Hb <10 at 13–32 weeks	Ferric carboxymaltose, 1000 mg	-	Ferrous sulfate, 60 mg bid	No	Maternal anemia (Hb <11) at 36 weeks	900
IVIDA2[36]	US NIH	Hb <10 & ferritin <30 at 24–28 weeks	Ferric derisomaltose, 1000 mg	-	Ferrous sulfate, 65 mg qd-tid	Yes	Maternal peripartum blood transfusion	746
IVON[37]	Nigeria Gates	Hb <10 at 20–32 weeks	Ferric carboxymaltose, 1000 mg	-	Ferrous sulfate, 65 mg tid	No	Maternal anemia (Hb <11) at 36 weeks	1056
RAPID IRON[38]	India Children Investment Fund Foundation (UK)	Hb <10 at 13–26 weeks	Ferric carboxymaltose, 1000 mg	Ferric derisomaltose, 1000 mg	Ferrous sulfate, 60 mg bid	No	Low birth weight Maternal anemia (Hb <11) at 30–34 weeks	4320
REVAMP-TT[40]	Malawi Gates	Hb <10 at 27–35 weeks	Ferric carboxymaltose, 1000 mg	-	Ferrous sulfate, 60 mg bid	No	Maternal anemia (Hb <11) at 36 weeks	590

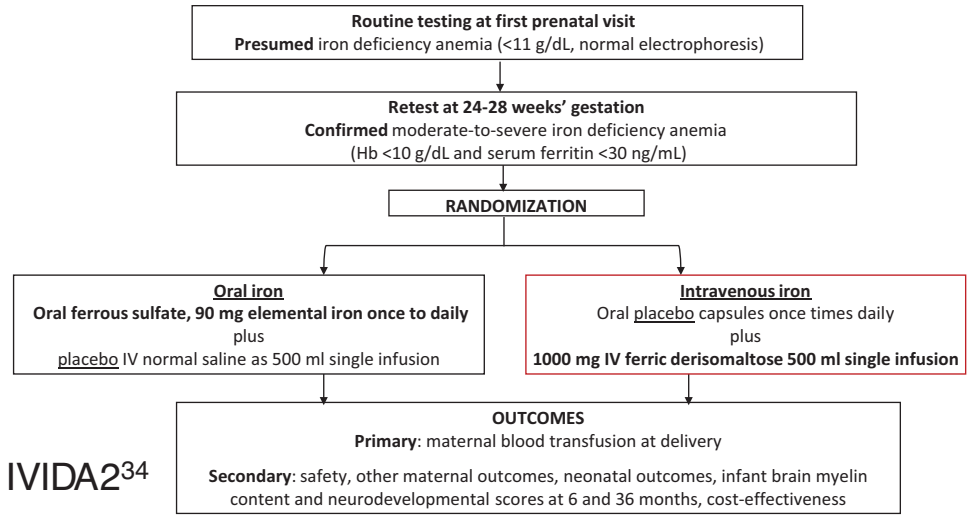

Figure 1. IVIDA2 trial flowsheet.[36]

mentation in pregnant people without anemia affects perinatal outcomes.[8] Thus, providing more than 27mg of elemental iron daily for pregnant people without anemia is not currently the standard of care.

If performed, evaluation for iron deficiency anemia during pregnancy by obstetric care providers is usually with serum ferritin only, as it is more easily interpretable compared with the full panel of iron studies,[3] despite being an acute phase reactant that may vary during normal pregnancy due to the physiologic rise in hepcidin levels.[9] Furthermore, ferritin is considered to be a more sensitive and specific marker for iron deficiency than other markers, including serum iron and transferrin saturation.[10,11] However, the WHO and ACOG have different thresholds of serum ferritin required to diagnose iron deficiency during pregnancy (Table 1). The WHO defines iron deficiency during the first trimester of pregnancy as serum ferritin <15 ng/L,[12] whereas ACOG defines iron deficiency during pregnancy as a serum ferritin <30 ng/L in any trimester.[3] We use the ACOG diagnostic thresholds for iron deficiency during pregnancy as the higher ferritin level has much higher sensitivity (92%) without a significant compromise of specificity (98%).[11]

Management of iron deficiency without anemia during pregnancy

By applying ACOG definitions of iron deficiency and anemia, we can define iron deficiency without anemia during pregnancy as a serum ferritin <30 ng/L but hemoglobin ≥11.0 g/dL.[3] Currently, ACOG does not recommend treatment for iron deficiency without anemia,[3] perhaps because there are no compelling data showing that maternal iron deficiency is associated with reduced fetal or neonatal iron stores or adverse childhood neurodevelopmental sequellae.[13,14] However, new approaches for evaluating iron homeostasis have prompted a call to change this paradigm.[15] Data generated from novel iron testing methods have suggested that neonates born to mothers with lower ferritins have significantly lower ferritins than neonates born to mothers with normal ferritins.[16] More research is needed to untangle the potential relationship between maternal iron deficiency without anemia and abnormal neonatal outcomes.

Furthermore, the iron demands of normal pregnancy may increase the risk of iron deficiency anemia later in pregnancy. As untreated iron deficiency anemia increases the risk of perinatal complications,[17] waiting for iron deficiency anemia to develop prior to initiating treatment may in fact cause harm. Data on interventions designed to prevent the development of iron deficiency anemia among pregnant people with iron deficiency and normal hemoglobin remain scant. A recent randomized controlled trial conducted in Denmark compared the efficacy of a single-dose (1000 mg) intravenous (IV) iron (ferric derisomaltose) with 100 mg daily oral iron (ferrous fumarate) in preventing anemia among 201 pregnant people at 14 to 21 weeks with iron deficiency (ferritin <30 ng/L).[18] Over the 18-week follow-up period, those receiving IV iron had a higher mean hemoglobin increase and were less likely to develop anemia compared with those receiving oral iron (9% vs 27%; 18% difference, 95% CI [10%, 25%]).[18] There was no significant difference between the 2 groups in treatment-related adverse events.[18] While these results are promising, 11% of the analytic population had iron deficiency anemia at randomization. Thus, these results may not be applicable to pregnant people with iron deficiency but not anemia. More high-quality data are urgently needed to clarify whether treating iron deficiency without anemia during pregnancy improves perinatal outcomes.

Treating iron deficiency anemia in pregnancy

Unlike the clinical conundrum of iron deficiency without anemia during pregnancy, iron deficiency anemia has been associated with increased rates of cesarean delivery, postpartum depression, and perinatal blood transfusion,[17,19,20] the major driver of the CDC's severe maternal morbidity composite quality metric.[21] Iron deficiency anemia is also associated with increased risk of low birth weight, preterm birth, and small-for-gestational-age neonates.[17,19] Moreover, those who are iron deficient during pregnancy are at risk of delivering iron-deficient neonates, who themselves are at risk for delayed growth and development even after postnatal iron repletion.[22] Fetal-neonatal iron deficiency has been linked to neurological impairments in infants[23] that may persist into adulthood.[22] Animal studies have identified

that iron has a crucial role in normal fetal and postnatal brain development, including myelination, dendritic growth, and synapse formation.[24-26] Thus, there is a clear association between untreated iron deficiency anemia in pregnancy and long-term adverse neurodevelopmental outcomes.

Oral versus intravenous iron for treating iron deficiency anemia in pregnancy

Iron supplementation is recommended for treating iron deficiency anemia during pregnancy, but the optimal route of delivery remains uncertain. Oral iron, administered in doses higher than found in prenatal vitamins, is the current standard for treating iron deficiency anemia during pregnancy in the United States.[3] Ferrous sulfate is the most commonly prescribed oral iron formulation as it is inexpensive, safe, readily available, and, when tolerated, effective. However, a meta-analysis of 43 randomized controlled trials reported that up to 70% of patients prescribed oral iron experienced significant gastrointestinal perturbation, decreasing adherence to therapy.[27] Because higher and more frequent doses have not been shown to improve iron uptake but increase adverse medication effects, lower iron doses and alternate-day dosing have been proposed to treat iron deficiency anemia during pregnancy.[28,29] Specifically, alternate-date oral iron supplementation has been shown to avoid hepcidin suppression, thereby increasing oral iron supplementation while likely reducing the prevalence of adverse medication effects.[30] Though this regimen is commonly used in parts of Europe and the United Kingdom, alternate-day dosing is not yet recommended during pregnancy in the United States.[3]

Intravenous (IV) iron is another option for treating iron deficiency anemia during pregnancy. IV iron is usually administered in the second or third trimesters, as there are no safety data for first-trimester use. All IV iron products currently on the market have similar safety and efficacy.[31] Thus, the choice of formulation is based on cost and administration burden. Formulations such as low-molecular-weight iron dextran, ferric derisomaltose, or ferric carboxymaltose that allow a complete replacement dose in a single visit are preferred to those that require multiple infusions such as ferumoxytol or iron sucrose, namely because they reduce costs associated with infusion and increase likelihood that the pregnant person receives full treatment for iron deficiency anemia.

ACOG currently recommends oral iron repletion as the first-line treatment for iron deficiency anemia during pregnancy, stating that IV iron "may be considered for those who cannot tolerate or do not respond to oral iron or for those with severe iron deficiency later in pregnancy."[3] However, some data suggest that IV iron may be superior to oral iron in rapidly correcting anemia and iron deficiency. Two meta-analyses of randomized trials found that compared with oral iron, IV iron was associated with significantly higher hemoglobin level following therapy among pregnant people with iron deficiency anemia.[32,33] One of these meta-analyses also evaluated maternal and neonatal outcomes.[33] Among 8 randomized controlled trials with these specific outcomes, IV iron was associated with higher neonatal birth weight (weighted mean difference 58.25 g [95% CI 5.57 g, 110.94 g]), higher neonatal ferritin levels (weighted mean difference 21.38 ng/mL [95% CI 5.50 ng/mL, 37.25 ng/mL]), and less frequent adverse effects (relative risk 0.34 [95% CI 0.20, 0.57]) and therapy discontinuation (0.02% with IV and 2% with oral iron).[33] However, the primary trials did not assess clinically meaningful maternal or neonatal outcomes and included small sample sizes (50-252).[33]

After these meta-analyses were published, two large randomized controlled trials—one in India[34] and another in Malawi[35]—were published. In the study from India, pregnant people at 20 to 28 weeks with a hemoglobin of 5-8 g/dL or at 29 to 32 weeks with a hemoglobin of 5-9 g/dL were randomly assigned to receive up to 5 divided doses of IV iron sucrose infusions or oral iron (100 mg elemental iron twice daily).[34] The primary outcome was a maternal composite outcome, defined as one of the following conditions: postpartum hemorrhage, blood transfusion during and after delivery, sepsis, shock, prolonged hospital stay and intensive care unit admission, or referral to higher centers.[34] The results showed no difference in the primary outcome, serious maternal adverse events, or serious fetal and neonatal adverse events.[34] However, this study is limited by using iron sucrose, which required multiple infusions, resulting in a wide range of IV iron doses infused (200-1600 mg), and the mean iron sucrose dose of 400 mg is subtherapeutic for treatment during pregnancy. In addition, this study is limited by the fact that the primary composite outcome includes multiple conditions not directly associated with anemia, such as sepsis.[34]

In the study from Malawi, pregnant people at 13 to 26 weeks with a hemoglobin of less than 10.0 g/dL and negative malaria rapid diagnostic test were randomized to a single dose of up to 1000 mg of ferric carboxymaltose or oral iron (60mg elemental iron twice daily for 90 days).[35] The primary outcome was anemia at 36 weeks' gestation (defined as hemoglobin <11.0 g/dL), and the primary neonatal outcome was birth weight. There was no difference in the primary outcome—or in rates of anemia or moderate-to-severe anemia 4 weeks posttreatment, at delivery, or 4 weeks postpartum—or primary neonatal outcome and no significant difference in adverse events.[35] However, those randomized to ferric carboxymaltose had lower rates of iron deficiency and iron deficiency anemia (defined as anemia with a ferritin <15 mg/L or <30 mg/L if C-reactive protein >5 mg/L) at 36 weeks' pregnancy, birth, and 4 weeks' postpartum compared with those randomized to oral iron. Importantly, only approximately 40% of the study sample had iron deficiency anemia, with a median ferritin at randomization slightly less than 30 mg/L, suggesting that approximately half of those randomized did not have iron deficiency. Of note, subgroup analyses limiting the analytic population to those with iron deficiency or iron deficiency anemia at randomized demonstrated no difference in maternal anemia at 36 weeks' gestation or neonatal birth weight. In addition, those randomized to oral iron were prescribed treatment for only 90 days after randomization,[35] which does not align with ACOG recommendations to continue oral iron supplementation until delivery.[3]

There is, therefore, an urgent need for a study to test the clinical effectiveness, safety, and cost-effectiveness of IV iron on clinically relevant maternal and neonatal outcomes among pregnant people with iron deficiency anemia. Our group and others are conducting such trials.[36-40] In our trial, the only multicenter, placebo-controlled, double-blinded randomized controlled trial, 746 pregnant people with moderate-to-severe iron deficiency anemia (hemoglobin <10 g/dL and ferritin <30 ng/mL) at 24 to 28 weeks' gestation are randomized 1:1 to either a single 1000 mg dose of intravenous ferric derisomaltose and oral placebo (1 to 3 times

daily) or a single placebo infusion with 1 to 3 times daily 325 mg ferrous sulfate tablets containing 65mg of elemental iron.[36] The primary outcome is peripartum blood transfusion, defined as blood transfusion from delivery to 7 days postpartum. This primary outcome is clinically meaningful and plausible. In our pilot randomized trial, there was a 100% reduction in blood transfusion among pregnant people with iron deficiency anemia treated with IV iron compared with oral iron (0% vs 15%).[41] Secondary outcomes include adverse medication reactions, maternal and neonatal hematologic indices, and offspring neurodevelopment as measured via nonsedated MRI and neurodevelopmental assessment at 6 months of life. Results from this and the other ongoing trials will help define the optimal route of iron repletion among pregnant people with iron deficiency anemia.

Summary

Anemia is common during pregnancy, and while most causes are physiologic, the most common pathologic cause is iron deficiency. ACOG recommends confirmation of iron deficiency anemia with iron studies when anemia is diagnosed during pregnancy but acknowledges that presumptive treatment for suspected iron deficiency anemia is common in practice. Currently ACOG does not recommend treating iron deficiency without anemia during pregnancy. Though the benefits of treating iron deficiency anemia during pregnancy are clear, the optimal route of iron repletion remains uncertain. Results of ongoing large randomized trials will help define the optimal route of iron treatment for pregnant patients diagnosed with iron deficiency anemia.

Conflict-of-interest disclosure

Adam K. Lewkowitz served on medical advisory boards for Shields Pharmaceutics in 2021 and Pharmacosmos Therapeutics Inc. in 2022.

Methodius G. Tuuli: no competing financial interests to declare.

Off-label drug use

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Management of pregnant women who have bleeding disorders

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Bleeding disorders, including von Willebrand disease (VWD), hemophilia, other coagulation factor deficiencies, platelet disorders, defects of fibrinolysis, and connective tissue disorders, have both maternal and fetal implications. Successful management of bleeding disorders in pregnant women requires not only an understanding of bleeding disorders but also an understanding of when and how bleeding occurs in pregnancy. Bleeding does not occur during a normal pregnancy with a healthy placenta. Bleeding occurs during pregnancy when there is an interruption of the normal utero-placental interface, during miscarriage, during an ectopic pregnancy, or at the time of placental separation at the conclusion of pregnancy. Although mild platelet defects may be more prevalent, the most commonly diagnosed bleeding disorder among women is VWD. Other bleeding disorders are less common, but hemophilia carriers are unique in that they are at risk of bleeding themselves and of giving birth to an affected male infant. General guidance for maternal management of a woman who is moderately or severely affected includes obtaining coagulation factor levels at a minimum in the third trimester; planning for delivery at a center with hemostasis expertise; and anticipating the need for hemostatic agents. General guidance for fetal management includes pre-pregnancy counseling; the option of preimplantation genetic testing for hemophilia; delivery at a tertiary care center with pediatric hematology and newborn intensive care; consideration of cesarean delivery of a potentially severely affected infant; and avoidance of invasive procedures such as scalp electrodes and operative vaginal delivery in any potentially affected infant.

LEARNING OBJECTIVES

- Understand when and how bleeding occurs in pregnancy
- Be able to provide general guidance for the maternal and fetal management of a woman with a bleeding disorder during pregnancy

Bleeding disorders

Bleeding disorders that the hematologist may encounter during pregnancy include von Willebrand disease (VWD), hemophilia carrier status with factor VIII or factor IX deficiency, thrombocytopenia, platelet dysfunction, defects of fibrinolysis, connective tissue disorders, and other coagulation factor deficiencies. The most commonly encountered inherited bleeding disorder during pregnancy, besides, perhaps, a mild undiagnosed platelet disorder, is VWD. Based on enrollment of symptomatic patients in hemostasis centers[1] and a Danish national registry,[2] the prevalence of VWD is only 1 in 10 000, and based on a population study of childbearing-age women, the prevalence of VWD is 1 in 4000.[3] In a study of patients with a history of bleeding or bruising, however, the prevalence is 1 in 1000,[4] and based on 2 population studies of personal bleeding symptoms, low von Willebrand factor levels, and family history of VWD, the prevalence of VWD is approximately 1%,[5,6] suggesting that the hematologist will encounter undiagnosed cases as well as diagnosed ones. As for the prevalence of other inherited bleeding disorders, the prevalence of hemophilia carrier status is approximately 1 in 3000,[7] and the prevalence of rare, severe inherited bleeding disorders is 1 in 500 000 to 2 million.[8] Although the range of bleeding disorders seen in specialized treatment centers may be skewed to more severe cases and therefore may not represent what would be encountered by the practicing hematologist,

surveillance of females in the Centers for Disease Control and Prevention's network of hemophilia treatment centers from 2012 to 2021 revealed that approximately 62% of enrollees had VWD, 7% were carriers of hemophilia with factor VIII (FVIII) or factor IX (FIX) deficiency, 11% had various other coagulation factor deficiencies, 19% had platelet disorders, and 2% had connective tissue disorders or disorders of fibrinolysis.[9] Although this review focuses primarily on inherited bleeding disorders, general principles in the management of bleeding disorders in pregnancy often apply to both inherited and acquired conditions.

Bleeding in pregnancy

Successful management of bleeding disorders in pregnant women requires not only an understanding of bleeding disorders but also an understanding of when and how bleeding occurs in pregnancy. Bleeding does not occur during a normal pregnancy with a healthy placenta. Bleeding occurs during pregnancy when there is an interruption of the normal utero-placental interface, during miscarriage, during an ectopic pregnancy, or at the time of placental separation at the conclusion of pregnancy, whenever that is. Almost all bleeding that occurs during pregnancy or after delivery arises from the small blood vessels within the uterus and can be referred to as obstetric bleeding. Obstetric bleeding is controlled by emptying the uterus and promoting uterine contraction. Most of the rest of the bleeding that occurs during pregnancy or after delivery arises from incisions, lacerations, ruptured vessels, or ruptured viscus, including the bleeding that accompanies birth trauma, cesarean delivery, or a ruptured ectopic pregnancy and can be referred to as surgical bleeding. Surgical bleeding is controlled by ligatures and sometimes embolization. Less than 1% of bleeding that occurs during pregnancy or after delivery is related to a coagulation defect.[10]

Identifying the pregnant woman at risk of bleeding

Every pregnant woman is at risk of bleeding during pregnancy and at the time of delivery. Almost always bleeding is due to obstetrical or surgical factors, but without a normal number of platelets, normal platelet function, normal levels of VWF, normal levels of coagulation factors, normal fibrinolytic activity, and normal collagen, a woman may be at risk for excessive bleeding. A patient may be referred to the hematologist for care with a known bleeding disorder or may be referred for evaluation of a possible bleeding disorder. A personal history that suggests an underlying bleeding disorder is well understood and includes:[11]

- Heavy menstrual bleeding especially since menarche
- Iron deficiency anemia requiring treatment or transfusion
- Prolonged bleeding from trivial cuts
- Nose bleeds
- Notable bruising without injury
- Bleeding from the oral cavity or gastrointestinal tract without an anatomic lesion
- Prolonged or excessive bleeding following dental extractions and/or procedures
- Unexpected bleeding during or following surgery
- Hemorrhage that required blood transfusion

It also includes other reproductive tract bleeding that may have been overlooked, such as:

- Hemorrhagic ovarian cysts
- Excessive bleeding at the time of miscarriage
- Delayed postpartum hemorrhage (PPH), occurring 24 hours or more after delivery

Immediate PPH, occurring less than 24 hours after delivery, is rarely associated with an underlying bleeding disorder. The coagulopathy that accompanies PPH is usually acquired and acute resulting from massive hemorrhage due to obstetrical or surgical factors.[12] Patients may be referred to the hematologist for evaluation of a possible bleeding disorder after a massive PPH, but when an underlying bleeding disorder is discovered, the patient is likely to have had other signs or symptoms of a bleeding disorder preceding the pregnancy.[13] So while patients with an underlying bleeding disorder are at risk of excessive bleeding during pregnancy or after delivery, isolated PPH, which accompanies 3% of births,[14] rarely indicates an underlying bleeding disorder.

Diagnosis of an underlying bleeding disorder during pregnancy is complicated by the fact that pregnancy is a hypercoagulable state. Not every aspect of this hypercoagulable state is understood, but during pregnancy and the early postpartum period, there in an increase in VWF,[15,16] FVIII,[15,16] fibrinogen,[17,18] and certain other coagulation factors,[18] a decrease in the natural anticoagulant protein S,[18] a decrease in fibrinolytic activity,[18] and a decrease in platelet number,[19] but an increase in mean platelet volume[20] and platelet reactivity.[21] Because of this hypercoagulable state, the final diagnosis of an underlying bleeding disorder should be made during baseline health conditions and not during pregnancy.[22]

Von Willebrand disease (VWD)

VWD, deficiency of normal von Willebrand factor (VWF), is characterized by mucocutaneous bleeding (nose bleeds, heavy menstrual bleeding, or easy bruising) as opposed to deep tissue bleeding (muscle or joint bleeds), although the latter may be seen in patients with VWD, particularly those with more severe disease. While women and men are equally likely to inherit VWD, women are disproportionately affected due to the bleeding challenges of menstruation, miscarriage, and childbirth and, as noted by Erik von Willebrand in his original paper,[23] are twice as likely to be diagnosed with the disease. Type 1 VWD, which is usually mild to moderate in severity, accounts for 80% of diagnosed VWD and is due to a reduced level of normal VWF (quantitative deficiency) with VWF antigen and VWF activity levels proportionally decreased. The threshold for diagnosis according to the latest American Society of Hematology (ASH), International Society on Thrombosis and Haemostasis (ISTH), National Hemophilia Foundation (NHF), and World Federation of Hemophilia (WFH) guidelines on the diagnosis of von Willebrand disease[22] is a VWF level ≤0.30 IU/mL regardless of bleeding history, and for patients with abnormal bleeding (or during pregnancy, we would add), a VWF level ≤0.50 IU/mL. Inheritance is autosomal dominant. Type 2 VWD, which is usually moderate in severity, accounts for most of the rest of diagnosed VWD cases and is due to a low level of functional VWF manifest by a reduced ratio of VWF activity relative to VWF antigen of less than 0.7.[22] Type 2 VWD is further subdivided into 4 different subclasses

depending on the mechanism leading to the qualitative dysfunction. Inheritance is usually autosomal dominant. Type 3 VWD, which is severe and extremely rare, affects approximately 1 in 1 000 000 persons and is due to undetectable levels of VWF with very low levels of FVIII.[22] Inheritance is autosomal recessive or codominant (as occurs in compound heterozygotes).[24]

While VWF and FVIII levels rise during pregnancy, in women with VWD, median levels are lower than levels in women without VWD and fall rapidly after delivery, approaching baseline by 1 week postpartum and reaching baseline by 3 weeks postpartum.[15] Overall, the risk of bleeding during pregnancy (antepartum bleeding, immediate PPH, severe PPH, perineal hematoma, and delayed PPH) is increased by 2- to 10-fold in women with VWD.[3,25] In a systematic review of 87 studies (71 case reports or case series and 16 cohort studies) of maternal bleeding complications in 811 deliveries in women with VWD, the primary PPH rate was 32% and the secondary PPH rate was 13% among the cohort studies, but only 3 of the studies were prospective and only 1 included a comparison group of unaffected women.[26] In this latter prospective study of women whose levels were at least 50 precent of normal or higher (≥0.5 IU/mL) at 36 weeks gestation, the risk of PPH was not significantly increased compared with unaffected women.[15]

Hemophilia

Hemophilia A is due to deficiency of FVIII and hemophilia B to deficiency of FIX. Hemophilia is characterized classically by deep tissue (muscle and joint) bleeding, although an increased risk of mucosal bleeding can also be seen. The inheritance of hemophilia is X-linked. Males with hemophilia have an abnormal gene for FVIII or FIX on their single X chromosome. The prevalence among males is about 1 in 5000. Females with an abnormal gene for FVIII or FIX on 1 of their 2 X chromosomes are considered carriers. Table 1 summarizes obligate versus possible carrier status in hemophilia. In a study of the pedigrees of families with hemophilia, there were 156 female carriers for every 100 males with hemophilia.[7] Female carriers are very rarely affected with severe hemophilia, but it is possible in the case of monosomy X (the presence of a single X chromosome), homozygosity or compound heterozygosity for two abnormal X chromosomes, or nonrandom X inactivation (the most common of these 3 mechanisms). Women who are heterozygous for an abnormal gene for FVIII or FIX on 1 of their 2 X chromosomes and have factor levels below the hemostatic range also meet the criteria for hemophilia.[27] Approximately 30% of carriers have levels in the hemophilia range.[23] The ISTH recently published a new nomenclature for hemophilia carriers based on factor levels and symptoms. For women and girls with FVIII or FIX >0.05 and <0.40 IU/mL, the classification would be mild; with FVIII or FIX 0.01-0.05 IU/mL, the classification would be moderate; and with FVIII or FIX <0.01 IU/mL, the classification would be severe hemophilia. If FVIII or FIX were ≥0.40 IU/mL, the classification would be symptomatic (with a bleeding phenotype) or asymptomatic (without a bleeding phenotype).[28]

Table 1. Obligate versus possible carrier status in hemophilia

	Obligate carrier	Possible carrier
Mother	• Of a son with hemophilia if there is another affected male relative • Of more than 1 son with hemophilia	Of only 1 son with hemophilia
Sister		• Of a male with hemophilia • Of a female carrier
Daughter	Of a man with hemophilia	Of a female carrier
Other relation		• Of a male with hemophilia • Of a female carrier

The risk of PPH in carriers is hard to estimate, but in case series and in 1 cohort study, the rate of PPH ranged from 13 to 22%.[29] Most of these reports were retrospective or based on patient recall, which is inherently inaccurate, but the suggestion is that the risk of PPH is increased.[29] In a systematic review of 17 case reports or case series and 11 cohort studies of maternal bleeding complications in 502 deliveries in hemophilia carriers, there was a PPH rate of 20% among the cohort studies.[30]

Unlike for patients with VWD, in whom genetic testing is less commonly performed and results will generally not affect the management of pregnancy or delivery, in carriers or potential carriers of hemophilia, identifying the underlying genetic variant prior to pregnancy is very valuable.[29] Once a likely causative variant is identified, carriers of hemophilia have the option of undergoing in vitro fertilization for preimplantation genetic testing of embryos; or during pregnancy of undergoing prenatal diagnosis of hemophilia by chorionic villous sampling (placental biopsy performed during the first trimester of pregnancy) or amniocentesis (amniotic fluid sampling performed during the second or third trimester of pregnancy). Regardless of previous genetic testing of a carrier or potential carrier of hemophilia, determination of the fetal sex should be performed by either ultrasound, serum cell free DNA, or invasive genetic testing (chorionic villus sampling or amniocentesis). If the fetus is female, there is a 50% chance she will be a carrier. If the fetus is male, there is a 50% chance he will have hemophilia.

Other inherited coagulation factor deficiencies

Factor XI (FXI) deficiency, while not as common as hemophilia A or B, is more common than the other inherited coagulation factor deficiencies. It is also more common among persons of Ashkenazi Jewish ancestry where heterozygosity approaches 1 in 11 individuals and homozygosity or compound heterozygosity approaches 1 in 450 individuals.[31] FXI is part of the intrinsic pathway and the kallikrein-kinin system. FXI deficiency is characterized by variable mucocutaneous bleeding that does not always correlate with the FXI level. Inheritance of FXI deficiency is autosomal dominant with severe forms following a recessive or compound heterozygous pattern. Heterozygotes typically have an FXI activity level of 20% to 60%.[31] Severe FXI deficiency is defined as an FXI activity level of less than 20%.[31] In a retrospective study of obstetric and perioperative management of patients with FXI deficiency, FXI levels did not change among 81 women whose levels were measured at least twice during pregnancy at different time points.[32] While the risk of bleeding in FXI deficiency is variable, the rate of PPH appears to be increased. The rate of PPH was found to be 11% in a cohort that included 143 vaginal and 63 cesarean deliveries,[32] and 18% in a systematic review of 498 deliveries.[33]

Factor VII (FVII) deficiency is considered the most common autosomal recessive coagulation factor deficiency. Like FXI deficiency, FVII deficiency is also characterized by variable mucocutaneous bleeding that does not always correlate with the FVII level. The ISTH classifies FVII deficiency as severe (FVII <10% with greatest risk for major spontaneous bleeding); moderate (FVII 10%-20% with risk for mild spontaneous or provoked bleeding); and mild (FVII 20%-50% with mild or no bleeding).[34] Patients with severe FVII deficiency, which has a prevalence of 1 in 500 000, are usually homozygotes or compound heterozygotes.

Congenital fibrinogen deficiency with either a reduction in the quantity of fibrinogen (afibrinogenemia, hypofibrinogenemia) or the quality of fibrinogen (dysfibrinogenemia) is very rare, affecting less than 1 in 1 000 000 individuals.[35] There is a strong correlation between fibrinogen levels and maternal bleeding and, unlike other coagulation factor deficiencies, a strong correlation between fibrinogen levels and bleeding at the utero-placental interface. Patients with afibrinogenemia experience spontaneous bleeds into muscles and joints and are at significant risk of intracranial hemorrhage. Patients with hypofibrinogenemia are usually asymptomatic but are vulnerable to bleeding after trauma. Patients with dysfibrinogenemia can have both spontaneous bleeding and spontaneous thromboses.[35] Levels that might not result in systemic bleeding, however, can still result in bleeding at the utero-placental interface, resulting in miscarriage or placental abruption as well as PPH.[35]

Factor XIII (FXIII) deficiency is very rare, affecting 1 to 2 per million individuals with the prevalence varying by country and the frequency of consanguinity. In 2 different European registries, FXIII deficiency accounted for 6%-7% of patients with rare bleeding disorders.[36,37] Severe FXIII deficiency (FXIII <10%) is associated with provoked bleeding, delayed wound healing, and repeated miscarriages.[37] In a systematic review of the literature, the rate of miscarriage was 66% and the rate of PPH 25%.[38] Successful pregnancies have been achieved with FXIII replacement.[37]

Platelet disorders

Platelet disorders are disorders of platelet number and function and range in severity from mild to very severe. Severe platelet disorders include Bernard-Soulier syndrome, a deficiency of the platelet glycoprotein receptor Ib/IX, and Glanzmann's thrombasthenia, a deficiency of the platelet glycoprotein receptor IIb/IIIa. Patients with both Glanzmann's and Bernard-Soulier are at high risk for PPH,[39,40] but they can also make alloantibodies to various platelet antigens, further complicating pregnancies with fetal/neonatal alloimmunization. In a review of patients with Glanzmann's from a single center, 3 of 9 neonates had severe thrombocytopenia, all of whom were born to mothers who were positive for antiplatelet antibodies,[41] and in a systematic review of patients with Bernard-Soulier, 6 neonates from 30 pregnancies were diagnosed with neonatal alloimmune thrombocytopenia, all of whom were born to mothers who were positive for antiplatelet antibodies.[40] Prevention of fetal/neonatal alloimmunization involves the use of antepartum intravenous immunoglobulin with or without the addition of steroids.[42]

Management of pregnant women with any bleeding disorder

There is some general guidance that applies to the management of pregnant women with any bleeding disorder. Women should be informed about what they can expect during pregnancy and childbirth, including the risk of increased bleeding complications at delivery and the chance that their infant will be affected. Ideally women will have been diagnosed before pregnancy, have had the opportunity for molecular testing if that is available, and have had the opportunity for preconception counseling with their hematologist and obstetric provider. When appropriate, a woman and her partner should be referred for genetic counseling. During pregnancy, at a minimum, women should have their coagulation factor levels checked at registration for prenatal care and again at 36 weeks' gestation. Additional levels in the second and early third trimester are of value in managing any bleeding during pregnancy and in anticipation of an early delivery. Women should refrain from taking low-dose aspirin for preeclampsia prevention. Women with a moderate or severe bleeding disorder, or those at risk of having a severely affected infant, should deliver in a tertiary care center with the requisite specialists and services—hemostasis expertise, maternal-fetal medicine, anesthesia, laboratory, pharmacy, blood bank, pediatric hematology, and newborn intensive care. For hospitals that have the infrastructure, formal multidisciplinary team review of cases can be helpful. Women with a moderate or severe bleeding disorder should have the opportunity to meet with a member of the anesthesia team prior to delivery to establish the option of neuraxial anesthesia or plan for an alternative. Those expecting a potentially severely affected infant should have the opportunity to meet with pediatric hematology prior to delivery. Anticipation of a mildly or moderately affected infant is not an indication for cesarean delivery, but while there is a risk of nonsevere fetal bleeding (as in the case of a woman with VWD or a hemophilia carrier), invasive procedures such as fetal scalp electrodes, and if possible, operative vaginal deliveries should be avoided. If a severely affected infant is anticipated, a cesarean delivery should be performed. (See the section "Management at the time of delivery: hemophilia" below.) The usual measures to prevent PPH (ie, uterotonic medication and active management of the third stage of labor) should be used. Obstetric and surgical bleeding should be managed aggressively. Intravenous tranexamic acid (TXA) can be safely used immediately after delivery (1 g repeated in 30 minutes if necessary).[43] Oral TXA can be used for prevention or treatment of delayed postpartum bleeding at a dose of 1 to 1.3 g every 8 hours. Regardless of the outcome of any testing during pregnancy, nonsteroidal anti-inflammatory drugs should be avoided postpartum. Umbilical cord blood should be obtained to test the infant. Circumcision of a male infant should be postponed until a bleeding disorder is ruled out or established and a suitable treatment plan made. Patients should have contact with a provider in the first week or two after hospital discharge.

Breastfeeding should be permitted in infants whose mothers require treatment with TXA, especially since TXA is given for only a short duration. In a prospective study of women receiving TXA at the time of cesarean delivery, the concentration in breast milk was only 1% of the maternal plasma concentration,[44] which is consistent with unpublished data from the manufacturer.[45] In the 20 060 subject Woman Trial, no adverse effects were noted in exposed infants.[43] In a small study of the long-term effects of infants exposed to TXA during lactation, no adverse effects were noted.[46]

Management at the time of delivery: VWD

Management at the time of delivery may include the cautious use of 1-deamino-8-D-arginine vasopressin (DDAVP or desmopressin) to induce endothelial secretion of VWF and FVIII, VWF concentrates (plasma-derived or recombinant), and antifibrinolytics, most commonly TXA. Specific therapy by VWD subtype is summarized in Table 2. In a multicenter prospective study of the postpartum management of VWD, 32 women with VWD were enrolled during 35 pregnancies. By the time of admission for delivery, approximately half of the women (17 women during 18 pregnancies) had VWF levels greater than 50% (0.5 IU/dL) and therefore were not treated. All these women had type 1 VWD. The other 15 women (during 17 pregnancies) who did not achieve VWF levels greater than 50% (0.5 IU/dL) by the time of admission for delivery (30% of those with type 1 VWD and all of those with type 2 VWD) were treated. Except for 2 women who received desmopressin and 1 woman who received no treatment after delivery, all the women who were treated received VWF concentrate before and after delivery.[15]

Desmopressin, if used at all at the time of delivery, must be used with caution. It is a synthetic analog of vasopressin (antidiuretic hormone), administered at delivery intravenously at a dose of 0.3 µg/kg, with a maximum dose of 25-30 µg, over 25 to 30 minutes, or intranasally 300 µg.[47] (Of note, intranasal desmopressin has limited availability in the US.) If additional treatment is required, VWF concentrates are recommended instead. Desmopressin is used in patients with type 1 VWD and some patients with type 2 (A, M, N) VWD who have a history of mild bleeding. Desmopressin is contraindicated in type 2B, as it may worsen thrombocytopenia, and in type 3 due to lack of response.[48] If desmopressin is to be used, a desmopressin trial should be performed prior to pregnancy to document efficacy. If a trial has not been performed prior to pregnancy, VWF concentrates and TXA should be used instead. A desmopressin trial should not be performed during pregnancy.[48] Since life-threatening hyponatremia, seizures, and neurological injury have occurred with the use of desmopressin,[47,49] the use of hypotonic fluids should be avoided (normal saline is favored), oral intake of water should be limited, and serial serum sodium measurements should be obtained. Oxytocin, administered to induce labor and administered postpartum, also has some antidiuretic activity. Furthermore, it is almost impossible to limit fluids during labor or at the time of delivery, when women routinely receive a minimum of 2 to 3 liters. Desmopressin is specifically contraindicated in patients with preeclampsia and those with active coronary artery disease, cerebrovascular disease, peripheral vascular disease, or increased risk of thrombosis.[48]

Alternatively, VWF concentrates may be safely used in any patient with VWD who requires treatment. VWF concentrates are either plasma derived or recombinant. The plasma-derived concentrates also contain FVIII. The usual initial dose is 40-80 VWF:RCo activity units/kg with maintenance doses of 20-40 VWF:RCo activity units/kg every 12 hours as needed for at least 3 days following vaginal delivery and at least 5 days following cesarean delivery.[15,50] While maintenance doses are often administered in bolus fashion, they can also be administered continuously at 2 VWF:RCo activity units/kg per hour, which allows for more constant VWF levels and facilitates the option of neuraxial anesthesia. In women with VWD for whom neuraxial anesthesia during labor is deemed suitable, the recent ASH ISTH NHF WFH guidelines suggest targeting a minimum VWF activity level of 0.50 IU/mL.[48] In women receiving repeated dosing of factor replacement it is important that VWF and FVIII activity be measured in real time so that adjustments can be made to avoid under- and over-dosing. In the systematic review by Punt et al., there were two cases of venous thromboembolism in women receiving factor replacement.[26] TXA can be used postpartum to help prevent delayed onset bleeding. The recent ASH ISTH NHF WFH guidelines suggest the use of oral TXA after delivery in all types of VWD.[48]

Given that VWF levels increase with pregnancy, use of nonpregnant normal levels to determine targets is likely not optimal. While there are ongoing studies in this area, target levels of 1.0 to 1.5 IU/dL have been suggested.[50,51] Levels should be maintained at >0.50 IU/dL for at least 3 days after vaginal delivery and at least 5 days following cesarean delivery.[50] While VWD in the fetus is not an indication for cesarean delivery, there is a risk of nonsevere fetal bleeding, so invasive procedures such as

Table 2. Specific therapy at the time of delivery for VWD subtypes

Subtype	Specific therapy for VWD
1	• Most will not require treatment. • If VWF levels <50% or 0.5 IU/mL at 36 weeks' gestation, treatment is required at the time of delivery. A higher threshold for treatment such as 0.8 IU/dL or 1.0 IU/mL has been adopted by some experts and may be considered. • VWF and FVIII levels should be followed to guide dosing. • Consider cautious use of desmopressin if a desmopressin trial was performed outside of pregnancy and efficacy was documented.
2	Expect that treatment will be required with VWF concentrates.
2A	• Most will require VWF concentrates. • Some may respond to desmopressin (requires previous desmopressin trial with documented efficacy).
2B	• Treat with VWF concentrates. • Desmopressin is contraindicated as it may worsen thrombocytopenia.
2M	• Most will require VWF concentrates. • Some may respond to desmopressin (requires previous desmopressin trial with documented efficacy).
2N	• Most will require VWF concentrates. • Some may respond to desmopressin (requires previous desmopressin trial with documented efficacy).
3	• Requires VWF concentrates.

fetal scalp electrodes and, if possible, operative vaginal deliveries should be avoided.[50] Neuraxial anesthesia is considered safe with VWF and FVIII activity levels ≥0.5 IU/mL. Levels ≥0.5 IU/mL should be maintained while the catheter is in place and for 6 hours after removal.[48]

Management at the time of delivery: hemophilia

For hemophilia carriers, management at the time of delivery requires attention not only to the risk of maternal bleeding but also to the risk of fetal bleeding. FIX levels do not increase significantly during pregnancy, and although FVIII levels do,[15,16] they likely do not increase to the same extent as FVIII levels do in women who are not hemophilia A carriers. While we have less data for hemophilia carriers than for patients with VWD, there are similar concerns regarding optimal target levels in carriers of hemophilia A, given that FVIII also increases during normal pregnancy. If a carrier's factor levels are less than 50% (<0.5 IU/mL) (or higher depending on evolving data and local practice) as delivery approaches (at 36 weeks' gestation, for instance), she would be at a greater risk of bleeding at delivery and postpartum. As is true in any bleeding disorder, most bleeding at the time of delivery is due to failure of the uterus to contract (obstetric bleeding) or to birth trauma (surgical bleeding), and while the risk of bleeding at delivery can be mitigated by routine obstetric measures, experts agree that women with factor levels less than 50% (0.5 IU/mL) (or higher depending on evolving data and local practice) should receive treatment with FVIII or FIX replacement at the time of delivery.[52] As is also true for women with VWD and other bleeding disorders, there are no randomized controlled trials to guide treatment; when required, treatment is recommended with virally inactivated plasma-derived or recombinant coagulation factor concentrates rather than cryoprecipitate or fresh frozen plasma,[52] and any recommendations regarding treatment threshold, product choice, dosing, or therapy duration are based on observational studies or expert opinion. Following delivery, concentrates should be administered to maintain factor levels above 0.5 IU/mL for at least 3 days following vaginal delivery and at least 5 days following cesarean delivery.[52] Factor VIII levels should be followed in real time to ensure adequate dosing and avoid overtreatment. Usual doses are 20-50 IU/kg.

An affected male infant has approximately a 3% risk of fetal or neonatal intracranial hemorrhage (ICH) if delivered vaginally and a 0.4% risk if delivered by cesarean.[53] We recommend that a woman expecting an affected or potentially affected male be delivered by cesarean before labor. While cesarean delivery does not completely eliminate the risk of ICH, the risk is reduced when cesarean is performed before labor compared with the risk when cesarean is performed after labor.[54] Also, although cesarean delivery generally increases risk of maternal bleeding and requires a longer hospital compared with vaginal delivery, this applies to the aggregate of cesarean deliveries—both planned and unplanned. A planned vaginal delivery includes the risk of an unplanned emergency cesarean delivery, which confers a greater risk of maternal bleeding and longer stay compared with a planned cesarean. In the 2 randomized trials of planned cesarean delivery versus planned vaginal delivery, planned cesarean delivery did not confer a greater risk of maternal bleeding or longer stay than planned vaginal delivery.[55,56] Various guidelines state that operative vaginal delivery (forceps or vacuum extraction), which greatly increases the risk of ICH,[54] should be avoided in the delivery of an affected male, but if the fetus is deep in the pelvis and delivery must be expedited, operative vaginal delivery may be unavoidable. In a large observational study of newborns with hemophilia, 4% of the 466 known obligate or possible carriers still had to be delivered by forceps or vacuum extraction.[54] The only certain way to avoid an operative vaginal delivery is to plan for a cesarean delivery. Following delivery, umbilical cord blood should be obtained and sent for factor levels. If the parents desire the infant to be circumcised, the procedure should be postponed until a diagnosis of hemophilia is ruled out or established and an appropriate treatment plan made.

Table 3. Treatment of other coagulation factor deficiencies

Deficiency	Treatment*
Factor VII deficiency	
20%-50%—mild	
10%-20%—moderate; at risk for mild or provoked bleeding	
<10%—severe	Low-dose rFVIIa or plasma
Factor XI deficiency	
20%-60%—variable risk for bleeding	
<20%—still variable risk for bleeding	Plasma or FXI concentrate where available
Fibrinogen deficiency	
<60mg/dL—at risk for miscarriage	Fibrinogen concentrates where available are the treatment of choice for patients with quantitative or qualitative deficiencies; otherwise, cryoprecipitate for antepartum as well as peripartum prophylaxis.
<100mg/dL—at risk for placental abruption	
<150mg/dL—at risk for placental abruption in labor and PPH	
Factor XIII deficiency	
<10%—severe; high risk for miscarriage and PPH	FXIII concentrate where available for antepartum as well as peripartum prophylaxis

*Includes the general guidance described under "Management of pregnant women with any bleeding disorder."

A woman expecting a female who is potentially a hemophilia carrier does not need a cesarean delivery. As in VWD, there is a risk of nonsevere fetal bleeding, so invasive procedures such as fetal scalp electrodes and, if possible, operative vaginal deliveries should be avoided.

Management of other inherited coagulation factor deficiencies and severe platelet disorders

Women with rare bleeding disorders, even those with mild deficiencies, are at increased risk of PPH.[57] The general guidance described in "Management of pregnant women with any bleeding disorder" applies to women with other inherited coagulation factor deficiencies and platelet disorders. Specific background regarding FVII, FXI fibrinogen, and FXIII deficiency is described in the individual sections on these conditions. Their treatment is summarized in Table 3. Bleeding or potential bleeding with severe platelet disorders has been managed with platelet transfusions and recombinant factor VIIa (rFVIIa).

Conclusions

Bleeding disorders increase the risk of maternal bleeding, particularly at the time of delivery, and in severely affected infants may result in fetal and neonatal ICH. Although mild platelet defects may be more prevalent, the most commonly diagnosed bleeding disorder among women is VWD. Other bleeding disorders are less common, but hemophilia carriers are unique in that they are at risk of giving birth to a severely affected male infant. General guidance for maternal management of an affected woman includes obtaining coagulation factor levels at least in the third trimester; planning for delivery at a center with hemostasis expertise; and anticipating the need for on-site laboratory testing and hemostatic agents. General guidance for fetal management includes prepregnancy counseling; the option of preimplantation genetic testing for hemophilia; delivery at a tertiary care center with pediatric hematology and newborn intensive care; consideration of cesarean delivery of a potentially severely affected infant; and avoidance of invasive procedures such as fetal scalp electrodes and operative vaginal delivery in any potentially affected infant. Future areas of research include determining the optimal factor level target levels for patients with VWD and hemophilia and considering alternative therapies for treatment.

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Off-label drug use

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Prevention, diagnosis, and management of PE and DVT in pregnant women

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Venous thromboembolism (VTE) is a leading cause of maternal morbidity and mortality worldwide. Despite the impact of VTE on pregnant and postpartum people and on society, guidelines addressing prevention, diagnosis, and management of VTE in pregnant and postpartum people frequently are based on recommendations from expert opinion and are extrapolated from data in nonpregnant populations. Pregnant individuals are frequently excluded from clinical trials, which is a barrier to providing safe, effective care. Anchoring to a case discussion, this review provides an update on recently published and ongoing randomized clinical trials (RCTs), prospective clinical management studies, and other research in this area. It highlights, in particular, the results of the Highlow RCT, which addresses optimal prevention of recurrence during pregnancy in people with prior VTE. Finally, we raise awareness of the impact of national and international clinical trial networks on the conduct of RCTs in pregnancy. We conclude, based on these data, that academic VTE clinical trials in pregnant women can and must be done.

LEARNING OBJECTIVES

- To understand the impact of VTE in pregnancy and the crucial importance of excellent prevention and diagnostic and management pathways
- To review the most recently published data and guidelines addressing optimal prevention, diagnosis, and management of VTE in pregnancy

CLINICAL CASE

It was February 2018. Aries was a 27-year-old clinical nurse specialist at 28 weeks' gestation in their first pregnancy. They were being cared for in the emergency department with suspected pulmonary embolism. They complained of left-sided pleuritic chest pain without breathlessness. Their respiratory rate was 16 breaths per minute, blood pressure was 111/70 mm Hg, heart rate is 84 beats per minute, and oxygen saturations were 99% on room air. They had no lower limb symptoms. I met them, and we discussed their suspected diagnosis.

The impact of venous thromboembolism (VTE) in pregnancy

VTE is a leading cause of death of pregnant and postpartum people.[1,2] Those who survive can have lifelong disability. VTE risk is higher during pregnancy than in the nonpregnant state and peaks postpartum: pooled incidence rates of 1.2 (95% confidence interval [CI]: 1.0-1.4) and 4.2 (95% CI: 2.4-7.6) per 1000 person-years have been reported during the antenatal and postpartum periods, respectively.[3]

I explained to Aries that we suspect pulmonary embolism. On one hand, Aries could appreciate the importance of not missing a pulmonary embolism diagnosis in pregnancy. However, they were worried about being exposed to radiation through diagnostic imaging. We had a discussion.

Radiation exposure during imaging for pulmonary embolism in pregnancy (Table 1)

A normal perfusion scan and a negative computed tomography pulmonary angiogram (CTPA) are considered effective for ruling out pulmonary embolism in pregnancy.[2] Sensitivity and negative predictive value of lung

Table 1. Fetal and maternal breast radiation exposure during diagnostic imaging for pulmonary embolism[2,6–8]

Test	Fetal radiation dose (mGy)	Maternal breast dose (mGy)
Chest X-ray	<0.01	<0.1
Perfusion lung scan:		
Low dose: ~40 MBq	0.02–0.20	0.16–0.5
High dose: ~200 MBq	0.20–0.60	1.2
Ventilation lung scan	0.10–0.30	<0.01
CT pulmonary angiography	0.05–0.5	~1–10[a] (lower with modern CTPA techniques)

[a]Modern advances in CT technology have greatly reduced maternal breast radiation exposure.[7,8] With breast shielding, further reductions in maternal breast absorbed dose can be achieved.[2,8]

MBq, megabecquerel.

scintigraphy and CTPA are reported to be high; however, large, adequately powered studies comparing methodologies are lacking.[4,5] Both maternal and fetal radiation exposure are low when modern imaging methods are used.[2] Low-dose perfusion scanning (estimated fetal radiation dose 0.02-0.20 mGy) and CTPA (estimated fetal radiation dose 0.05-0.5 mGy) expose baby to doses far below the threshold for fetal radiation complications (which is accepted to be 50-100 mGy).[2,6] Moreover, advances in CT technology have reduced radiation exposure through methods that include reduced kilovoltage, contrast-monitoring component, and anatomic coverage of the scan, and using iterative reconstructive techniques.[2,7,8] We recently reported that additional breast radiation dose reduction can be achieved by combining low-dose CTPA with breast shields in pregnancy without impacting image quality: shielding reduced surface breast radiation dose by 66% (to 0.5±0.3 mGy) in an anthropomorphic phantom and by 48% (to 0.7±0.2 mGy) in study participants.[8]

A prospective clinical management study, "OPTICA" (Optimised Computed Tomography Pulmonary Angiography [CTPA] in Pregnancy, Quality and Safety; NCT04179487) aims to validate the safety of such an optimized low-dose CTPA protocol as part of local algorithms for evaluation of suspected pulmonary embolism in pregnancy. The primary outcome is the incidence of VTE at 3 months in people in whom the baseline CTPA excluded pulmonary embolism[6] (Figure 1).

Diagnosis of pulmonary embolism in pregnancy

Aries asked, "Do I really need to have a scan?" They knew that diagnostic algorithms that combine pretest probability scores with D-dimers can rule out pulmonary embolism in nonpregnant patients.[2] At the time of their assessment, these algorithms were not validated in pregnancy; however, studies were ongoing, which have since been completed and published.

First, a UK prospective cohort study augmented with additional cases of confirmed pulmonary embolism did not demonstrate diagnostic utility for D-dimers or clinical decision rules in people with suspected pulmonary embolism during pregnancy.[9,10] In this study, objective pulmonary embolism diagnostic imaging and clinical diagnosis were permitted and there was no fixed diagnostic algorithm. Subsequently, 2 multicenter prospective diagnostic management outcome studies were published. In the first, the "CT-PE-Pregnancy" study (ClinicalTrials.gov: NCT00740454), pulmonary embolism was excluded without CTPA imaging in pregnant people with non-high revised Geneva pretest probability score and a negative D-dimer (defined as <500 ng/L).[11] The primary outcome, symptomatic VTE at 3 months, occurred in 0.0% (95% CI: 0.0%-1.0%) of untreated people; 11.7% did not require diagnostic imaging. Bilateral compression ultrasound (CUS) was mandated in people qualifying for CTPA but had a low diagnostic yield.

A second multicenter prospective management study with a similar design (the Artemis study[12]) evaluated an algorithm termed "YEARS" (Figure 2), adapted for pregnancy. Pulmonary embolism was excluded in people with no "YEARS" items and a D-dimer level <1000 ng/mL, or ≥1 "YEARS" item and D-dimer <500 ng/mL. CUS was performed if there were clinical signs of deep vein thrombosis (DVT). At 3-month follow-up, only 1 participant developed a popliteal DVT (0.21%; 95% CI: 0.04%-1.2%). Exposure to diagnostic imaging could be avoided in 39% (95% CI: 35%-44%) of patients. The diagnostic yield of targeted CUS was 7%.

The hospital in which Aries was a patient was a recruiting site for the Artemis[12] study. Had Aries been a participant in this trial, it would have been noted that they had one "YEARS" item (pulmonary embolism most likely diagnosis) at the time of recruitment, meaning that a CTPA would be required (rather than rule out without diagnostic imaging) with a D-dimer test >500 ng/mL. Their D-dimer subsequently returned as 1200 ng/mL. A CTPA revealed a left lower lobe pulmonary embolism.

The studies described previously impacted the 2019 European Society of Cardiology (ESC) Guidelines on Diagnosis and Management of Acute Pulmonary Embolism,[2] which now state that D-dimer measurement in conjunction with clinical prediction rules "should be considered" during investigation of suspected pulmonary embolism in pregnancy (as summarized by the algorithm in figure 3). The ESC guidelines define the statement "should be considered" (which indicates a Class IIa recommendation) as "weight of evidence/opinion is in favour of usefulness/efficacy."

The pregnancy-adapted "YEARS" algorithm was subsequently externally validated using data from the "CTPE-pregnancy" study in a post hoc analysis.[13] The pulmonary embolism prevalence was 6.5%; 91 people had no "YEARS" items, and 280 had one or more items. Of 371 people, 77 met criteria for pulmonary embolism exclusion and would not have undergone CTPA according to the "YEARS" algorithm (which includes risk-adapted D-dimer assessment, as discussed previously). The failure rate was 0%, although this is an imprecise estimate (0.77; 95% CI 0.0-3.9%).

Moreover, a recent individual patient data meta-analysis including data from 893 patients from the CT-PE-pregnancy[11] and Artemis[12] studies supported the use of noninvasive diagnostic strategies in pregnant people with suspected pulmonary embolism, as pulmonary embolism could be ruled out based on non-high clinical probability and a normal D-dimer in up to 40% of people.[14] Point estimates of the failure rates were acceptably low, applying a safety threshold dependent on pulmonary embolism prevalence at baseline. For the YEARS

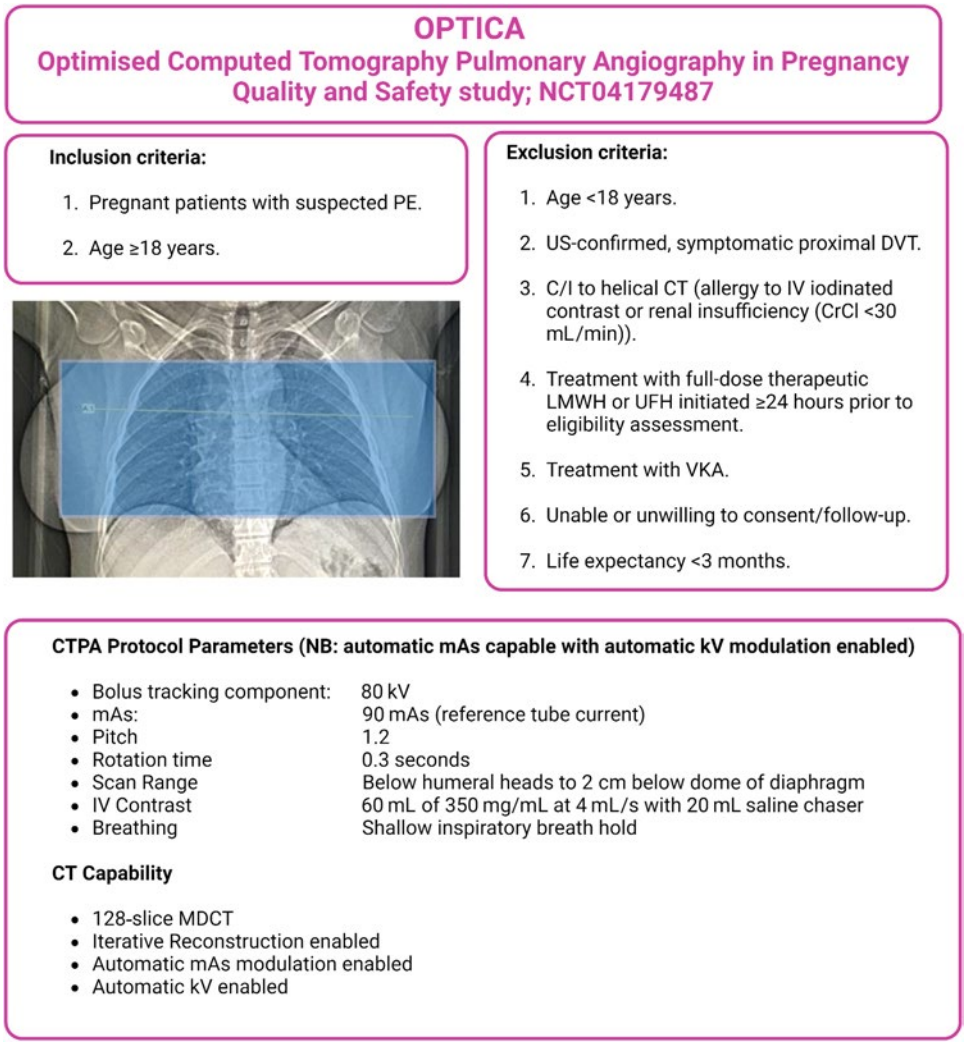

Figure 1. OPTICA study (NCT 04179487) overview, outlining inclusion and exclusion criteria, CTPA protocol parameters, and settings. *Inset:* The scan range for the OPTICA study extends from below the humeral heads to approximately 2 cm below the lowest dome of diaphragm. C/I, contraindication; CrCl, creatinine clearance (calculated by Cockroft-Gault equation); CT, computed tomography; PE, pulmonary embolism; UFH, unfractionated heparin; US, ultrasound; VKA, vitamin K antagonist.

algorithm, the sensitivity, failure rates, and efficiency (number of CTPA scans avoided) were 98% (95% CI 88%-100%), 1.4% (95% CI 0.49%-3.3%), and 43% (95% CI 40%-46%), respectively. The efficiency of CUS in patients without DVT symptoms was low, at 0.79% (95% CI 0.16%-2.4%), but 10-fold higher in those with DVT symptoms, at 7.9% (95% CI 3.9%-15%). The baseline pulmonary embolism prevalence was 5.4%.

Efforts to further improve pulmonary embolism diagnosis in pregnancy are ongoing, including the recent derivation of a novel pretest probability score: the pregnancy-adapted Geneva (PAG) score.[15] In contrast to previous rules, the PAG score includes only objective items that are relevant to pregnant people, excluding items such as age >65 years or cancer. The authors derived the PAG score using data from the CT-PE-Pregnancy study.[11] The area under the curve of the PAG and the original Geneva pretest probability scores were 0.795 (95% CI 0.690-0.899), and 0.684 (95% CI 0.563-0.805), respectively.

CLINICAL CASE (continued)

As a nurse, Aries was interested to know whether similar studies were evaluating algorithms for suspected DVT in pregnancy.

Diagnosis of DVT during pregnancy

D-dimers and clinical prediction rules are not currently validated for DVT exclusion in pregnancy, and diagnostic imaging is essential. The LEFt clinical decision rule shows promise in evaluating pregnant people with suspected DVT. Points are given for Left leg symptoms (1 point), Extremity swelling (≥2 cm difference in calf circumference; 1 point) and First-trimester symptom onset (1 point). People with 0 or 1 point have an "unlikely" clinical probability, and those with >1 point a "likely" clinical probability. In retrospective analyses of 2 cohort studies, the diagnostic failure rate of the LEFt rule was

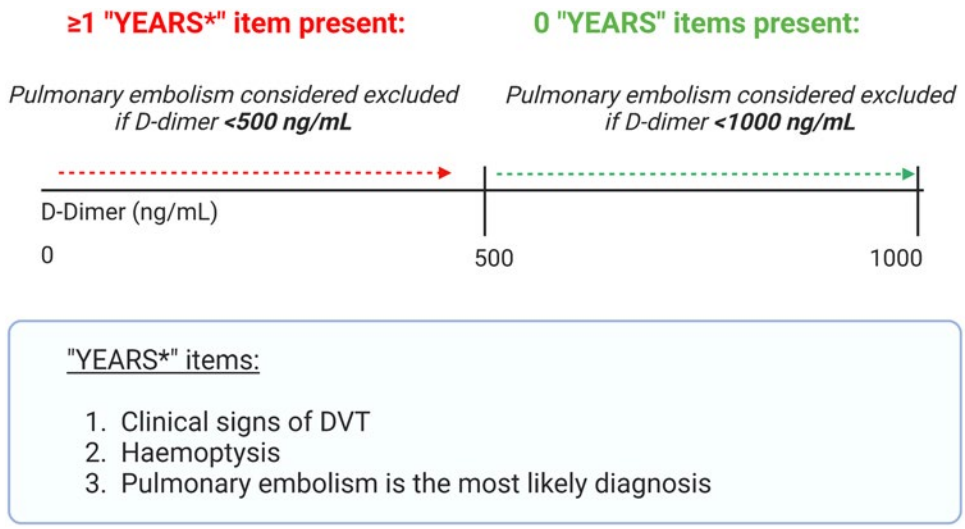

Figure 2. The "YEARS" items, which were included in the Artemis study diagnostic algorithm (Netherlands Trial Register number, NL5726).[12] In this study, pulmonary embolism was excluded in people with no YEARS items and a D-dimer level <1000 ng/mL, or ≥1 YEARS item and D-dimer <500 ng/mL. CUS was performed if there were clinical signs of DVT.

3.1% (95% CI: 0.8%-7.7%)[16] and 0% (95% CI: 0%-8.2%),[17] respectively, highlighting the need for more data. The ongoing LEaD study (Safely Ruling Out Deep Vein Thrombosis in Pregnancy With the LEFt Clinical Decision Rule and D-Dimer: A Prospective Cohort Study; NCT02507180) aims to prospectively evaluate the performance of the diagnostic algorithm.

Back to the case; management of acute VTE in pregnancy

In line with 2019 ESC guidelines,[2] Aries's antenatal and peripartum care was guided by a multidisciplinary team with experience in pulmonary embolism management in pregnancy. In the Rotunda Hospital, Dublin, Ireland, where Aries was being cared for, written plans are jointly agreed on by a multidisciplinary team and discussed with the patient themselves. This approach is now also endorsed by the authors of a recent expert consensus toolkit from the Foundation for Women and Girls with Blood Disorders Thrombosis Subcommittee on the multidisciplinary care of pregnant people with VTE or at risk of VTE,[18] with recommendations being provided on the roles of individual team members in the care of pregnant people with VTE. Low-molecular-weight heparin (LMWH) is recommended by international guidelines and by these consensus recommendations for the treatment of VTE during pregnancy, and direct oral anticoagulants are contraindicated.[2,18] Importantly, the Foundation for Women and Girls with Blood Disorders Thrombosis Subcommittee expert consensus toolkit[18] makes recommendations on the specific contents of a delivery plan, which should include the timing, route, and location of delivery; an intrapartum anticoagulation plan (with guidance on the time to discontinue anticoagulation in the event of a planned or unplanned delivery and whether bridging anticoagulation is required); the timelines required for eligibility for neuraxial anesthesia; and, where relevant, a postpartum anticoagulation plan (with advice on options based on the infant feeding plan).

Management of therapeutic LMWH in the peripartum period for pregnant people lacks high-quality supporting data.[18] Guidelines consider competing risks and benefits when making recommendations on the timing of peripartum regional analgesia.[2,19] These guidelines suggest that regional analgesia should be avoided unless LMWH has been discontinued at least 24 hours before delivery (assuming normal renal function and including risk assessment at extremes of body weight). ESC guidelines recommend that "LMWH should not be given for at least 4 hours after removal of the epidural catheter; the decision on timing and dose should consider whether the epidural insertion was traumatic and take into account the risk profile of the (pregnant person)."[2] For example, if a shorter time interval between the removal of the epidural catheter and commencement of the first LMWH is selected following this risk assessment, the first dose could initially be a prophylactic one. Indeed, UK guidelines[20] make the following suggestion: "A thromboprophylactic dose of LMWH . . . should be given 4 hours postoperatively (at least 4 hours after removal of the epidural catheter, if appropriate) and the treatment dose recommenced 8 to 12 hours later."[1] Importantly, LMWH can be given to breastfeeding people.

Postpartum hemorrhage (PPH) is also a leading cause of maternal death.[21] As we manage acute VTE during pregnancy with anticoagulation, the potential increased bleeding risk is therefore highly relevant.[2,22] A recent systematic review sought to characterize the risk of bleeding in pregnant people managed with therapeutic LMWH.[23] The authors noted variability in bleeding definitions used in individual studies. Because of this limitation, only a descriptive report of outcomes was possible. The authors reported major bleeding in 2.9%-5.0% and PPH risk of 12%-30% in people receiving therapeutic anticoagulation. Importantly, the authors highlighted the lack of high-quality data despite this critical fact: both VTE and bleeding are global health priorities that kill thousands of pregnant people every year.[21,24] In a 2019 systematic review only 34% of pregnant

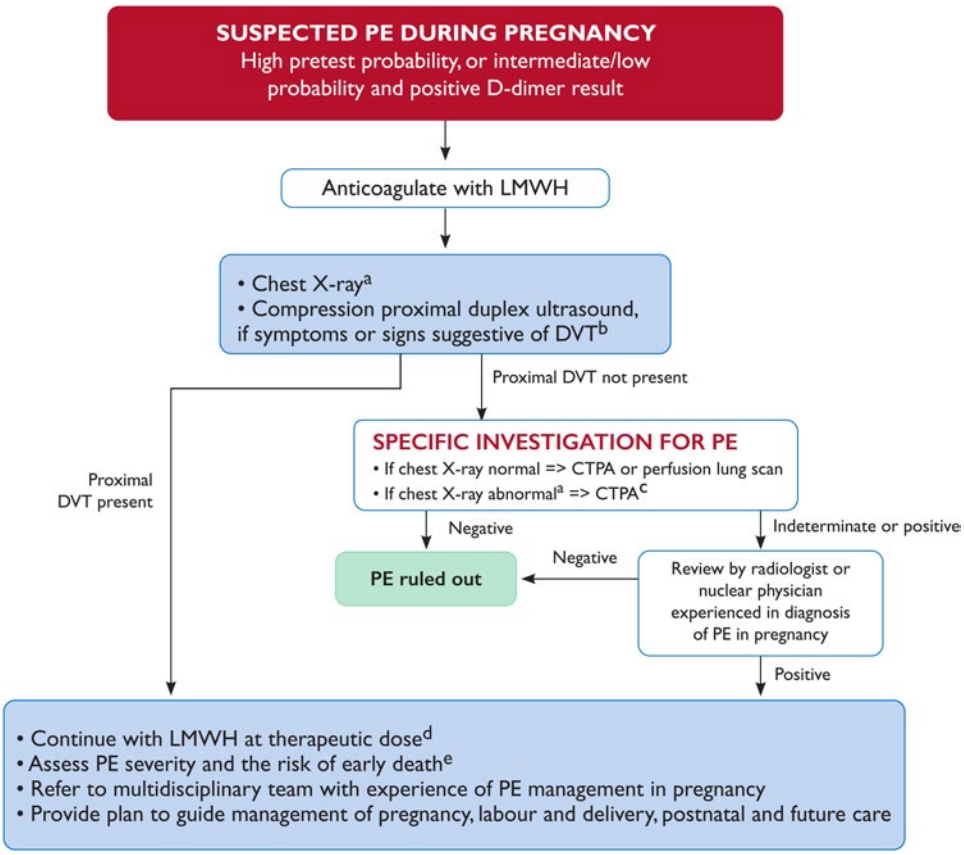

Figure 3. European Society of Cardiology (ESC) algorithm for diagnostic workup and management of suspected pulmonary embolism during pregnancy and up to 6 weeks postpartum. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS). https://doi.org/10.1093/eurheartj/ehz405. (a) If chest X-ray is abnormal, consider also alternative cause of chest symptoms. (b) DVT in pelvic veins may not be ruled out by CUS. If the entire leg is swollen, or there is buttock pain or other symptoms suggestive of pelvic thrombosis, consider magnetic resonance venography to rule out DVT. (c) CTPA technique must ensure very low fetal radiation exposure (see Table 1). (d) Perform full blood count (to measure hemoglobin and platelet count) and calculate creatinine clearance before administration. Assess bleeding risk and ensure absence of contraindications. (e) See Konstantinides and Meyer.[2] High, intermediate, and low PE pretest probability as defined in Konstantinides and Meyer.[2] CTPA, computed tomography pulmonary angiography; CUS, compression ultrasonography; PE, pulmonary embolism. Reproduced with permission from Konstantinides and Meyer.[2]

people included in an LMWH trial had bleeding events prospectively recorded using a standardized definition.[25] Arising from this unmet clinical need, a new classification of bleeding during and after pregnancy for use in clinical trials has been proposed by the Scientific and Standardization Subcommittee on Control of Anticoagulation of the International Society on Thrombosis and Haemostasis (ISTH)[25] (Figure 4).

High-quality data are eagerly anticipated from ongoing cohort studies. For example, the prospective, multicenter "PREP and GO" (**PR**ospective **E**valuation of **P**eripartum **A**nticoagulation mana**G**ement for thromboembolism; NCT05756244) study will evaluate peripartum anticoagulation management among pregnant people with VTE and its impact on patient outcomes using standardized definitions and adjudicated outcomes. The primary objective is to estimate the combined incidence of major and clinically relevant nonmajor bleeding up to 6 weeks postpartum for the most common 6 antepartum strategies (Table 2).

Furthermore, the ongoing Pregnancy AND Anticoagulation (PANDA) study, being conducted via the "Venous thromboEmbolism Network U.S." (VENUS) national VTE research network is a prospective observational cohort of 250 pregnant people who require anticoagulation. The primary objective is to compare the incidence of pregnancy complications associated with anticoagulation around the time of delivery between pregnant people treated with either unfractionated heparin or LMWH around the time of delivery. The Pregnancy AND Anticoagulation study has a composite endpoint of cesarean delivery, labor induction, inability to give epidural or spinal anesthesia, postpartum hemorrhage, and venous thrombosis from 36 weeks to 6 weeks postpartum.

Back to the case; postpartum management

Aries recovered well and continued therapeutic LMWH until 6 weeks postpartum, in line with guideline and consensus

A

		Major bleeding	CRNMB	Minor bleeding
Antepartum and secondary postpartum periods				
Vaginal bleeding	Prompting a face-to-face evaluation			
	Leading to hospitalization			
	Leading to antithrombotic therapy modification			
	Related to early pregnancy loss[c]			
Placenta praevia				
Placenta abruption				
Fetal or neonatal death (for example, bleeding because of placenta abruption)				
Any sign or symptom of hemorrhage[c], including bleeding found by imaging alone				
	Prompting a face-to-face evaluation			
	Leading to hospitalization or increased level of care			
	Requiring medical intervention by health-care professional			
Acute clinically overt bleeding				
	Leading to death			
	That occurs in a critical organ: intracranial, intraspinal, intraocular, retroperitoneal, pericardial, intra-articular, intramuscular with compartment syndrome			
	Associated to a fall in hemoglobin level of 2 g/dL or more			
	Leading to transfusion of two or more units of whole blood or red cells to			

[a]Early pregnancy loss before the 13th gestational week (first trimester).

[b]Placenta previa requiring delivery.

[c]Defined as any overt, actionable sign of hemorrhage (vaginal and nonvaginal) that does not fit the criteria of major bleeding (including spontaneous subcutaneous hematoma >25 cm^2 or >100 cm^2 if provoked).

Figure 4. Proposed definition of bleeding events in studies evaluating antithrombotic therapy in pregnant (individuals) from ISTH Scientific and Standardization Subcommittee on Control of Anticoagulation (reproduced from[45] with permission from Elsevier. License no. 5518820689792; License date 30/03/2023. Colors correspond to the criteria selected for each class of bleeding: red for major bleeding, orange for clinically relevant nonmajor bleeding, and green for minor bleeding, respectively. (A) Proposed classification for antepartum and secondary postpartum (24 h to 6 weeks after delivery) periods. (B) Proposed classification for primary postpartum (first 24 h of delivery) period.

statement recommendations favoring limited duration anticoagulation (no fewer than 3 months in total and usually continuing for at least 6 weeks postpartum) for a pregnancy-provoked VTE event.[2,26]

CLINICAL CASE (continued)

It is now January 2023, and Aries presents to the outpatient clinic, 6 weeks into their second pregnancy. They are keen to understand how they can optimally protect themselves from experiencing VTE recurrence. We discuss this and the results of the recently published landmark Highlow randomized controlled trial (RCT).[27]

Aries is aware that people with previous VTE, particularly an unprovoked or hormone-provoked event, are at higher risk of recurrence during pregnancy than outside pregnancy.[1,11,28–30] In contrast, pregnancy-associated VTE recurrence risk is lower (1.0%; 95% CI: 1.9%-5.7%) in people with a previous VTE provoked by a *major nonhormonal* transient risk factor.[31] Consequently, there had been, prior to 2022, a consistent recommendation in international and society guidelines for pharmacologic thromboprophylaxis during pregnancy and for 6 weeks postpartum in individuals in these higher-risk categories[32–34] (Figure 5). However, the optimal LMWH dose for recurrent VTE prevention was not known.

This situation was rectified by the publication in late 2022 of the multicenter, multinational academic Highlow RCT.[27] This RCT recruited 1110 pregnant individuals aged ≥18 years and ≤14 weeks' gestation who had experienced prior objectively confirmed VTE that was either unprovoked or provoked by a hormonal (or pregnancy-related) risk factor; 70 hospitals from

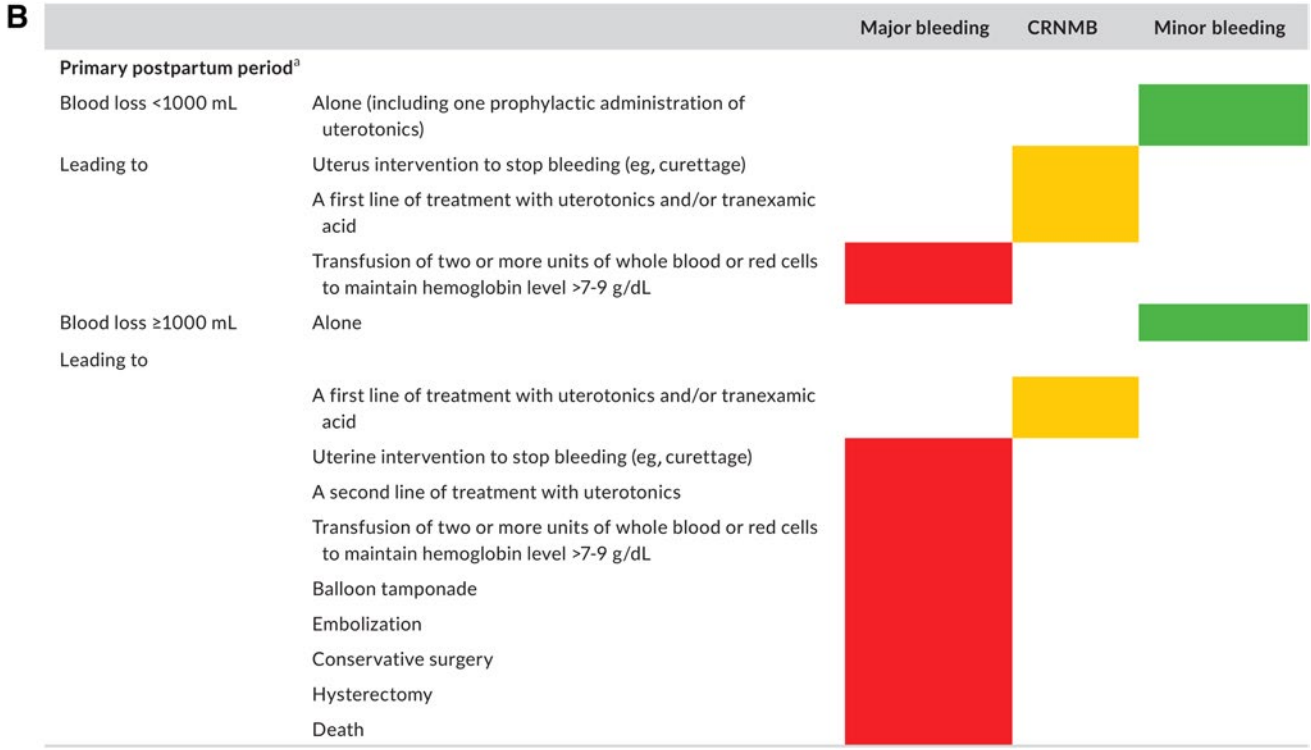

Figure 4. Continued

Table 2. Anticipated common antepartum management strategies based on intention, used in the "PREP and GO" (PRospective Evaluation of Peripartum Anticoagulation manaGement for thromboembolism; NCT05756244) multicenter prospective cohort study

Prophylactic-dose LMWH strategies	More-than-prophylactic-dose (intermediate/therapeutic dose) LMWH strategies
Prophylactic-dose LMWH with expectant management (held with contractions)	More-than-prophylactic-dose LMWH with expectant management (held with contractions)
Prophylactic-dose LMWH and IOL (held for 12 hours)	More-than-prophylactic-dose LMWH and IOL (held for 24 hours)
Prophylactic-dose LMWH and cesarean delivery (held for 12 hours)	More-than-prophylactic-dose LMWH and caesarean delivery (held for 24 hours)
Switched to prophylactic-dose UFH[a]	Switched to intermediate/therapeutic-dose UFH

[a]Typically switched between 37–38 weeks' gestation.

The primary objective is to estimate the combined incidence of major and clinically relevant nonmajor bleeding up to 6 weeks postpartum for the most common 6 antepartum strategies. Of the 8 strategies listed above, the investigators expect 6 predominant strategies. If other possible strategies are used other than those listed above (eg, continuing anticoagulation throughout labor intentionally or stopping anticoagulation early at 37–38 weeks' gestation), they will also be recorded. Prophylactic-dose LMWH: enoxaparin 40 mg daily, dalteparin 5000 IU daily, tinzaparin 4500 IU daily, nadroparin 2850 IU daily; more-than-prophylactic-dose LMWH: Anything higher in dose than what is listed above, including intermediate-dose and therapeutic-dose LMWH.

IOL, induction of labor; UFH, unfractionated heparin.

9 countries participated. Individuals were randomized to either weight-adjusted intermediate-dose or fixed low-dose LMWH. There was no significant difference between the 2 groups in the primary efficacy outcome (recurrent, objectively confirmed, centrally adjudicated VTE up to 6 weeks postpartum), which occurred in 3% and 2% in the low- and intermediate-dose groups, respectively (relative risk [RR] 0.69 [95% CI 0.32–1.47]; $P = 0.33$). The primary safety outcome (major bleeding) occurred in 4% of each of the intermediate-dose and low-dose groups (RR 1.16 [95% CI 0.65–2.09]), demonstrating that low-dose LMWH is the appropriate dose for prevention of pregnancy-related recurrent VTE. Interestingly, postpartum VTE recurrence occurred more frequently in people receiving low-dose than intermediate-dose LMWH (2% and 1%, respectively). Although it is important to point out that this was a *post hoc* analysis, for which the study was not powered, it suggests a potentially interesting hypothesis that an intermediate postpartum LMWH dose could result in reduced VTE rates in

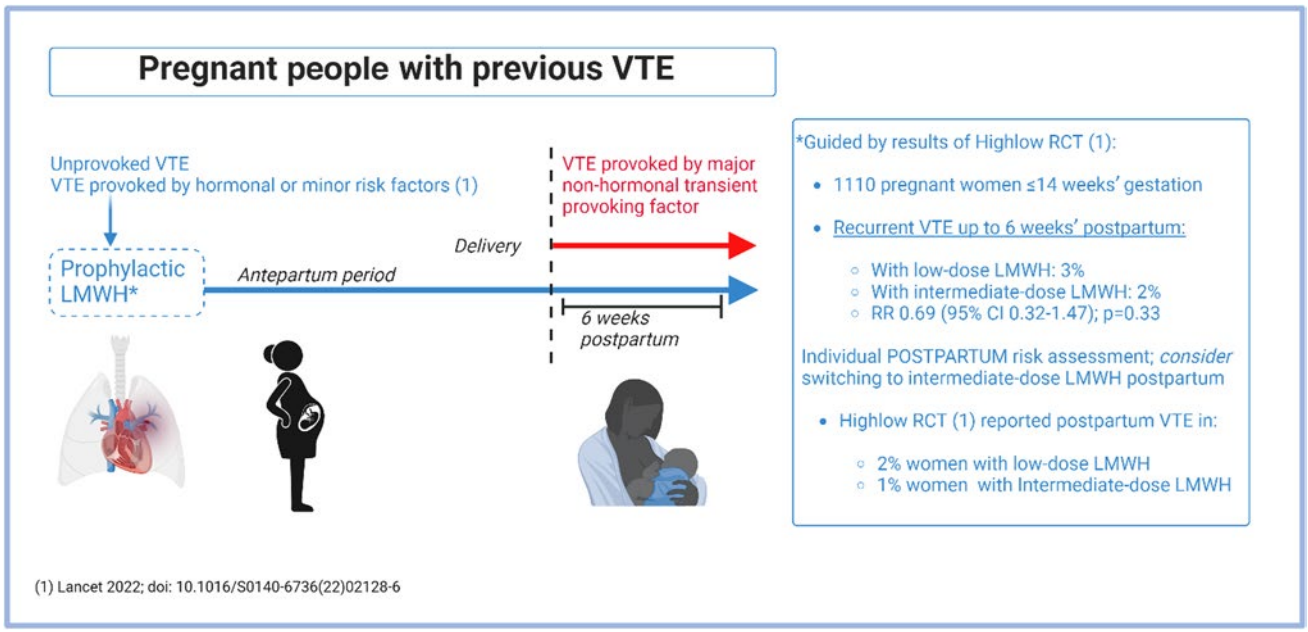

Figure 5. How we approach VTE prevention in pregnant people with a prior VTE history. "Unprovoked VTE; VTE provoked by hormonal or minor risk factors" is an abbreviated reference to those patients who should receive both antepartum and postpartum LMWH. This group is described in the inclusion criteria for the Highlow study as "Patients with previous objectively confirmed VTE, either unprovoked, in the presence of use of oral contraceptives or estrogen/progestagen use, or related to pregnancy or the postpartum period, or minor risk factors (e.g., long distance travel, minor trauma)."[27]

the postpartum period. However, to definitively answer this question, an adequately powered study for this outcome is required.

Primary VTE prevention

We know that prior VTE is an important risk factor for VTE during pregnancy and postpartum (and that people with prior VTE should receive postpartum pharmacologic thromboprophylaxis [Figure 5]), but that this risk factor is identified in only 1%-2% of pregnant individuals.[35] How should VTE prevention be optimized postpartum in individuals with *other, more commonly occurring risk factors and combinations of these risk factors*?

Identifying people at increased risk of developing postpartum VTE may allow for targeted intervention.[32] In postpartum individuals with high VTE risk, the benefits of thromboprophylaxis may outweigh the risks.[1] VTE risk factors are common: in an Irish study including 21,019 postpartum VTE risk assessments,[36] we reported that 78% of pregnant people had at least one VTE risk factor and that one-fifth of people developed new VTE risk factors in the peripartum period that would not have been identified antenatally,[35] highlighting the crucial importance of VTE risk assessment not only during pregnancy but also postpartum.

This question remains one of the most urgent knowledge gaps in obstetric and VTE practice internationally. Despite its importance, there is a striking lack of data to guide either antepartum and particularly postpartum thromboprophylaxis, at a time when VTE risk is highest and when risk assessment can be challenging. International guideline recommendations vary widely and are based on expert consensus because there are insufficient data to make evidence-based recommendations.[1,37] A major issue has been that the use of LMWH injections limits the feasibility of a large RCT, as seen in the experience of the pilot PROSPER trial (LMWH vs placebo among postpartum people with VTE risk factors). Among eligible people refusing consent, 27.2% were uncomfortable with LMWH injections.[38]

However, there is hope on the horizon (Table 3). Aspirin has shown promise in VTE prevention in nonobstetric populations, notably following hip or knee arthroplasty.[39,40] Although these data cannot, at this time, be extrapolated to VTE prevention in pregnancy and postpartum, the use of an oral drug could hypothetically improve patient acceptance of a postpartum trial intervention. Low-dose aspirin (ASA) is considered safe during breastfeeding.[32]

The pilot PARTUM multicenter, randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov Identifier: NCT04153760), now nearly complete, randomizes eligible individuals at elevated VTE risk to low-dose oral aspirin or placebo daily for 6 weeks. The primary outcome of this pilot trial is to determine the feasibility of a full multicenter RCT by determining the mean recruitment rate per center per month, calculated over 6 months.

The single-center "PP-HEP" pilot trial ("Preventing postpartum venous thromboembolism with low-molecular-weight heparin: a feasibility randomized controlled trial") in Geneva (NCT05878899) has also recently shown that approximately 1 in 4 people deemed to be at intermediate risk of VTE were willing to participate in a pragmatic, open-label trial of a 10-day postpartum LMWH course. Data from this pilot trial were presented at the International Society on Thrombosis and Haemostasis congress 2023. One hundred twenty-two participants were

Table 3. Ongoing or recently completed (since 01/2023) interventional postpartum pilot RCTs addressing (principally) primary VTE prevention in people with combinations of VTE risk factors in the postpartum period

Trial	Pilot PARTUM (NCT04153760)	PP-HEP (NCT05878899)	LEAP (NCT05058924)
Status	Ongoing, recruiting	Closed (March 2023)	Ongoing, recruiting
Sponsor	University of Calgary	University Hospital Geneva	Mount Sinai Hospital, Canada
Study design	Multicenter, multinational, placebo-controlled, double-blind pilot RCT	Single-center pilot open-label RCT	Single-center pilot open-label RCT
Intervention	ASA (81 mg once daily) vs placebo once daily for 6 weeks	Enoxaparin 20–60 mg once daily (according to body weight) for 10 days vs no treatment	3 weeks of prophylactic LMWH[a] followed by 3 weeks of ASA (81 mg once daily) vs prophylactic LMWH[a] for 6 weeks
Inclusion criteria (summarized)[a]	ONE (or more) First Order Criterion: 1. Known inherited thrombophilia prior to enrollment 2. Antepartum immobilization (strict bedrest) for ≥7 days. OR TWO (or more) Second Order Criteria: 1. Prepregnancy BMI ≥30 kg/m^2 2. Smoking ≥5 cigarettes/day prepregnancy 3. Previous clinical history of superficial vein thrombosis 4. Pre-eclampsia 5. Current pregnancy ending in stillbirth (>20/40) 6. Emergency cesarean birth 7. Small-for-gestational-age infant at time of delivery 8. Postpartum infection 9. Postpartum hemorrhage (>1000 mL)	Postpartum women within 48 h of delivery, with at least ONE of: 1. Emergency cesarean section 2. Prepregnancy BMI ≥35 kg/m^2 3. Known low-risk thrombophilia 4. Preeclampsia 5. Preterm delivery 6. Peripartum systemic infection 7. Intrauterine growth restriction AND/OR at least 2 of: 1. Age ≥35 years 2. Pre-pregnancy BMI 30.0–34.9 kg/m^2 3. Current smoking 4. Elective cesarean section 5. Postpartum hemorrhage 6. Antenatal immobility	>18 years of age AND: 1. Personal history of unprovoked VTE prior to pregnancy or hormone associated VTE and not prescribed therapeutic anticoagulation. OR 2. Family history (first-degree relative) of VTE and antithrombin deficiency, protein C or protein S deficiency OR 3. Combined thrombophilia or homozygous for the factor V Leiden mutation or prothrombin gene mutation, and family history of VTE (first-degree relative)
Exclusion criteria (summarized)[a]	1. >48 hours since delivery of the placenta at randomization. 2. Received >2 doses of LMWH since delivery of the placenta 3. Need for postpartum LMWH. prophylaxis/systemic anticoagulation[b] 4. Need for postpartum ASA[b] 5. Contraindication to ASA[a] 6. <18 years of age 7. Unable or refused consent	1. Indication for therapeutic anticoagulation 2. High risk of postpartum VTE 3. Increased bleeding risk 4. Contraindication to heparin 5. Age <18 years	1. Preexisting indication for therapeutic LMWH 2. Contraindication to ASA[a] 3. Contraindication to LMWH[a] 4. Active bleeding, excluding physiologic vaginal bleeding 5. Bleeding disorders 6. Known severe hypertension
Pilot trial primary objective	Mean recruitment rate per center per month, calculated over 6 months	Recruitment rate (number of study inclusions per month over 6 months) and proportion of participation[a]	Enrollment rate, consent rate, adherence to prescription, withdrawal of consent rate, rates of contamination[a]
Target sample size	384	100–200	50

[a]Full criteria are available for the relevant trials on clinicaltrials.gov.

[b]As judged by physician and/or local investigator.

ASA, aspirin; × /40, × weeks' gestational age.

randomized to enoxaparin 40-60mg per day for 10 days or no treatment. The overall recruitment rate was 12.8 per month,[41] providing further evidence that recruitment of people to postpartum LMWH trials is possible, even if projected VTE rates are low, and that regional or country-specific variations in recruitment rates may be important.

Furthermore, an ongoing pilot single-center RCT presented at the American Society of Hematology Meeting 2022 (NCT05058924)[42] randomized postpartum individuals deemed to be at elevated VTE risk to either prophylactic LMWH for 3 weeks followed by low-dose aspirin for the following 3 weeks (treatment A) or standard-care prophylactic-intensity LMWH for 6 weeks (treatment B). Recruitment and adherence appear promising, with an enrollment rate reported at American Society of Hematology of 69.2% (18/26) and treatment adherence rates of 98.2% and 94.1% in groups A and B. At 6 weeks quality-of-life scores (measured by the Duke Anticoagulation Satisfaction Scale) improved by 33.3% in group A compared with group B (P=0.01).

There is a similar dearth of RCT evidence guiding optimal antepartum primary VTE prevention. No VTE risk assessment model has been sufficiently validated. However, the research question has been prioritized. A multicenter study performed by the French STRATHEGE investigators compared VTE and

placental vascular complication rates pre- and postimplementation of a risk scoring system (including but not limited to prior VTE events), which was used to determine thromboprophylaxis strategies in 2085 people.[43] Vascular events were reported in 190 (19.2%) people before and 140 (13%) after implementation of risk score-driven prophylaxis (RR 0.68 [95% CI 0.55; 0.83]) and the risk of pregnancy-associated VTE was reduced following implementation (RR 0.47 [95% CI 0.27; 0.81]). PPH occurred in 3.2% of people before and 4.5% after implementation (RR 1.38 [95% CI 0.89; 2.13], *P*=0.15).

CLINICAL CASE (continued)

Aries was struck by the importance of conducting high-quality studies in pregnancy to improve the care delivered to pregnant people. We had discussed with them the challenges faced by clinicians and patients: despite the very high stakes, pregnant people are often excluded from participation in clinical trials.[44]

International networks and collaboration are central to the success of RCTs addressing VTE in pregnant people, who have traditionally been excluded. Both the Highlow and PARTUM trials have been endorsed by the International Network of Venous Thromboembolism Clinical Networks (www.invent-vte.com). Participating National VTE networks include CanVECTOR (Canada), INNOVTE (France), INViTE (Ireland), Dutch Thrombosis Network (Netherlands), Center for Thrombosis and Hemostasis (Germany), TRIP (Italy), Norwegian Thrombosis Network, THANZ (Australia and New Zealand), VENUS (United States), CURES (China), and UK-TReN (United Kingdom).

Concluding remarks

Aries elected to commence prophylactic LMWH throughout their pregnancy and chose to increase their dose to an intermediate intensity postpartum, having discussed the remaining data limitations. Their journey demonstrates the crucial importance of prioritization of high-quality RCTs and prospective clinical management studies for the prevention, diagnosis, and management of VTE in pregnant people.

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Conflict-of-interest disclosure

Barry Kevane reports honoraria unrelated to this project from Bayer (advisory board membership) and Leo Pharma.

Fionnuala Ní Áinle reports grants for investigator-initiated studies paid to her institution and unrelated to this project from Sanofi, Daiichi Sankyo, and Bayer and acting as a consultant (membership of a trial executive committee, paid to institution) for Boston Scientific.

Off-label drug use

Barry Kevane: Nothing to disclose.
Fionnuala Ní Áinle: Nothing to disclose.

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How to avoid early mortality in acute promyelocytic leukemia

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Acute promyelocytic leukemia (APL), a phenotypically and genotypically unique subtype of acute myeloid leukemia, has seen unprecedented advances in its management since the introduction of all-*trans* retinoic acid (ATRA) and arsenic trioxide. However, the phenomenal pharmacologic conversion of this once highly fatal disease to one with a long-term survival exceeding 90% among patients who survive induction remains impaired by the significant incidence of early death (ED) reaching 30% in some real-world studies. The key driver for ED in APL is catastrophic hemorrhage with a proclivity for cranial sites. Most EDs in APL are currently considered preventable. Here, we discuss the concept of early death in APL and its characteristics. Importantly, we outline implementable strategies to reduce the incidence of ED. Early recognition of APL underpins these preventive measures as significant delays in the diagnosis increase the likelihood of ED. While early administration of ATRA is often taught to all hematology trainees, this lifesaving intervention is only possible if providers, including those in emergency departments and urgent/immediate care settings, are trained to have a high index of suspicion and competence to recognize the morphologic and clinical characteristics of the disease. Other proposed strategies tackle the complications that can be present at diagnosis or arise during induction therapy and address the issues of expert consultation and protocol adherence in the management of these patients. While some of these measures appear intuitive and others aspirational, widespread adoption could bring about an era of cure for almost every patient with APL.

LEARNING OBJECTIVES

- Describe the concept of early death in acute promyelocytic leukemia (APL), its characteristics, and its key drivers
- Propose strategies to prevent early death in APL

CLINICAL CASE

A 32-year-old man with a 2-week history of headaches and easy bruising sought medical attention in the emergency department. Complete blood count showed a white blood cell count of 60 000/μL, hemoglobin of 5.9 g/dL, and platelet count of 14 000/μL. The prothrombin was 22.8 seconds, activated partial prothrombin time 32.2 seconds, and fibrinogen 60 mg/dL. He received packed red blood cells, platelets, and cryoprecipitate units. The peripheral blood smear was suggestive of acute promyelocytic leukemia (APL). All-*trans* retinoic acid (ATRA) was started. A computed tomography scan of the head showed a small subarachnoid hemorrhage. A bone marrow aspiration/biopsy was consistent with the diagnosis of APL. Cytoreduction was initiated with hydroxyurea. Peripheral blood testing for PML::RARα returned positive, and arsenic trioxide and gemtuzumab ozogamicin were added to ATRA. The coagulopathy was managed with supportive transfusions. However, dyspnea and fever developed 7 days into his treatment. Examination showed edematous lower extremities and weight gain of 5 kg since admission. Oxygen saturation was 88% on ambient air, and he required 2 L of supplemental oxygen. Dexamethasone was administered at 10 mg twice daily for differentiation syndrome, and APL therapy was continued. There was improvement within 36 hours, and supplemental oxygen was weaned.

Introduction: Distinguishing features of APL

APL is the most curable subtype of acute myeloid leukemia (AML) in adults. The morphologic features of the leukemia cell are unique; the molecular pathogenesis has been

deciphered; the clinical manifestations, primarily life-threatening bleeding, are characteristic; and the treatment is completely different from all other subtypes of AML.[1] The disease is associated with a t(15;17) (q24;q21), resulting in the formation of the PML::RARα fusion transcript that blocks differentiation. Unlike other subtypes of AML in which leukemia cell genetics drive prognosis, the initial white blood cell (WBC) count at presentation is the most important prognostic factor in APL. The biologic basis for the relationship between a high WBC and prognosis is enigmatic. The WBC count is likely a surrogate for an as yet unidentified genetic marker. Almost miraculously, the vitamin A derivative ATRA and arsenic trioxide (ATO) induce differentiation and apoptosis of the promyelocytic leukemia cells. When administered together in low-risk patients without chemotherapy or in high-risk patients with either an anthracycline or the immunoconjugate gemtuzumab ozogamicin (GO), approximately 98% and 90%, respectively, are cured of their disease.[2-6] This is provided that the patient survives induction since there is essentially no primary resistance in the ATRA-ATO era. Although cure in AML always depends on surviving induction, this is a particularly important concept in patients with APL since the major clinical manifestation, and one of the most distinguishing features, is a unique, complex, and potentially fatal bleeding diathesis. In fact, the major cause of treatment failure is not resistant disease, as is true of all other subtypes of AML, but rather early death (ED). ED in the context of APL is defined as death occurring within the first 30 days of diagnosis (although some studies define ED as within 30 days of admission or start of induction).[7] Avoiding ED in patients with APL has emerged as the last frontier in the cure of all patients.

Early death in APL: Scope of the problem

Hemorrhage was recognized as a dominant feature of APL in the earliest report by Hillestad[8] in 1957 that established APL as a distinct clinical entity. This description highlighted key characteristics: rapidly fatal course of a few weeks' duration, severe bleeding tendency due to fibrinolysis and thrombocytopenia, and normal erythrocyte sedimentation rate (ESR) probably due to reduced plasma fibrinogen. Now, more than 60 years after these observations, bleeding remains the major cause of ED and therefore the major cause of treatment failure.

Historically, in large cooperative group trials of newly diagnosed patients treated with ATRA plus chemotherapy, the ED rate is between 5% and 10%, but the percentage of EDs attributable to bleeding is between 30% and 70%.[9-13] However, these numbers reflect the outcome of younger patients generally without comorbidities who survive long enough to complete administrative requirements for enrollment on a clinical trial. In population-based studies, the ED rate is considerably higher and varies between 15% and 30%.[7,14-17] The Swedish Acute Leukemia Registry reported an ED rate of 13% in younger patients (aged <35 years) but 25% overall with no change from 1997 to 2013.[7] However, Jamy and colleagues[16] reported significant progress, particularly in younger patients. Analysis of the Surveillance, Epidemiology, and End Results Registry revealed that ED rates have decreased significantly over time in younger adults, with a reduction in the ED rate from 27.4% between 1992 and 1995 to 5.4% between 2012 and 2015, but remained high in patients aged >40 years in whom the ED rate decreased only modestly from 35.2% between 1992 and 1995 to 22.2% between 2012 and 2015 (Figure 1). This is important since 30% of patients in the Swedish registry were older than 65 years.[7] Bewersdorf and colleagues[17] found that the inpatient mortality rate was 14% among 1464 admissions. Furthermore, 86% of low-risk patients were treated in accordance with National Comprehensive Cancer Network (NCCN) guidelines, whereas only 64.6% of high-risk patients were treated as recommended (Figure 2). This interesting observation may set the stage for a strategy to decrease the ED rate discussed below. The contribution of "delayed or missed diagnosis" to the ED rate is difficult to quantify as even population-based reports do not capture the patients who die early in emergency departments before a diagnosis is made. However, early identification of patients with APL must be a priority as it is key to the prevention of ED.

Characteristics of early death in APL

In a contemporary series, Gill et al[18] reported an ED rate of 15.6% with ATRA administered ≥24 hours after presentation

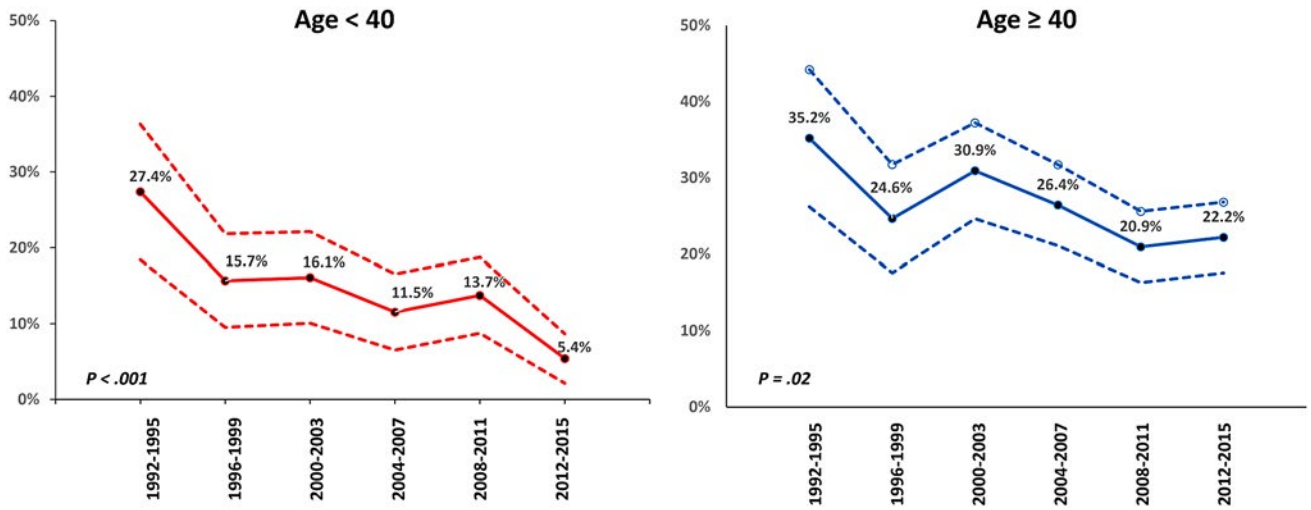

Figure 1. Graphical representation of early mortality trends by age.[16]

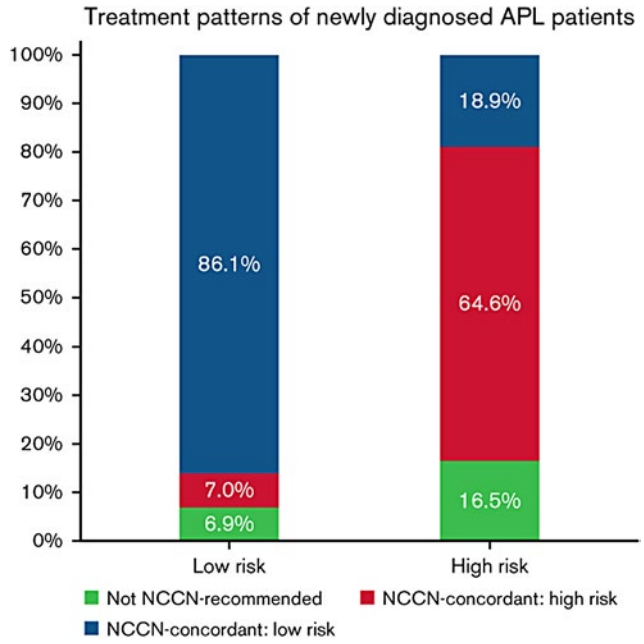

Figure 2. Treatment patterns of newly diagnosed patients with APL by risk group based on concordance with the NCCN guidelines.[17]

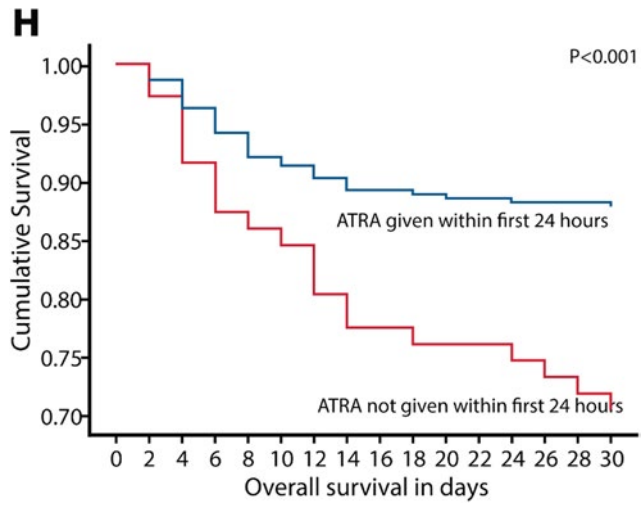

Figure 3. Impact of timing of ATRA administration on 30-day survival.[18]

19.8% of the time. Those patients for whom ATRA was administered within the first 24 hours had a better outcome than those receiving ATRA >24 hours from admission (Figure 3).[18] This is an important observation since ED most commonly occurs within the first 4 days, particularly in the first 24 hours, and almost all EDs occur within 2 weeks.[7,18] Bleeding is by far the most frequent cause of ED in APL. The Swedish registry reported 46% of EDs were attributable to bleeding.[7] While intracranial hemorrhage is the most common presentation, life-threatening hemorrhage also can occur in the gastrointestinal tract and lungs.[7] Other less common causes of ED include thrombosis, differentiation syndrome (DS), and infection.[18] However, bleeding should be considered the most preventable cause of ED. Sepsis is the most commonly identified nonhemorrhagic cause of death in patients with APL, as demonstrated by the Swedish registry, which found it responsible for 15% of observed mortality.[7] While it may seem counterintuitive, thrombosis is an often-unrecognized cause of morbidity and ED in APL.[19,20] The pathogenesis of this apparent paradox is explained below. DS is a cardiorespiratory distress syndrome caused by ATRA, ATO, or both.[21] The effect of differentiating agents such as ATRA and ATO on blast cells and promyelocytes results in increased production of proinflammatory cytokines (including tumor necrosis factor α and interleukins 1, 6, and 8), increased adhesivity of the blasts, and release of cathepsin G.[22] These clinically manifest as systemic inflammation (fever, hypotension, tachycardia, and tachypnea) and increased vascular permeability with endothelial injury (pulmonary edema, peripheral edema, and weight gain).[23] If unchecked, progression to multiorgan failure and death can result. However, with either prophylactic or early institution of corticosteroids, the syndrome may be less frequently problematic with very low mortality rates.

Factors predictive of ED have been reported. In addition to delays in ATRA administration, Gill et al[18] reported that hypofibrinogenemia, WBC count ≥10 000/μL, and male sex predicted ED. The concept of high-risk disease (presenting WBC count ≥10 000/μL) should not be confused with high risk for ED as the former is designed to predict the risk for relapse following frontline therapy and is a marker for overall worse outcome. Bewersdorf and coworkers[17] found that patient age, high-risk disease, and NCCN guideline nonconformant treatment were associated with higher odds of death or discharge to hospice. In a multivariate analysis of 5 clinical trials, WBC count >20 000/μL was the only independent predictor of hemorrhagic ED.[24] The Swedish registry reported the highest rate of ED among patients with a WBC count in the 5000 to 10 000/μL range.[7] Österroos and colleagues[15] observed a linear increase in risk of ED with either age ≥50 years or WBC count ≥2200/μL. Importantly, there was an increased risk of ED even in patients with leukopenia. They divided age, WBC counts, and platelet counts into subgroups with assigned points to develop a risk score. Other groups have developed similar scoring systems.[25] However, such scoring systems have limited practical value and have not influenced treatment strategies. Since ED may occur in patients with low-risk disease, the use of a different treatment approach in this group vs those with high-risk disease does not appear to be justified.

Pathogenesis of the coagulopathy: Bleeding and thrombosis

APL causes distinctive changes in the coagulation and anticoagulation systems, resulting in a coagulopathic state that can manifest as hemorrhage, thrombosis, or both (Figure 4).[26] It has been proposed that APL causes bleeding by mechanisms that include primary and secondary fibrinolysis. Primary fibrinolysis is mediated by increased expression of annexin II and plasminogen activators (PAs) by malignant promyelocytes, resulting in increased levels of plasmin and consequently hypofibrinogenemia and elevated fibrin split products.[27,28] Simultaneously, there is a decrease in the levels of circulating α2-antiplasmin, a potent antifibrinolytic protein, resulting in unchecked fibrinolysis. Secondary fibrinolysis is driven by an increased production of endothelial tissue PAs.[29] Intracranial hemorrhage is also mediated by increased endothelial expression of annexin II and effects of increased levels of PAs in the brain.[30] Podoplanin, an aberrantly

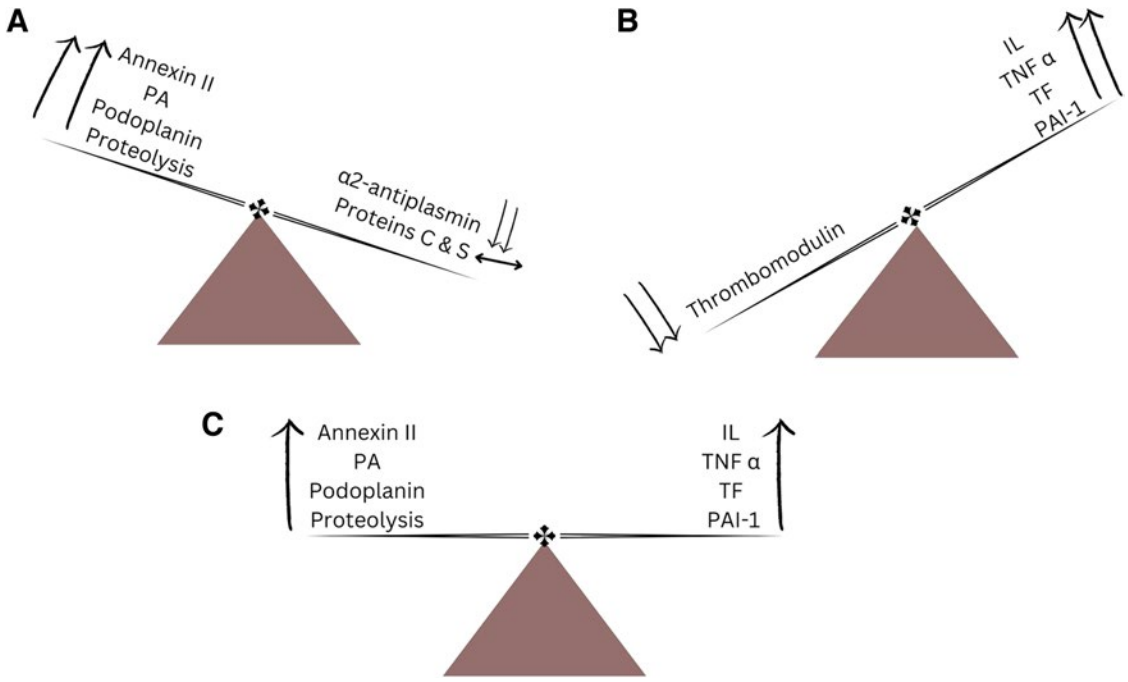

Figure 4. The dynamic interplay between the procoagulant and anticoagulant agents/mechanisms resulting in (A) hemorrhage, (B) thrombosis, and (C) concomitant hemorrhage and thrombosis. IL, interleukin; PAI-1, plasminogen activator inhibitor 1; TF, tissue factor; TNFα, tumor necrosis factor α.

expressed surface protein, contributes to thrombocytopenia and bleeding by promoting platelet aggregation and consumption; ATRA downregulates the expression of podoplanin and decreases risk of bleeding.[31] Proteolysis of procoagulant proteins is another proposed mechanism.[27] It was initially thought that the coagulopathy in APL is merely disseminated intravascular coagulation. However, fibrinolysis and proteolysis also play integral roles. APL appears less characterized by the formation of microvascular thrombi, and there are more marked derangements in coagulation parameters such as prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, fibrin split products, and D-dimer while levels of anticoagulant proteins, such as proteins C and S, are preserved.[32]

Thrombosis also can be a manifestation of disordered coagulation in APL, albeit far less commonly compared to hemorrhage.[19] This may be attributable to hypercoagulability promoted by cytokines such as interleukins and tumor necrosis factor α, which enhance the activity of tissue factor and PA inhibitor 1 while decreasing the production of the endothelial anticoagulant thrombomodulin.[26]

Treatment of high-risk APL: Focus on prevention of early death

The modern treatment of APL is anchored on aggressive management of the coagulopathy and early initiation of ATRA. Differentiating agents, ATRA and ATO, have become the backbone of the treatment of APL.

Patients with high-risk APL should be treated according to one of several protocols for high-risk disease, initially aimed at controlling the WBC count and therefore perhaps decreasing the risk of ED. One approach is the addition of an anthracycline to ATRA and ATO. The APML4 study, which incorporates idarubicin in the induction phase of therapy on days 2, 4, 6, and 8 with ATRA and ATO, as well as ATRA and ATO in consolidation for 2 cycles and maintenance, results in 5-year disease-free survival and overall survival rates of 95% and 87%, respectively.[4] An alternative strategy is the addition of GO, 9 mg/m^2, given on day 1 of induction therapy, to ATRA and ATO in high-risk patients, which results in a 5-year disease-free survival and overall survival of 89% and 86%, respectively.[2] Other clinical trials have also shown the benefit of adding GO in this patient population.[11,33]

Our approach to the management of patients with high-risk APL involves the combination of ATRA and ATO with GO. Consolidation therapy is given with 4 courses of ATRA and ATO without maintenance. The addition of an anthracycline, often idarubicin, is a very acceptable alternative if this is the clinician's preference or GO is unavailable. The use of GO may preserve cardiac function and decrease long-term cardiac toxicity, desired goals in both younger and older patients.[34]

In addition to early initiation of ATRA, proactive management of coagulopathy is vital and should be considered as important as ATRA. Due to the high risk of bleeding, there is essentially no role for prophylactic or therapeutic anticoagulation (including low-dose unfractionated heparin, which has historically been used in this patient population), and it should be avoided except possibly in the very rare setting of life-threatening thrombosis, an event the authors have never had to confront.

Although the benefit is not clearly established, we administer prophylactic steroids to all patients with APL given the minimal toxicities and would have done so in the clinical case presented. The choice of steroid and dose should be as administered in the regimen being followed. In patients managed without prophylactic steroids but who develop DS, the use of dexamethasone 10 mg every 12 hours until resolution has become the standard

of care. Considering the risk of ED from infectious complications, especially in older adults treated with chemotherapy, the immunologic risks associated with use of prophylactic steroids should be carefully weighed against their anticipated benefit by the consultant hematologists and other experts involved in the care of these patients, particularly those with established infections.

The patient in the case described achieved complete remission with induction therapy and as anticipated had a favorable long-term outcome. The prompt initiation of ATRA and aggressive use of blood products to manage the coagulopathy likely prevented progression of intracranial hemorrhage. Prompt recognition of DS allowed early initiation of dexamethasone and allowed uninterrupted treatment with ATRA and ATO.

How to avoid early death in APL: A practical and aspirational guide

Given the natural history of the disease, there will always be some patients who come to medical attention with catastrophic bleeding whose fatal outcome cannot be prevented. However, for most patients whose outcome can be influenced, the following measures, some intuitive and others aspirational, are a guide to reduce the ED rate (visual abstract).

Practical measures

1. Promptly identify patients with APL as early recognition is key. Characteristic features include large ecchymoses, epistaxis, oral mucosal bleeding, and heavy menstrual bleeding. It is not usual for patients to report oral bleeding while brushing their teeth or flossing. The bleeding is often out of proportion to the degree of thrombocytopenia. It is important that the emergency department—often the first-contact health care professionals—reflexively obtain peripheral blood smear and coagulation profile (ie, PT, aPTT, D-dimer, and fibrinogen) for patients with an abnormal WBC count (typically neutropenia and/or blast cells) and/or thrombocytopenia. Every hematologist should be familiar with the unique morphologic features of APL on the peripheral blood smear (Figure 5).
2. Administer ATRA at the very earliest and slightest suspicion of APL in the emergency department with no delay for transfer to the inpatient floor or for pathology or genetic confirmation of the disease. Exposure to several days of ATRA is associated with very little or no toxicity if the diagnosis is not APL.

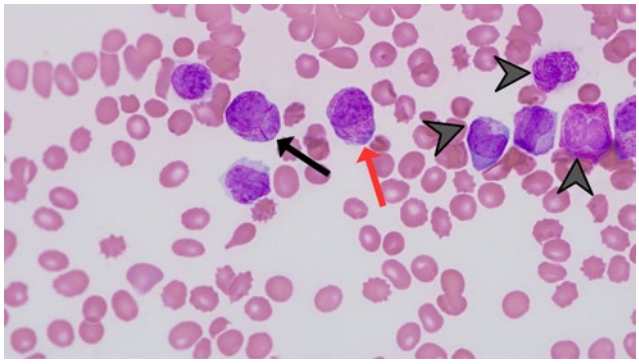

Figure 5. Photomicrograph (magnification 60×) depicting morphologic features of APL, including abundant primary azurophilic granules (red arrow), multiple Auer rods (black arrow), and reniform or bilobed nuclear contours (arrowheads).

3. Be aggressive in blood product (cryoprecipitate and platelet units) support for the coagulopathy with cryoprecipitate and platelets with the goal to maintain the fibrinogen ≥150 mg/dL and platelet count ≥50 000/μL. Admittedly, both thresholds are arbitrary. Coagulation studies (ie, PT, aPTT, D-dimer, and fibrinogen) can be checked 2 to 3 times a day for the first 3 to 4 days.
4. Be vigilant for the detection of thrombosis. Clinicians should have a low threshold for imaging if there is a suspicion for thrombosis. Avoid the prophylactic use of anticoagulation.
5. Administer steroids in an effort to prevent or ameliorate DS. Use caution in patients with active infections.
6. Follow written guidelines (such as NCCN) for management in consultation with an expert in the field for day-to-day care, particularly early in the disease course. Choose 1 published regimen and follow it through without "mixing and matching" regimens to achieve optimal outcomes. In 2004, the American Society of Hematology established the International Consortium on Acute Promyelocytic Leukemia with its purpose to improve outcomes of APL in developing countries, including Brazil, through a uniform clinical protocol and establishment of a network for communication and collaboration.[35] The success of this approach has been demonstrated. Historically in Brazil, the ED rate in APL was 32%.[36] After the adoption of a uniform clinical protocol, the ED rate decreased to 15% in the participating countries.[36]

Aspirational measures

1. Emergency medicine training should include teaching about APL and its presentation clinical and laboratory characteristics. The curriculum should emphasize the key role of emergency medicine physicians in the prevention of ED, and trainees should achieve competency in early recognition of patients with the disease.
2. Emergency department management algorithms should be updated to include reflexive and STAT orders in patients for whom complete blood count reveals blast cells, abnormal neutrophils, and/or platelet counts.
3. Hospital standard operating procedures should ensure ATRA is immediately available. While many facilities see few patients with APL and therefore believe that maintaining stock is not cost-effective and a prohibitive administrative burden, they should establish a network with the nearest hospital that keeps ATRA on the shelf.
4. Although ED rates may improve over time without change in treatment approach, we recommend clinicians seek immediate consultation with a colleague with particular expertise in the disease. Jillella and colleagues[37] have implemented a simple yet very effective strategy. A network of 7 experts across the United States available any time with brief, written, and practical management guidelines was established. Treatment was based on well-recognized standards of care with dose modifications for older adults and those with comorbidities. A decrease in the ED rate to 3.5% was observed, a level comparable to that observed in the recent Surveillance, Epidemiology, and End Results rates in younger patients, and this strategy serves as a paradigm that could be widely adopted as a way to provide free telemedicine physician-to-physician consultations regarding the care of patients with APL who are not already being treated by experts.

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Oluwatobi Odetola: no competing financial interests to declare.
Martin S. Tallman: no competing financial interests to declare.

Off-label drug use
Oluwatobi Odetola: 6-mercaptopurine and methotrexate as maintenance in some protocols.
Martin S. Tallman: 6-mercaptopurine and methotrexate as maintenance in some protocols.

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How to manage ITP with life-threatening bleeding

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While immune thrombocytopenia often presents with mild bleeding manifestations or surprising findings of thrombocytopenia on routine complete blood counts in patients without symptoms, some patients can present with new thrombocytopenia and life-threatening bleeding. Emergent assessment and treatment are needed to prevent substantial morbidity and even mortality. These patients present to the emergency room with bleeding, and hematologists are subsequently consulted. Understanding the approach to making the diagnosis and excluding other life-threatening illnesses is essential, as is rapid initiation of treatment in the bleeding patient even when the diagnosis of immune-mediated thrombocytopenia is tentative. Using a case-based format, we review how to approach and treat patients presenting with new thrombocytopenia and bleeding.

LEARNING OBJECTIVES

- Evaluate adult patients who present with new thrombocytopenia and life-threatening bleeding
- Determine the best treatment plan for adults who have newly diagnosed ITP and life-threatening bleeding

Introduction

Adult patients who present with new isolated thrombocytopenia can be challenging to manage, especially if they have more symptoms than mucosal bleeding or petechiae. Immune thrombocytopenia (ITP) is a diagnosis of exclusion, as no test can positively confirm a diagnosis of ITP, yet patients may require urgent or emergent treatment due to bleeding manifestations on presentation. Although bleeding events are rare in patients with primary ITP, they can occur and when they do, they can be life-threatening.[1] Past reports from a pooled analysis suggested a 5-year mortality from bleeding of 49% in patients over the age of 60, although this predated the use of thrombopoietin receptor agonist (TPO-RA) and rituximab in the treatment of ITP.[2] The approach to a patient with new findings of thrombocytopenia accompanied by bleeding requires a rapid clinical assessment and the institution of treatment aimed at determining the underlying etiology.

Antecedent medical history can be informative, including a history of recent infections, the use of antibiotics known to be associated with thrombocytopenia,[3] or a history of disorders associated with ITP such as collagen vascular disorders, especially lupus and rheumatoid arthritis, and inflammatory bowel disorders.[4] Here we discuss the approach to the differential diagnosis of new-onset thrombocytopenia associated with bleeding. Standardized definitions of bleeding in patients with ITP, assessment of the need for urgent or emergent treatment, and treatment strategies that can be used up front to mitigate bleeding and restore hemostasis are reviewed.

CLINICAL CASE

A 67-year-old woman presents to the emergency department (ED) with a severe headache. Her past medical history is notable for hypertension, hyperlipidemia, and hypothyroidism due to a remote history of Hashimoto's thyroiditis; she has been taking lisinopril, rosuvastatin, and levothyroxine for over 3 years. She has noted some bruises on her legs but thought they might be due to playing with her grandchildren. She denies any recent infections, including COVID-19 or diarrheal illnesses. Her grandchildren have been healthy. A physical exam in the ED reveals normal vital signs and no acute distress, clear lungs, regular rate and rhythm with no murmur, and no hepatosplenomegaly. There are a few small 1- to 2-cm bruises on bilateral legs, distal lower extremity petechiae she thought were due to an allergy to sunscreen, and a bruise near her watch on her left forearm. A complete blood cell count (CBC) and chemistry panel are sent, and she undergoes noncontrast head computed tomographic imaging. The computed tomographic radiologist pages you with findings of a moderate-sized subdural hematoma.

The lab pages with results of the CBC because her platelet count is 3000/μL. Her hemoglobin level is 12.7 gm/dL with a normal mean corpuscular volume (MCV). A chemistry panel reveals normal creatinine and normal transaminases. The ED pages you, the hematologist on call.

Differential diagnosis

In a patient presenting with new thrombocytopenia, the platelet count and presenting symptoms inform the speed of evaluation and treatment. The patient in this case has not only significant thrombocytopenia but also serious bleeding in a critical location that must be addressed now, as compared to a patient who, on routine yearly physical exam, is found to have a platelet count of 40 000/μL and no bleeding manifestations. Presenting symptoms and recent history are important clues that can aid in the diagnosis. She has had no recent fevers, viral infections, gastrointestinal illness with diarrhea, or recent use of antibiotics or new medications, though other symptoms, such as transient neurologic symptoms like her headache, are important. Few disorders other than ITP are associated with a platelet count of less than 10 000/μL, but this platelet count can also be seen in other life-threatening disorders such as immune thrombotic thrombocytopenic purpura (iTTP), atypical hemolytic syndrome (aHUS), or acute leukemia, which are important to rule out. Risks for viral infections such as hepatitis C and HIV should be assessed, as these infections are associated with the development of immune-mediated thrombocytopenia. A history of autoimmune thyroid disease has been associated with ITP while lupus and RA have a strong association with the development of ITP. This patient, given her remote history of Hashimoto's thyroiditis, may be at increased risk for other autoimmune disorders such as ITP. Other disorders such as chronic lymphocytic leukemia and even immune checkpoint inhibitor treatment can be associated with ITP; however, these should be evident by the history, or in the case of chronic lymphocytic leukemia, the CBC.

In addition to the history, a thorough physical exam is needed, as well as laboratory tests, including a review of the peripheral blood smear. With a normal white blood cell count and differential, hemoglobin, and hematocrit, aplastic anemia and hematologic malignancies such as acute leukemia can be excluded. Acute kidney injury, as manifest by elevated creatinine, and new anemia suggest diagnoses other than ITP. The peripheral blood smear should be assessed for schistocytes, the presence of which can indicate iTTP, aHUS, or disseminated intravascular coagulation (DIC). The platelets are usually larger than average with ITP. Although an elevated MPV is indicative of large platelets, it is often not reliably measured or reported when the platelet count is low; however, if previous CBCs are available for review and an elevated MPV was seen, it can lend some degree of certainty to the diagnosis of ITP. A "normal" recent CBC can also exclude the possibility of the sudden presentation of an inherited thrombocytopenia, although this is very unlikely at age 67. The reticulated platelet count, or immature platelet fraction, is a promising assay that can help distinguish between peripheral platelet destruction and decreased marrow platelet production, as the immature platelet fraction is increased in disorders such as ITP but is not routinely reported in many clinical laboratories and has not yet been validated in the differential diagnosis of severe thrombocytopenia.[5,6] A review of the peripheral smear is also important to rule out pseudo-thrombocytopenia, although this is unlikely in this patient because she has bruising and bleeding.

Other laboratory tests that can aid in excluding diagnoses such as iTTP and HUS include creatinine, the transaminases, total and direct bilirubin, and the coagulation tests prothrombin time, activated partial thromboplastin time, and fibrinogen. In this patient all these tests are normal, suggesting that this is not iTTP, aHUS, or DIC. Chronic liver failure can contribute to ITP not only through the development of splenomegaly and splenic sequestration but also due to decreased TPO production, as TPO is synthesized in the liver. This patient would have to have experienced a history of chronic liver disease or findings in physical exam and laboratory assessments (such as low albumin) to implicate chronic liver disease as the etiology for thrombocytopenia.

In this older patient, a diagnosis of ITP is highly likely based on the above findings. If the patient were much younger and had a slightly higher platelet count, congenital thrombocytopenia should also be considered in the differential diagnosis. Patients with known chronic ITP can also present with life-threatening bleeding, and the treatment strategies below can be used, although knowledge about the patient's current and prior ITP treatments may help tailor treatment.

Severity of bleeding

Although the platelet count can drop to very low levels in patients with ITP, significant bleeding events in adults are rare, with intracerebral hemorrhage (ICH) occurring in just 1.4% (95% CI, 0.9-2.1) of adults with chronic ITP and severe non-ICH bleeding in 9.6% (95% CI, 4.1-17.1) with acute or chronic ITP in one meta-analysis.[1] This report, like others, uses terms such as "severe" to define bleeding events, yet there has been variability in definitions of bleeding across studies in patients with ITP. To address this lack of standardized definitions, a large panel of experts from multiple specialties as part of the International Society on Thrombosis and Hemostasis Scientific and Standardization Committee on Platelet Immunology recently published a consensus definition of critical bleeding: "A critical ITP bleed was defined as: (a) a bleed in a critical anatomical site including intracranial, intraspinal, intraocular, retroperitoneal, pericardial, or intramuscular with compartment syndrome; or (2) an ongoing bleed that results in hemodynamic instability or respiratory compromise."[7] The authors note that patients with ITP and critical bleeding meeting this definition require treatment. While it is generally thought that critical bleeds rarely occur even with a platelet count less than 20 000/μL, the authors of this standardized definition and others note that factors such as the use of anticoagulants or anatomic lesions can result in critical bleeds at higher platelet count thresholds.

Other less severe forms of bleeding have been variably categorized as minor, including bruising or skin petechiae, mucosal bleeds such as epistaxis or oral bleeding, mild hematuria, or increased hemorrhoid bleeding. Some patients present with bleeding that falls in between minor and critical. In these cases, individual patient assessment is required to determine the urgency for addressing the etiology of thrombocytopenia and instituting treatment. In general, patients with platelet

Table 1. Initial treatment of critical bleeding in patients with ITP

Agent	Mechanism of action	Onset of effect	Risks
IVIG	Interferes with FcR-mediated opsonization of antibody-coated platelets by macrophages	Hours	Volume overload Headache Fever
Glucocorticoids	Inhibits macrophage-mediated platelet clearance Decreases IgG production	Days	Elevated blood glucose Hypertension Steroid psychosis
Platelet transfusions	Immediate supply of platelets	Immediate	Short duration of effect Transfusion reaction Bacterial infection from contaminated products
Tranexamic acid	Stabilizes hemostatic clots	Immediate	Headache Nausea

counts greater than 30 000/μL and no bleeding manifestations can be treated as outpatients.[8] Factors such as age,[9] comorbid disease, including renal insufficiency, and medications affect the risk of bleeding and the need for hospitalization and urgency for treatment.

The patient in this case meets the definition of critical bleeding. She requires not only hospital admission but also urgent treatment to prevent further life-threatening bleeding.

Initial treatment

Treatments for the patient with presumed new-onset or recently diagnosed ITP with critical bleeding require rapid results and are therefore limited to intravenous immunoglobulin (IVIG), steroids, and platelet transfusions (Table 1). In patients with critical bleeding, we use both IVIG and glucocorticoids together. In cases of critical bleeding manifest as ICH, we also use platelet transfusion until IVIG takes effect. These strategies for rapidly increasing the platelet count can also be used for those with critical bleeding and chronic ITP who are on minimal or no treatment.

Platelet transfusion

Urban legends about the use of platelet transfusions in ITP are many; however, platelet transfusion is the fastest method to increase the platelet count. Unlike other disorders such as iTTP or HIT, in which there is concern that platelet transfusions will exacerbate the underlying disease process, there is no such concern with ITP. The problem is that the duration of effect is short, as antibody-mediated clearance of both autologous and transfused platelets generally occurs. A 1-hour posttransfusion platelet count is important not only to gauge the need for further transfusions but to help confirm that peripheral platelet destruction is occurring, supporting the diagnosis of ITP. Common strategies to combat the rapid platelet clearance in patients with ITP include continuous platelet transfusion,[10] transfusion of 2 bags of apheresis platelets or 2 bags of pooled donor platelets every 5 to 6 hours (one author's strategy, JMC), or concomitant platelet transfusions with the administration of IVIG.[11] Platelet transfusions carry a risk of common transfusion-associated reactions, they must be ABO compatible, and they can lead to bacterial contamination because they require storage at 20 °C to 24 °C.

While platelet transfusions are appropriate in this setting, they should be used to achieve hemostasis (>20,000/μL) and not to achieve an artificial threshold for intervention (such as a request for a platelet count of 100,000/μL to go to surgery). If patients do not respond one or two units of transfused platelets, repeat transfusion of additional units is unlikely to be effective and puts the patient at higher risk of transfusion-associated complications. Platelet transfusion is best considered part of a multi-modality hemostatic strategy, along with antifibrinolytics, while waiting for glucocorticoids and IVIG to work.

Intravenous immunoglobulin

IVIG works in ITP by blocking crystallizable fragment–mediated opsonization of antibody-coated platelets, the usual mechanism of peripheral platelet destruction in ITP.[12] Its effects can be rapid, with an increase in platelet count seen as early as 24 hours after administration. It is usually accompanied by a continued increase over the next 24 to 48 hours. The original dosing scheme was 0.4 gm/kg/d for up to 5 days; however, similar results were found when given as 1 gm/kg/day for 1 or 2 doses.[13] We usually use the larger dose of 1 gm/kg/d, and if there is no or minimal improvement in the platelet count 24 hours later, we give a second dose of 1 gm/kg/d. However, in older patients or those with impaired cardiac or renal function, the lower dose may prevent acute volume overload. As with the 1-hour posttransfusion platelet count, which can support the diagnosis of ITP, a response to IVIG can help confirm the diagnosis of ITP.[14]

Glucocorticoids

Glucocorticoids are the mainstay of ITP treatment for newly diagnosed patients who do not require admission and can be managed in the outpatient setting, but they can also be used in those with bleeding. The onset of action is slower than with IVIG, although response at 2 days has been reported, and an increase in the platelet count in response to glucocorticoids alone usually occurs between 5 and 10 days. Much has been written about the type of glucocorticoid and dosing strategy—pulse dexamethasone at 40 mg/d for 4 days, a fixed dose of IV prednisolone at 1 gm/d, or a weight-based dose of oral prednisone of 0.5 to 2.0 mg/kg/d—with a number of randomized controlled trials (RCTs) comparing dexamethasone and prednisone in patients with ITP without critical bleeding, including 1 meta-analysis.[15] Dexamethasone has been found to yield higher platelet counts at 7 days, although there does not appear to be a difference at 30 days. For patients with ICH or other critical

bleeding, a dose of 1 gm IV methylprednisolone is warranted, with subsequent doses based on response. For less severe bleeding, pulse dexamethasone is associated with a higher platelet count at 7 days, which may simply reflect the higher steroid dose at the outset. Side effects, however, need to be considered and managed, including infection, blood glucose in patients with diabetes, and steroid psychosis with high doses in the elderly.

Subsequent treatments

It is hoped that a combination of the above treatments will be rapidly effective in those that present with critical bleeding due to ITP. In cases refractory to these treatments, other approaches may be needed as long as the diagnosis is certain; aside from continued reassessment to consider alternatives, this usually requires a bone marrow examination to ensure that the megakaryocytes are at least normal in number.

Thrombopoietin receptor agonists (TPO-RA) mimetics have significantly improved the treatment options for patients with chronic ITP but are currently considered by the American Society of Hematology guidelines for the management of ITP to be second-line therapy for those not responsive to glucocorticoids after 3 months.[8] The use of TPO-RA for newly diagnosed ITP is more controversial, with little data available to support up-front use and certainly not as stand-alone initial therapy as it can take 1 to 3 weeks to see effects. If a patient with newly diagnosed ITP is refractory to initial treatment with IVIG, corticosteroids, and transfusion or has a very short duration of the increase in platelet counts, we consider starting TPO-RA after 2 to 3 days of treatment in patients who also have critical bleeding. We start at the maximum dose in patients with ITP and critical bleeds as one can more easily titrate the dose down than wait to see effects and then increase after 1 or more weeks of treatment. Although any of the available TPO-RA approved by the US Food and Drug Administration should work, romiplostim as a parenteral agent is more frequently available in inpatient formularies at this time, and it is more easily titrated than the oral agents eltrombopag and avatrombopag. Also, in a sick patient giving it parenterally eliminates any question of absorption. Romiplostim typically has a faster onset of action than eltrombopag, making it more suitable for use in ITP with life-threatening bleeding.

Rituximab, an anti-CD 20 monoclonal antibody, can result in remission of ITP. It works by targeting B lymphocytes, resulting in cell death and therefore decreasing the production of antiplatelet antibodies. It is considered a second-line treatment for ITP by the American Society of Hematology.[8] It takes a while for rituximab to be effective, with no difference seen at 30 days in the RCT of patients treated with glucocorticoids plus rituximab vs glucocorticoids alone.[16] Thus, it is not useful in the initial emergent management of patients with ITP and critical bleeding.

Anti-D immunoglobulin is not frequently used in adult patients. The patient must be Rh positive. It works by causing red cell destruction and therefore interfering with reticuloendothelial uptake of platelets; however, its effects are not easily controlled. Moderate to severe hemolysis can occur that can result in cardiovascular instability and acute kidney injury due to free hemoglobin, although its effects may be additive with IVIG.

Splenectomy has been used in patients with acute ITP wishing to avoid other treatments, in those who are refractory to initial treatments, and in those with chronic ITP not responding or losing response to treatment. The platelet count going into surgery can be low; surgeons report that once the splenic artery is clamped, the platelet count rises rapidly as the reticuloendothelial destruction of platelets ceases. However, over time the reticuloendothelial activity in other organs can take over and ITP can recur. The shortest duration of effect of splenectomy seen by 1 author is 6 weeks, with a rise in platelet count to over 400 000/μL, only to drop below 20 000/μL 6 weeks later. Emergency splenectomy can be an option for some patients with ITP and critical bleeding if they are candidates for anesthesia. Emergency splenectomy may be appropriate for ITP patients with life-threatening bleeding who do not respond to initial therapies.

Although vincristine was a mainstay of therapy in the 1970s and 1980s, it is rarely used currently in the management of ITP. Its main drawback was neurologic side effects with multiple doses, especially in the elderly, as well as a good but only transient response. However, it is platelet sparing and can be effective when other treatments have proved ineffective when used for only 1 or at most 2 doses, as was recently described in cases of ITP associated with SARS-CoV-2 vaccines.[17] Early addition of vincristine (1–1.5 mg) has been studied and found to be safe and effective in emergency treatment of ITP. Vincristine has rapid onset of action and may augment response to IVIG and steroids if these agents do not initially rapidly raise the platelet count.[18]

Adjunctive hemostatic treatments

As with any form of bleeding, antifibrinolytic agents would appear to have efficacy at preventing clot dissolution. Although the use of tranexamic acid in RCTs for bleeding in trauma and after cesarean birth have shown positive results, RCTs have demonstrated no efficacy in cases of gastrointestinal bleeding, ICH, or bleeding due to thrombocytopenia associated with hematologic malignancies.[19-22]

Although tranexamic acid and aminocaproic acid are not well-studied in ITP patients with life-threatening bleeding, their use has become commonplace. Their safety has been established in numerous settings, so their benefit likely outweighs their risk in ITP patients. When used, patients with life-threatening hemorrhage should receive an intravenous bolus (e.g., epsilon-aminocaproic acid 5 grams) followed by a continuous drip until hemostasis is achieved (e.g., 1 gram per hour for 8 hours). If prothrombin time, activated partial thromboplastin time, and fibrinogen are normal, there is no role for factor concentrates, such as prothrombin complex concentrates, that are not activated. Similarly, activated recombinant factor VII likely has no role in treating bleeding associated with ITP, as evidenced in a case report from Germany in 2021 in which despite using all modalities of treatments, including rVIIa, fatal bleeding could not be prevented.[23]

Summary

Patients who present with thrombocytopenia and critical bleeding require urgent assessment and management. Excluding other diagnoses and rapidly instituting treatment for those with isolated thrombocytopenia with initial use of IVIG, glucocorticoids, and platelet transfusions may prevent serious morbidity and fatal outcomes. Early recognition, exclusion of alternative

diagnoses, and rapid institution of fast-acting treatment agents are required to successfully treat these patients.

Conflict-of-interest disclosure

Steven Fein: speakers bureau for Amgen and Sobi.
Jean Connors: Nothing to disclose.

Off-label drug use

Steven Fein and Jean Connors: Rituximab used for treatment of ITP.

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Inpatient recognition and management of HLH

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Hemophagocytic lymphohistiocytosis (HLH) is one of the life-threatening emergencies that a hematologist may be called upon to diagnose and manage. It is a hyperinflammatory process that develops in patients with genetic abnormalities, hematologic malignancies, chronic inflammatory states, or infections. The main clinical challenges are recognizing HLH, determining whether the immune response is aberrant or appropriate, and deciding upon therapy. Patients may present with fever, central nervous system symptoms, cytopenias, or elevated liver enzymes.

Recognizing HLH is challenging because its features overlap with numerous systemic disorders, thus requiring a high level of suspicion and timely investigations to confirm the diagnosis and detect the underlying trigger. Once HLH is diagnosed, careful consideration of immunosuppressive therapy's potential benefit versus harm is necessary. Such therapy can sometimes be tailored to the underlying trigger. In the acute setting, the competing pressures of completing a thorough diagnostic process (including evaluation for the presence of lymphoma and infection) and the need for expedited treatment must be balanced. During the management of an HLH patient, continuous vigilance for the presence of as-yet unrecognized disease triggers, monitoring response, and identifying emerging complications is critical. This review will discuss the recognition and management of HLH in the inpatient setting.

LEARNING OBJECTIVES

- Recognize the HLH syndrome in the inpatient setting
- Identify HLH disease triggers and their appropriate treatments
- Determine when to start immunosuppressive therapy for suspected HLH

CLINICAL CASE

A 45-year-old healthy man presented with a 3-day history of fever of up to 38.2°C, headache, and gait instability. His general physical exam was unremarkable, and neurologic examination revealed normal motor, sensory, and cerebellar functions. Complete blood count and serum chemistries were normal, and the C reactive protein (CRP) level was elevated at 9 mg/dL. A brain CT scan was normal. Lumbar puncture showed clear cerebrospinal fluid (CSF) with 10 white blood cells, 90% mononuclear cells, no red blood cells, and an elevated protein level of 63 mg/dL. A presumptive diagnosis of aseptic meningoencephalitis was made.

Magnetic resonance imaging revealed an increased pontine signal, suggesting an inflammatory process. Syphilis, West Nile virus, HIV, herpes virus, and SARS-coronavirus type 2 infections were excluded. Autoimmune and paraneoplastic CSF antibody panels were negative. The patient's headache worsened, with no response to intravenous hydration, caffeine, or analgesic medications. Empiric therapy with methylprednisolone 1 g/d led to rapid clinical improvement. Upon corticosteroid cessation, the temperature increased to 39.2°C, and the headache recurred, accompanied by saddle anesthesia. The CRP increased to 18 mg/dL; new cytopenias developed: platelets were 135 k/μL and hemoglobin was 8 g/dL. Creatinine increased to 1.5 mg/dL, triglycerides were elevated at 661 mg/dL, and ferritin was 1275 mg/dL.

Differential diagnosis and initial evaluation

HLH is a hyperinflammatory syndrome characterized by a unique constellation of clinical and laboratory features:

Table 1. The differential diagnosis of hemophagocytic lymphohistiocytosis (HLH), including triggering and mimicking conditions

Malignant	Infectious	Inflammatory	Storage/metabolic	Vascular	Iatrogenic
BCL	EBV	SJIA	Gaucher's disease	TTP	CAR-T
HL	CMV	Still's disease	LPI	HUS	BITES
TCL	HIV	SLE	Wolman's disease	HELLP	ICB
CLL	*Leishmania*	ALPS	Lysosomal acid lipase deficiency	DIC	DRESS
MDS	COVID-19	Sarcoidosis	GSD type 1		
AML	Atypical infections*	GCA	Niemann-Pick disease		
ALL	West Nile virus	Kawasaki disease			
CML	Tuberculosis	PIMS			
Myelofibrosis	*Aspergillus*	Kikuchi disease			
HCC	Bacterial sepsis				

*Atypical infections: *Rickettsia*, *Leptospira*, *Bartonella*, *Brucella*, and *Ehrlichia*.

ALPS, autoimmune lymphoproliferative syndrome; AML, acute myeloid leukemia; BCL, B-cell lymphoma; BITE, bispecific T-cell engager; CART, chimeric antigen receptor T cell therapy; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; CMV, cytomegalovirus; DIC, disseminated intravascular coagulation; DRESS, drug reaction with eosinophilia and systemic symptoms; EBV, Epstein Bar virus; GCA, giant cell arteritis; GSD, glycogen storage disease; HCC, hepatocellular carcinoma; HELLP, emolysis, elevated liver enzymes and low platelet count; HL, Hodgkin lymphoma; HUS, hemolytic uremic syndrome; ICB, immune checkpoint blockade; LPI, lysinuric protein intolerance; MDS, myelodysplastic syndrome; PIMS, pediatric inflammatory multisystem syndrome; SLE, systemic lupus erythematosus; SJIA, systemic juvenile arthritis; TCL, T-cell lymphoma; TTP, thrombotic thrombocytopenic purpura.

fever, neurologic symptoms, splenomegaly, cytopenias, elevated triglycerides, decreased fibrinogen, elevated liver enzymes, and increased ferritin and soluble CD25 (sCD25). These may present in combination or individually, which may be particularly pertinent in instances of unexplained fever or liver enzyme elevations. Patients with HLH are frequently among the most severely ill in the hospital, often encountered in intensive care units with multiorgan dysfunction. The nonspecific nature of disease phenotypes in HLH makes the distinction from other causes of severe multisystem disorders, particularly sepsis, difficult.[1] Characteristically, HLH patients have an unremitting fever accompanied by severe cytopenias, particularly thrombocytopenia. Early assessment of ferritin and soluble CD25, typically markedly increased in HLH, may expedite the diagnosis. Recently, a specific T-cell phenotypic signature has been suggested to discriminate between HLH and sepsis: CD38/HLA-DR bright $CD8^+$ T cells were increased in a cohort of children with familial HLH but not in children with a confirmed diagnosis of bacterial sepsis; the presence of as few as 7% of cells with this unique phenotype was sufficient to distinguish between children with HLH from those with sepsis, although in HLH levels are usually higher.[2-4] HLH may mimic several conditions that must simultaneously be considered possible mimics or true triggers of HLH (Table 1). In a rapidly deteriorating HLH patient, prompt evaluation of these conditions is necessary. Novel serologic biomarkers, such as C-X-C motif chemokine ligand 9 (CXCL9)[5] and interleukin 18,[6] are emerging as markers with the potential to distinguish between HLH and clinical syndromes with overlapping features.

HLH is not an autoimmune disorder, but rather an aberrant inflammatory response characterized by excessive and maladaptive T-cell activation. Hence, when a critically ill patient has a widespread inflammatory response, it is imperative to establish a conceptual framework that distinguishes between an appropriate immunologic response, such as one to a serious bacterial or viral infection, and a disordered and unregulated response, which characterizes HLH. Seminal advances in understanding the molecular and cellular underpinnings of HLH have been critical in this regard. The mechanism of dysregulated immune response in HLH (Figure 1) was first described in a mouse model of the genetic version of the disease, familial HLH (F-HLH). The underlying genetic defects of lymphocyte cytotoxicity cause inadequate control of T-cell activation and failure to clear infections, particularly viral. This results in persistent CD8+ T–cell activation with massive cytokine elaboration, commonly called a *cytokine storm*. Preeminent among these is interferon-gamma (IFN-γ), the main driver of the disease phenotype. IFN-γ activates macrophages associated with hemophagocytosis and tissue infiltration and damage.[7,8]

HLH is commonly defined when a patient meets 5 or more of the 8 enrollment criteria from the HLH-2004 study, developed as entry criteria for a clinical trial in F-HLH.[9] The appropriateness of these criteria for diagnosing other types of HLH is uncertain.[10] Furthermore, while some of these features are driven by inappropriate immune activation in F-HLH,[7] their presence in the context of cancer may represent nonimmunologic effects of the malignant clone itself, such as infiltration of the marrow or spleen. Thus, some patients fulfilling the HLH-2004 criteria mimic F-HLH, and not only may such patients not benefit from HLH-directed therapy, but they may even be harmed by it.[11] Regardless of whether therapy may be helpful, if it obscures a critical diagnosis, such as prompt identification of malignancy, then it is potentially harmful.

How to approach a patient with suspected HLH

Once HLH is suspected, the provider should pose 3 questions:

1. Does my patient have HLH syndrome?
2. What is the underlying cause (genetic?) and trigger (infection, malignancy)?
3. Will my patient benefit from immunosuppressive therapy?

Does my patient have the HLH syndrome?

All patients fulfilling the required number of HLH diagnostic features of HLH-2004 diagnostic criteria[9] or HScore[12] (Table 2)

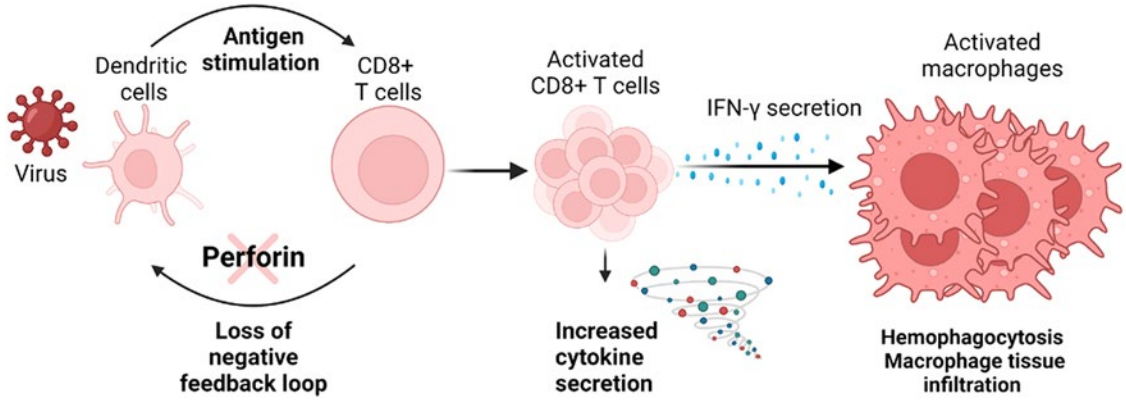

Figure 1. The pathogenesis of familial hemophagocytic lymphohistiocytosis (HLH). Patients with familial HLH (F-HLH) often have defects of the perforin cytotoxic pathway. Experimental studies have demonstrated that this pathway provides critical feedback regulating CD8+ T–cell activation. Defects result in excessive antigen presentation by dendritic cells, leading to excessive T-cell activation and overproduction of IFN-gamma, which results pathologic systemic macrophage activation. Thus, clinical HLH results from a primary defect of immune regulation leading to largely IFN-γ driven immunopathology. Created with BioRender.com

may potentially have HLH syndrome. However, not all individuals fulfilling these criteria have a hyperinflammatory process and may not benefit from immune suppression. Such patients should be considered to have an HLH mimic instead of HLH disease. For example, while a patient with spontaneous familial HLH or another with macrophage activating syndrome (MAS) associated with a rheumatologic disease would be considered to have HLH disease, a patient with disseminated tuberculosis with the requisite number of features resulting from an appropriate immune response would best be thought of as having an HLH mimic. Thus, establishing an HLH diagnosis begins with recognizing HLH syndrome followed by exclusion of HLH disease mimics (Figure 2).[11]

Serum ferritin is the most widely available biomarker for HLH and can be measured immediately upon suspicion of the process. However, hyperferritinemia is common in critically ill patients with sepsis, liver disease, and hematological malignancies,[13] conditions that may mimic or trigger HLH. Lachman et al recently demonstrated that 4 rather than 5 of the HLH-2004 criteria with optimized values (serum ferritin >3000 µg/L and fever >38.2°C) and an HScore of 168 improve the sensitivity and specificity of HLH diagnosis.[14] This application of the HLH diagnostic criteria in critically ill hyperferritinemic patients may facilitate the timely diagnosis of HLH.

Another important test, albeit not widely available globally, is sCD25. This assay and ferritin have a high negative predictive value; when both are normal, hyperinflammation is excluded (Figure 3). The only exception is infants under 1 year of age in whom HLH may present with a low but rising ferritin, necessitating serial measurement.

What is the underlying trigger?

F-HLH should be considered in all children and young adults with HLH (especially in male patients because some defects are X linked). A genetic panel should be sent as soon as possible, but treatment initiation should not be delayed until results are received. Lesions in genes affecting perforin function (*UNC13D, STX11, STXBP2, RAB27A, LYST*) or effector T-cell function (*SH2D1A, ITK, CD27*) are associated with HLH, as are those associated with inflammasome activation, such as defects in the *XIAP* and *NLRC4* are also genes. A recent whole exome–based study has expanded the list of potentially HLH-associated genes.[15] Adult HLH is not associated with germline mutations but may be associated with clonal hematopoiesis and somatic mutations.[16]

In adults, malignancies are the most common trigger of HLH and may occur in 3 settings. The first is HLH presenting concurrently with malignancy (M-HLH).[17] While the mechanism of this syndrome is unknown, patient outcome is especially poor, with a 5-year overall survival of only 10%-20%. Hematologic malignancies are the most common cancers associated with this form of HLH. They may be particularly difficult to recognize given the clinical similarity between the malignancy and HLH, which frequently begs whether the patients truly have HLH disease or an HLH mimic.[18] M-HLH can be accurately identified by the Optimized HLH Inflammatory Index, which comprises the combined elevation of sCD25 >3900 U/mL and ferritin >1000 ng/mL (Table 2).[19] An increased sCD25 to ferritin ratio has been reported to suggest an underlying lymphoma.[20] HLH consensus recommendations strongly support positron emission tomography–guided imaging, repetitive tissue sampling, and consultation with a lymphoma reference pathologist in adult patients with HLH (Figure 3).[21]

In the second scenario, HLH develops after chemotherapy that results in immune compromise, marrow suppression, and/or associated infection; the condition is best thought of as HLH arising in the context of immune compromise (IC-HLH). The final context is iatrogenic HLH resulting from immune-activating therapies (Rx-HLH).[22] Rx-HLH is always caused by (intentional) immune hyperactivation and thus should be considered "HLH disease" (Figure 2). These treatment modalities have in common the activation of T-cell responses against a variety of hematologic malignancies and include chimeric antigen receptor (CAR) T cells, bi-specific T-cell engager antibodies, and checkpoint inhibitors, the latter also being associated with Rx-HLH in solid organ tumors.[23] Recently, delayed onset Rx-HLH following CD22-directed CART cell administration characterized by

Table 2. General and context-specific diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH)[9,12,19,39]

<table>
<tr>
<th>Familial HLH</th>
<th>Reactive HLH</th>
<th>Context-specific</th>
</tr>
<tr>
<td>HLH-2004[9]

If criterion 1 or 2 is fulfilled.

1) A molecular diagnosis consistent with HLH

2) 5 of the 8 criteria:

• Fever

• Splenomegaly

• Cytopenias: 2 out of 3 lineages
o Hemoglobin <9 g/dL
o Platelets <100×10⁹/L
o Neutrophils <1.0×10⁹/L

• Triglycerides ≥265 mg/dL

OR

Fibrinogen ≤150 mg/dL

• Hemophagocytosis in bone marrow or spleen or lymph nodes. No evidence of malignancy

• Low or no NK cell activity

• Ferritin ≥500 µg/L

• sCD25 ≥2400 U/mL</td>
<td>H-Score[12] >169 points is the optimal diagnostic threshold for HLH. Addition of the points according to:

<table>
<tr><th>Variable</th><th>Categories</th><th>Points</th></tr>
<tr><td>Known underlying immunosuppression*</td><td>No
Yes</td><td>0
18</td></tr>
<tr><td>Temperature, °F (°C)</td><td><101.1 (<38.4)
101.1–102.9 (38.4–39.4)
>102.9 (>39.4)</td><td>0
33
49</td></tr>
<tr><td>Organomegaly</td><td>No
Hepatomegaly or splenomegaly
Hepatomegaly and splenomegaly</td><td>0
23
38</td></tr>
<tr><td>Number of cytopenias**</td><td>1 lineage
2 lineages
3 lineages</td><td>0
24
34</td></tr>
<tr><td>Ferritin, ng/mL (µg/L)</td><td><2000
2000–6000
>6000</td><td>0
35
50</td></tr>
<tr><td>Triglyceride, mg/dL (mmol/L)</td><td><132.7 (<1.5)
132.7–354 (1.5–4)
>354 (>4)</td><td>0
44
64</td></tr>
<tr><td>Fibrinogen, mg/dL (g/L)</td><td>>250 (>2.5)
≤250 (≤2.5)</td><td>0
30</td></tr>
<tr><td>AST, U/L</td><td><30
≥30</td><td>0
19</td></tr>
<tr><td>Hemophagocytosis features on bone marrow aspirate</td><td>No
Yes</td><td>0
35</td></tr>
</table>
*HIV positive or receiving long-term immunosuppressive therapy (ie, glucocorticoids, cyclosporine, azathioprine).

**Defined as hemoglobin ≤9.2 g/dL and/or WBC ≤5×10⁹/L and/or platelets ≤110×10⁹/L.</td>
<td>Malignancy-associated optimized HLH inflammatory (OHI) index[19]

sCD25 >3,900 U/mL

AND

Ferritin >1,000 ng/mL

Systemic juvenile arthritis associated with EULAR/ACR/PRINTO for MAS[39]

Serum ferritin level >684 ng/mL

AND

Any 2 of the following:

• Platelet count ≤181×10⁹/L

• AST >48 units/L

• Triglyceride >156 mg/dL

OR

Fibrinogen ≤360 mg/dL</td>
</tr>
</table>

AST, aspartate aminotransferase; EULAR/ACR/PRINTO, European League Against Rheumatism/American College of Rheumatology/Pediatric Rheumatology International Trials Organization; NK, natural killer; sCD25, soluble CD25/soluble interleukin-2 receptor alpha; WBC, white blood cell.

extreme hyperferritinemia[24] has been described and is termed "immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS)."[25]

Infections may either trigger or mimic HLH. Therefore, testing for common HLH-associated viruses by polymerase chain reaction (PCR) should be routine in HLH patients. Epstein-Barr virus (EBV) and cytomegalovirus are two of the most common infectious HLH triggers. EBV is associated with a worse prognosis than other subtypes.[26] Hyperinflammation in COVID-19 patients has proven to have common immunologic profiles with HLH disease and may be considered on this spectrum.[4] Atypical infections that may lead to cytopenias, elevations of inflammatory markers, and other features of HLH include visceral leishmaniasis; disseminated atypical/tuberculous mycobacteria; histoplasmosis; *Ehrlichia*, *Bartonella*, and *Brucella* species; disseminated adenovirus; and disseminated herpes simplex. In most cases, these infections should be considered as HLH mimics, and specific antimicrobial treatment is preferable to HLH-directed immune suppression because the HLH syndrome does not appear to have a largely immune-mediated etiology in these patients. Importantly, some patients with atypical infections benefit from immunosuppressive therapies.[11]

HLH in the context of rheumatologic diseases is termed MAS or rheumatologic (R)-HLH and deserves special consideration. Since patients with chronic inflammation have intrinsically elevated platelets and markers of inflammation, the European League Against Rheumatism/American College of Rheumatology/Paediatric Rheumatology International Trials Organization consortium developed unique diagnostic criteria for patients with juvenile systemic arthritis presenting with MAS (Table 2). Similarly, patients with SLE may have underlying cytopenias making MAS difficult to identify. These patients may present with neutrophilia rather than neutropenia and have other unique clinical symptoms such as arthritis and rash. Evidence from preclinical and clinical studies suggests that interleukin 18 distinguishes MAS from F-HLH.[6]

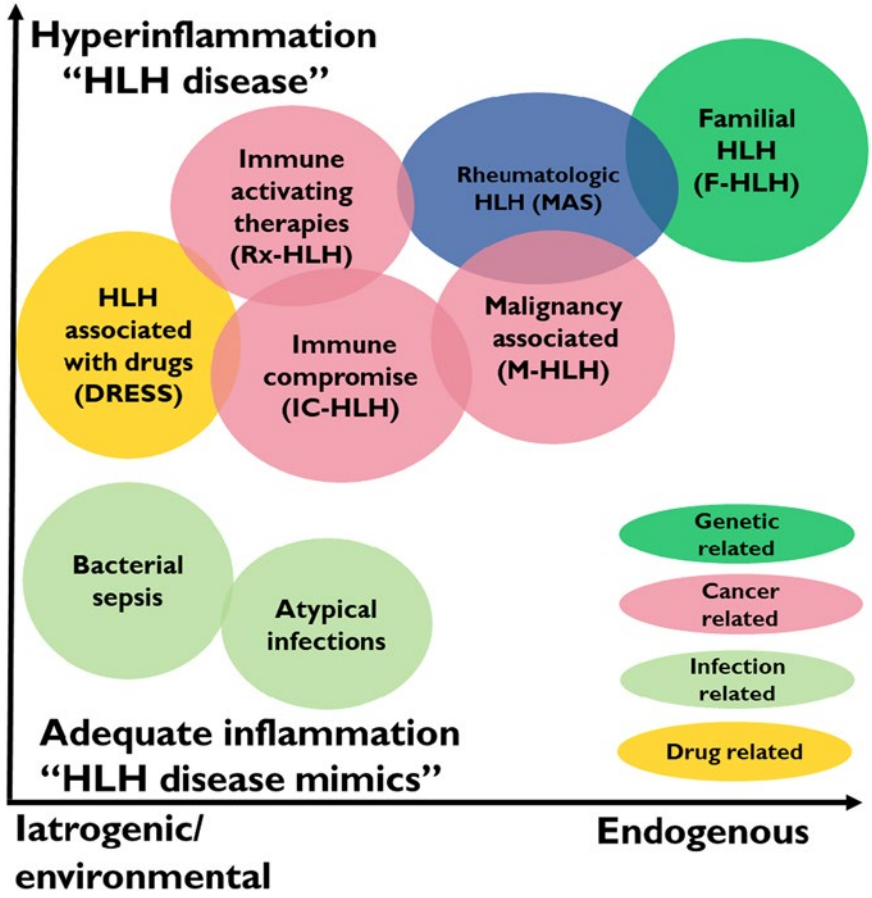

Figure 2. Conceptualizing hemophagocytic lymphohistiocytosis (HLH) syndrome. HLH syndrome includes all conditions meeting HLH diagnostic criteria. This syndrome includes conditions of hyperinflammation ("HLH disease," conditions close to the upper side) that would benefit from HLH-directed immunosuppressive therapies, and those conditions with adequate inflammation ("HLH disease mimics," conditions close to the lower side) that would not benefit from such therapy. These conditions can be endogenous (those close to the right) or iatrogenic (those close to the left). DRESS, Drug Reaction with Eosinophilia and Systemic Symptoms; MAS, macrophage activation syndrome.

Will my patient benefit from immunosuppressive therapy?

After determining that the patient meets the criteria for the HLH syndrome (Table 2), appears to have these features due to a hyperinflammatory process (Figure 2), and has been tested for underlying triggers (Figure 3), the clinician should consider whether the patient would likely benefit from immunosuppressive therapy. Our patient was started on steroids because of an ongoing uncontrolled inflammatory process without a definite diagnosis; though not optimal, this is a very common real-life scenario.

CLINICAL CASE (continued)

Following the elevated ferritin test, sCD25 was obtained and was elevated at 8390 U/mL, and a bone marrow biopsy showed hemophagocytosis. HLH syndrome was established based on fever, elevated sCD25, ferritin, bicytopenia, triglycerides, and hemophagocytosis.

The trigger for HLH was sought: positron emission tomography–guided imaging demonstrated increased splenic[18] fluorodeoxyglucose uptake and no lymphadenopathy. Testing for rheumatologic disorders was negative. EBV PCR was elevated with 51,000 copies/mL. A presumptive diagnosis of EBV-associated HLH was made. Genetic testing did not reveal any known F-HLH-associated mutation.

The patient was treated using the HLH-94 protocol; in addition, 3 doses of rituximab as EBV directed therapy were added. After the third dose, virus elimination was confirmed by PCR testing. During treatment, the clinical and laboratory abnormalities gradually resolved.

Approach to therapy

Because patients with F-HLH and often other forms of HLH may deteriorate rapidly, prompt and aggressive therapy is critical in improving outcomes and may require treatment initiation before the conclusion of diagnostic testing.[21] While the backbone of therapy is etoposide, dexamethasone, and other immunosuppressive agents, treatment should be individualized according to specific triggers such as the presence of malignancy or active viral infection. Though it may obscure the diagnosis

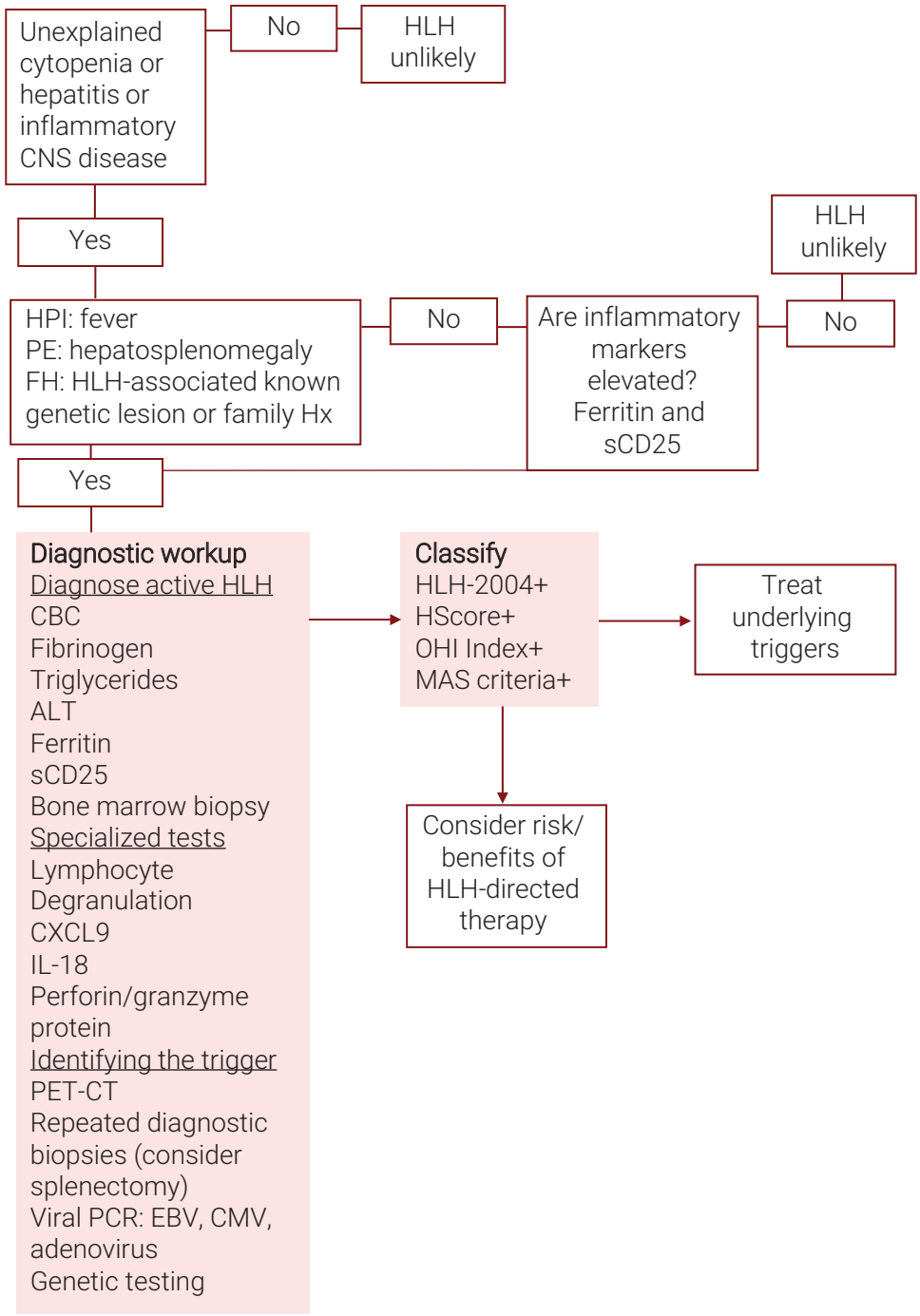

Figure 3. Algorithm for the evaluation of suspected hemophagocytic lymphohistiocytosis (HLH). ALT, alanine transaminase; CBC, complete blood count; CXCL9, C-X-C motif chemokine ligand 9; HPI, history of present illness; PE, physical examination; sCD25, soluble CD25 or soluble interleukin-2 receptor alpha.

of lymphoma or leukemia, early corticosteroid administration should be considered, particularly if organ dysfunction occurs.[27]

F-HLH is treated according to the HLH-94 protocol (Figure 4[28]): 8 weeks of etoposide and dexamethasone treatment followed by allogeneic hematopoietic stem cell transplantation (HSCT). Patients with refractory or recurrent disease or who cannot tolerate etoposide treatment (eg, because of severe cytopenias) can be treated with emapalumab, an anti-IFN-γ monoclonal antibody. Other targeted therapies are currently being tested in clinical trials. These include the JAK inhibitor ruxolitinib in a phase 2 trial (NCT04551131),[29] and a phase 3 trial of Tadekinig alpha, a recombinant interleukin-18 binding protein (r-hIL-18BP) in patients with deleterious *NLRC4* and *XIAP* mutations which are associated with autoinflammatory syndromes (NCT03512314).

Rx-HLH should be treated with anticytokine therapy, which preserves immunotherapy's efficacy, with tocilizumab and anak-

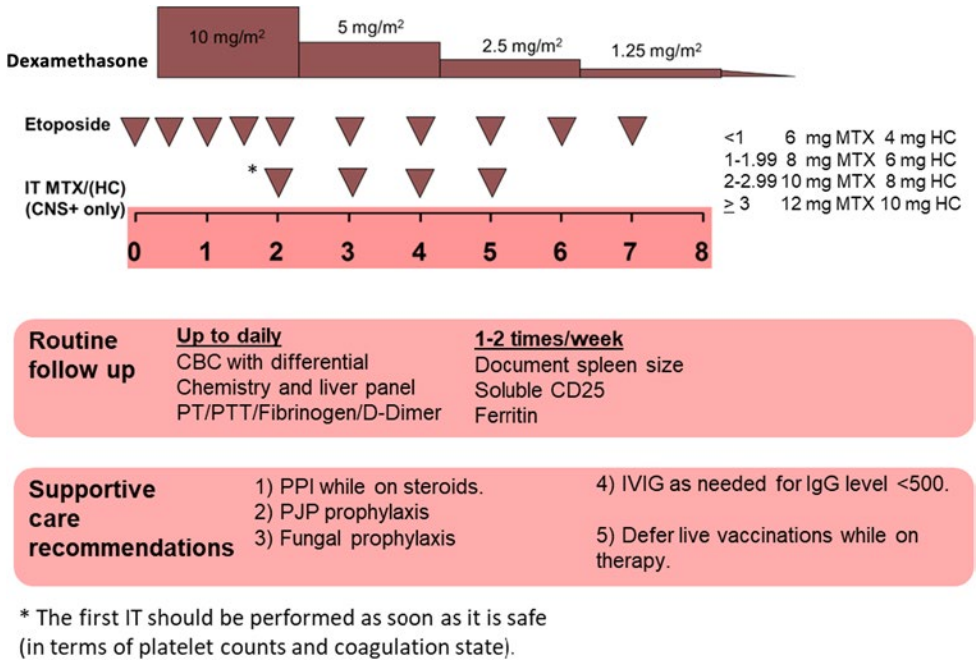

Figure 4. Etoposide/dexamethasone therapy for HLH (based on HLH94): initial therapy (weeks 1–8) for patients with familial/idiopathic HLH. CBC, complete blood count; CNS, central nervous system; HC, hydrocortisone; IgG, immunoglobulin G; IT, intrathecal therapy; IVIG, intravenous immunoglobulin; MTX, methotrexate; PJP, pneumocystis jirovecii pneumoni; PPI, proton pump inhibitors; PT, prothrombin time; PTT, partial thromboplastin time.

inra being the preferred agents.[25,30] Data regarding the efficacy of emapalumab[27] in this setting are emerging.

The optimal treatment for M-HLH treatment is unknown.[22] Currently, a 2-phase approach is advised.[22,31] First, quench the uncontrolled inflammation using corticosteroids or specific anticytokine therapy, sometimes with etoposide. Evidence is also emerging for ruxolitinib as successful therapy for inflammation control in these patients.[32,33] Second, provide tumor-specific antineoplastic therapy, possibly augmented with etoposide, as soon as feasible.

Treatment of IC-HLH requires thorough evaluation to identify possible bacterial, fungal, and viral triggers (including testing for viral triggers with PCR), which, if present and treated expeditiously, may lead to the resolution of the syndrome. In addition, anti-inflammatory therapy with steroids and anticytokine agents is sometimes appropriate.

MAS treatment is based predominantly on corticosteroids and rarely requires etoposide. Recent reports have shown a good response to emapalumab in refractory patients, and a long-term follow-up trial is ongoing.[34] Anakinra, a recombinant IL-1 receptor antagonist, and agents targeting IL-6 have been reported as beneficial in MAS patients.[35]

Allogeneic HSCT is the standard of care for patients with F-HLH, patients with refractory disease, and patients with M-HLH that have no alternate definitive therapy. Reduced-intensity conditioning regimens have reduced toxicities and improved survival rate[36] but are often complicated by mixed chimerism.[37] Excellent outcomes of HSCT in 21 patients with adult HLH have been reported with a 3-year OS of 75% (95% CI, 51%-89%), suggesting the need to consider this strategy more widely among these patients.[38]

Conclusions

Without rapid diagnosis and appropriate treatment, the natural course of HLH disease may be fatal. Due to the rarity, diversity, and complexity of the HLH syndrome, diagnosis is difficult and is often delayed. Increasing awareness of HLH in recent years has improved early diagnosis but may carry the risk of overdiagnosis and inappropriate immunosuppression in mimicking conditions. Early measurement of serum ferritin and sCD25 levels, particularly in M-HLH, can expedite diagnosis with a high degree of specificity. Increasing the availability and awareness of these important diagnostic tests, along with comprehensive testing to identify an HLH trigger, such as malignancy or viral infection, is crucial for improving patient outcomes.

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Adi Zoref-Lorenz: received consulting fees and research support from Sobi.
Martin Ellis: no competing financial interests to declare.
Michael B. Jordan: received consulting fees and research support from Sobi.

Off-label drug use

Adi Zoref-Lorenz: Off-label use of ruxolitinib, emapalumab, and anakinra is discussed.
Martin Ellis: Off-label use of ruxolitinib, emapalumab, and anakinra is discussed.

Michael B. Jordan: Off-label use of ruxolitinib, emapalumab, and anakinra is discussed.

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How to assess hemostasis in patients with severe liver disease

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Patients with advanced liver diseases frequently acquire profound alterations in their hemostatic system. Simultaneous changes in procoagulant and anticoagulant systems result in a reset in the hemostatic balance with a relatively neutral net effect, although there are notable hypocoagulable and hypercoagulable features in the hemostatic system in patients with liver disease. Laboratory and clinical studies have demonstrated that patients have a relatively well-preserved hemostatic system even though routine diagnostic tests of hemostasis (prothrombin time, platelet count) suggest a bleeding tendency. Routine diagnostic tests of hemostasis are unsuitable to assess the hemostatic status of patients with liver disease, as these tests are insensitive for the concurrent prohemostatic and antihemostatic changes in these patients. These tests are, however, frequently requested in patients with liver disease, as they are well established indicators of severity of liver disease. This paper will discuss commonly used diagnostic and research-type hemostatic tests and will outline how test results should be interpreted in patients with liver disease.

LEARNING OBJECTIVES

- To identify limitations of common laboratory tests performed in patients with complex hemostatic alterations as a result of liver disease
- To differentiate use of hemostatic tests in patients with liver disease as markers of disease severity from use in prediction of bleeding

CASE

A patient with chronic liver disease related to alcohol abuse is hospitalized for acute decompensation with massive ascites. The patient has severe liver disease, evidenced by a model of end-stage liver disease score of 20 and a Child-Pugh score C10. A paracentesis is scheduled, but the treating physician is concerned about the patient's coagulopathy with an international normalized ratio of 1.8, a plasma fibrinogen level of 1.4 g/L, and a platelet count of 61 000/μL.

Why assess hemostasis in severe liver disease?

Advanced chronic liver disease is commonly associated with substantial changes in the hemostatic system. Frequent findings include thrombocytopenia, low plasma levels of coagulation factors, inhibitors of coagulation, and proteins involved in the fibrinolytic system. The decreased platelet count and decreased plasma levels of hemostatic proteins likely relate to a combination of compromised synthetic failure of the diseased liver, decreased platelet production in part related to decreased thrombopoietin production, consumption of platelets and hemostatic proteins as a result of low-grade activation of the hemostatic system, and sequestration of platelets in an enlarged spleen. The net result of the complex hemostatic changes in these patients is surprisingly benign (Figure 1). Due to a simultaneous decline in both prohemostatic and antihemostatic factors, the hemostatic balance appears to remain in balance, as evidenced by laboratory studies using advanced hemostatic tests and clinical observations.[1] Specifically, such laboratory studies included 3 main categories. One category was studies of platelet adhesion from whole blood to adhesive proteins, such as collagen, under conditions of flow.[2] These studies have shown that highly elevated levels of the platelet adhesive protein von Willebrand factor (VWF) compensates for the decreased platelet count.

The second category of laboratory studies was thrombomodulin-modified thrombin generation tests.[3] Such assays are sensitive for levels of procoagulant proteins and

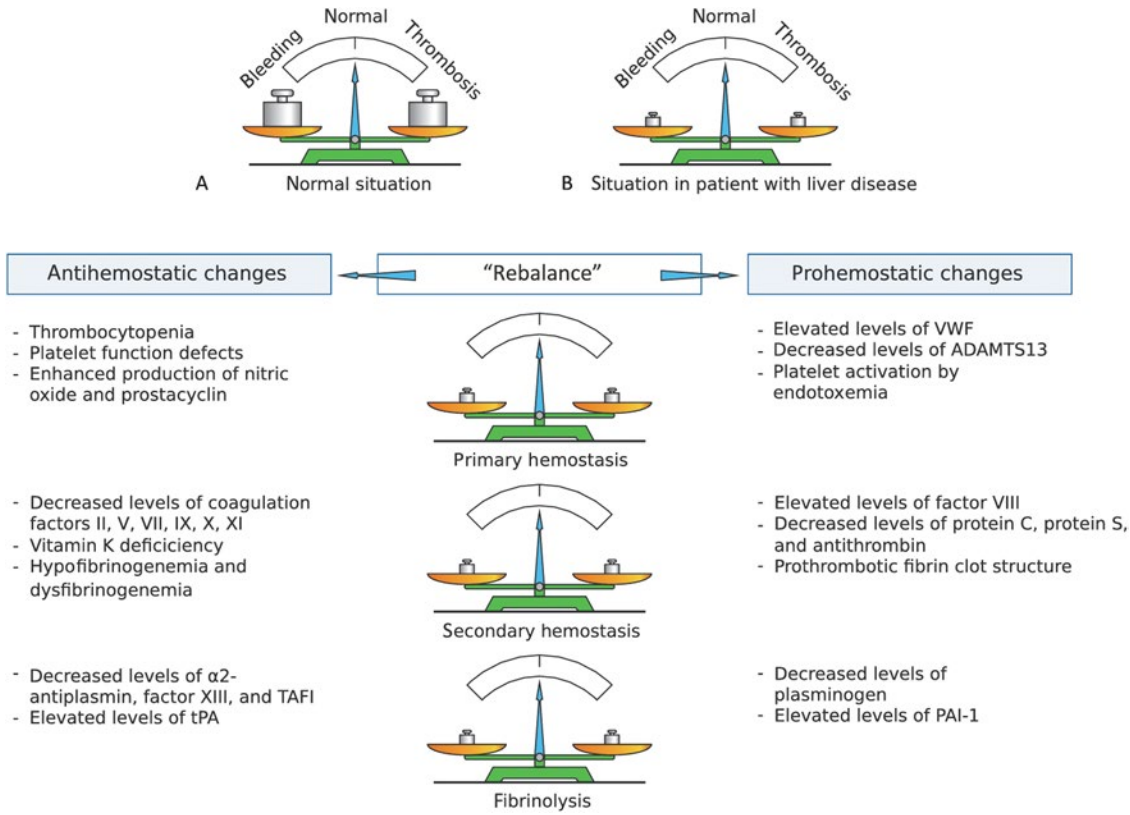

Figure 1. Schematic presentation of the rebalanced hemostatic system in patients with liver disease. In healthy individuals (A), the hemostatic system is in solid balance. In patients with liver disease (B and table) both prohemostatic and antihemostatic changes result in a "rebalance" of the hemostatic system. This new balance is characterized by specific hypocoagulable and hypercoagulable features. ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; PAI-1, plasminogen activator inhibitor-1; TAFI, thrombin activatable fibrinolysis inhibitor; tPA, tissue plasminogen activator; VWF, von Willebrand factor. Reprinted from van den Boom & Lisman with permission.[46]

for levels of anticoagulant drivers, including the protein C system and antithrombin. Due to a simultaneous decline in procoagulant and anticoagulant proteins, thrombomodulin-modified thrombin generation test results are normal or even show enhanced thrombin generation in patients with liver disease. Additional studies have demonstrated that low levels of procoagulant proteins are compensated for by low levels of protein C and antithrombin and elevated levels of factor VIII.[4-6]

The third category of studies is plasma-based fibrinolytic tests that demonstrate normal fibrinolytic potential in many patients with liver disease due to simultaneous changes in profibrinolytic and antifibrinolytic proteins.[7] Clinical observations in support of the concept of rebalanced hemostasis in liver disease include low transfusion requirements in many contemporary liver transplantation programs; also included is the notion that clinically relevant bleeds in patients with cirrhosis are often independent of hemostatic failure and are instead driven by portal hypertension (eg, variceal bleeds) or mechanical injury to blood vessels (eg, bleeding following invasive procedures) (Figure 2).[8-11] Importantly, these nonhemostatic bleeds require different strategies to treat or prevent. As in the case described herein, there is a seemingly paradoxical disconnect between routine diagnostic tests of hemostasis that suggest a bleeding tendency, the rebalanced hemostatic status identified by more sophisticated tests of hemostasis, and clinical bleeding which is often unrelated to hemostatic failure.

Recent clinical guidance documents suggest that routine diagnostic tests of hemostasis are not helpful in predicting spontaneous or procedure-related bleeding in patients with advanced chronic liver disease.[8-11] Some of these documents even advise not using such tests prior to invasive procedures. However, this advice is ignored in clinical practice for 2 important reasons. First, routine diagnostic tests of hemostasis are useful indicators of severity of liver disease. The prothrombin time (PT) or international normalized ratio (INR) are part of clinical scores for the severity of liver diseases such as the Child-Pugh and model of end-stage liver disease scores, as they are indicators of the synthetic capacity of the liver. The platelet count is a well-established indicator of severity of portal hypertension,[12] which relates to the decrease in platelet count caused by portal hypertension–related splenomegaly, which itself increases platelet sequestration in the spleen. The PT/INR and platelet count are thus helpful in staging patients with liver disease, and longitudinal assessment of these tests can be used to monitor disease progression. Second, it is commonly perceived that baseline PT/INR values and platelet counts are useful to guide hemostatic management in case bleeding occurs.

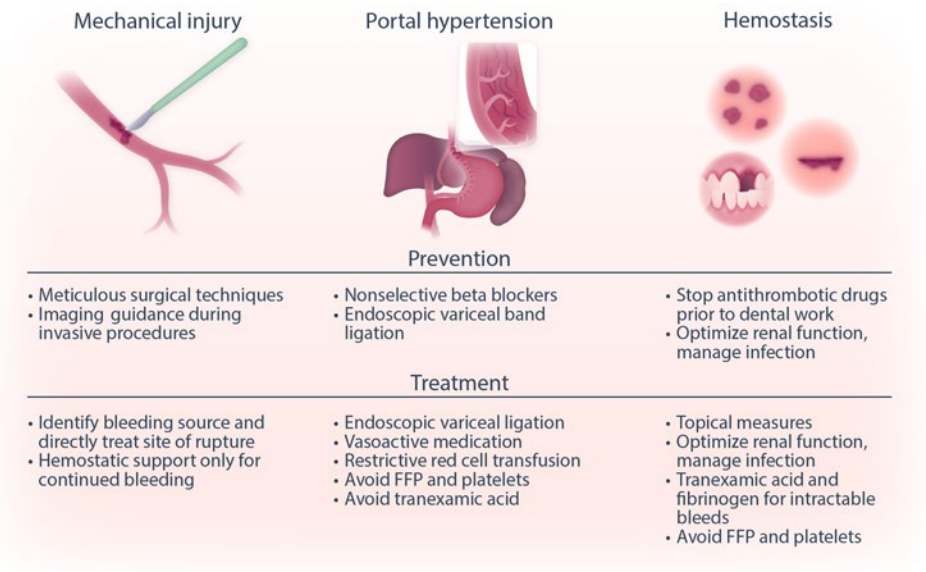

Figure 2. Categories of bleeding in liver disease. Bleeding in patients with liver disease may be due to mechanical injury (by inadvertent laceration of a vessel during surgery or a minor invasive procedure), portal hypertension-related causes (eg, variceal bleeding), or hemostatic failure (bleeding following dental extraction, bruising, and bleeding from puncture wounds). Shown are strategies to prevent and treat these different types of bleeding complications. Reprinted from Lisman with permission.[47] FFP, fresh frozen plasma.

Thus, hemostatic testing is commonly used to help assess severity of liver disease and to establish baseline values in case patients bleed during follow-up. In the presented case, however, there is concern that the abnormal INR and platelet count indicate a bleeding risk, even though there is generous evidence that these tests are unsuitable to assess the hemostatic status in patients with liver disease. Although the concept of rebalanced hemostasis proposes that patients with liver diseases, even those that are critically ill,[13] remain in hemostatic balance, there may be conditions in which the hemostatic balance in individual patients becomes hypocoagulable.[14-16] Such conditions include acute kidney injury, infection, and development of acute-on-chronic liver failure.[17] There is very limited evidence that, in these cases, the development of a net hypocoagulable state increases the risk for hemostasis-related bleeding.[16,18]

In this paper, I will discuss how routine diagnostic tests of hemostasis should be interpreted in patients with liver disease. In addition, I will examine whether more advanced hemostatic tests are helpful in assessing hemostatic capacity in these patients.

What do routine tests of hemostasis in patients with severe liver disease tell us?

This section will discuss the interpretation of routine diagnostic hemostasis tests in patients with liver disease, their limitations, and the relation to bleeding. Table 1 summarizes key aspects of this section.

PT/INR

The PT is a functional coagulation test that assesses the functional activity of coagulation factors V, VII, X, prothrombin, and fibrinogen. The PT or INR will thus be prolonged when there are qualitative or quantitative defects in one or more of these 5 procoagulant proteins. This test thus is useful to diagnose isolated

Table 1. Summary of how routine diagnostic hemostasis tests should be interpreted in liver disease patients

Laboratory test	Sensitive for	Does not assess	Utility in liver disease
PT/INR	Factors VII, X, V; prothrombin, and fibrinogen	Other procoagulant factors and anticoagulant proteins	Reflects synthetic capacity of the liver but has no relation to hemostatic status or bleeding
APTT	Prekallikrein; high-molecular weight kininogen; factors XII, XI, IX, VIII, X, and V; prothrombin; and fibrinogen	Factor VII and anticoagulant proteins	Has no relation to hemostatic status or bleeding
Fibrinogen	Fibrinogen level and function	Polymerisation defects due to increased sialic acid content, altered physical properties of the fibrin clot	Relation between low fibrinogen and bleeding in liver disease unclear
Platelet count	Platelet number	Compensation of thrombocytopenia by high VWF levels	Reflects severity of portal hypertension; relation between low platelet count and bleeding risk debated

APTT, activated partial thromboplastin time; INR, international normalized ratio; PT, prothrombin time; VWF, von Willebrand factor.

or combined deficiencies or defects in these coagulation factors, which, for example, include hereditary factor deficiencies or acquired defects related to vitamin K antagonist use.

Crucially, the PT is insensitive for variations in anticoagulant proteins, which include tissue factor pathway inhibitor, proteins C and S, and antithrombin. Per definition, therefore, the PT cannot be used to assess global hemostatic status. In patients with advanced liver disease, a prolonged PT is a useful indicator of hepatic synthetic function but not of global hemostatic capacity due to the simultaneous decline in procoagulant and anticoagulant proteins in these patients.[19] Indeed, a recent meta-analysis, which included 29 studies with a total of 13 276 patients, demonstrated no relation between the PT/INR and procedural bleeding risk in patients with cirrhosis.[20]

Activated partial thromboplastin time

Similar to the PT, the activated partial thromboplastin time (APTT) is sensitive for a discrete number of procoagulant proteins (prekallikrein; high-molecular weight kininogen; factors XII, XI, IX, VIII, X, V; prothrombin; and fibrinogen), and a prolongation of the APTT will signal a qualitative or quantitative defect in either of these factors. Notably, an isolated deficiency of 1 of the contact factors (prekallikrein, high-molecular weight kininogen, factor XII) is not associated with clinical bleeding, even though the APTT is prolonged by these deficiencies. In patients with advanced liver disease, the APTT is commonly prolonged, but not as severely as the PT. High levels of coagulation factor VIII (FVIII) are the reason the APTT is only mildly prolonged. Unlike most coagulation proteins that are synthesized in hepatocytes, FVIII is synthesized in both intrahepatic and extrahepatic endothelial cells.[21] Chronic endothelial cell activation with highly elevated plasma levels of the FVIII carrier protein von Willebrand factor (VWF) result in elevated FVIII levels in patients with liver disease. Like the PT, the APTT is insensitive to anticoagulant proteins and therefore does not adequately represent hemostatic capacity in patients with liver disease; hence, the APTT should not be used to assess bleeding risk.

Fibrinogen

Fibrinogen levels vary widely in patients with liver disease. In mild liver disease, fibrinogen levels may be increased,[22] which likely relates to the fact that fibrinogen is an acute phase protein. In more advanced liver disease, fibrinogen levels decrease on average, but the range of fibrinogen levels is much wider than in healthy individuals.[23] In patients with advanced cirrhosis, a subset has normal fibrinogen levels, whereas other patients have fibrinogen levels that are clearly below reference ranges. It is, however, unusual to find fibrinogen levels lower than 1 g/L.

It has been argued that low fibrinogen levels in critically ill patients with cirrhosis contribute to clinical bleeding.[24] However, although there is a relation between fibrinogen levels and bleeding risk, this relation may be indirect. Most cases of bleeding in critically ill cirrhosis patients are related to portal hypertension,[25] and the relation between low fibrinogen and portal hypertensive bleeds may thus reflect a relation between severity of disease and bleeding rather than a relation between hemostatic failure and bleeding. Indeed, a recent retrospective study demonstrated that (prophylactic) administration of cryoprecipitate with the aim to increase plasma fibrinogen levels did not reduce bleeding or mortality in critically ill cirrhosis patients.[26] It is therefore questionable whether low fibrinogen levels in patients with cirrhosis signal a clinically relevant hemostatic defect. Importantly, it has been demonstrated that the quality of fibrin clots in patients with cirrhosis is normal to thrombogenic, despite reduced fibrinogen plasma levels.[27] Thrombogenic posttranslational modifications in the fibrinogen molecule, notably oxidation, have been proposed to promote thrombogenicity of fibrin clots in these patients.[27]

Platelet count

Thrombocytopenia is a common finding in patients with cirrhosis, but it is unusual for the platelet count to drop below 50 000/μL, except in patients who are critically ill. Whether thrombocytopenia in these patients is accompanied by a platelet function defect is unclear: some studies find platelet function defects,[28] whereas others find hyperfunctional platelets in cirrhosis.[29,30]

Although thrombocytopenia in the absence of an underlying liver disease may be associated with a bleeding risk, the situation is more complex in patients with cirrhosis. Laboratory studies have demonstrated that the highly elevated levels of VWF in cirrhosis patients compensate for the low platelet count.[2]

It has been argued that a low platelet count reduces thrombin generation in patients with cirrhosis.[31] However, since the thrombin-generating capacity in platelet-poor plasma is preserved or even hypercoagulable, and since platelets are not the only cellular surfaces that can promote thrombin generation, this conclusion may be too simplified.

Whether a low platelet count is associated with a bleeding risk in patients with cirrhosis has been controversial: some studies report a clear association,[24] whereas other studies do not find an increased bleeding risk with decreased platelet count.[32]

Pros and cons of global hemostasis tests in patients with severe liver disease

The repertoire of diagnostic or research-type hemostasis tests is much larger than the PT/APTT/fibrinogen/platelet count test panel. Global tests of hemostasis are increasingly used to assess hemostasis or guide clinical management in patients with isolated or complex hemostatic disorders. Such tests include whole blood viscoelastic tests, platelet function tests, thrombin generation tests, and plasma-based fibrinolysis tests. Opportunities and caveats of these tests will be discussed in this section. Findings are summarized in Table 2.

Whole blood viscoelastic tests

Whole blood viscoelastic tests, such as thromboelastography (TEG) and rotational thromboelastography (ROTEM), and variants, such as the ClotPro device and the Quantra, are gaining popularity as rapid point-of-care devices to assess hemostasis in patients with bleeding related to trauma, childbirth, liver disease, and other medical situations associated with bleeding. In liver disease, multiple studies reported the use of kaolin-induced TEG,[33,34] while for ROTEM, multiple studies report a panel of EXTEM, INTEM, FIBTEM, and APTEM.[35,36] The advantage of these techniques is that clot formation is assessed in whole blood and thus interactions between cellular components and the plasma environment are taken into account. Viscoelastic tests in patients with liver disease give a more accurate representation of hemostatic status than routine diagnostic tests such as the PT

Table 2. Summary of how global diagnostic or research-type hemostasis tests should be interpreted in liver disease patients

Laboratory test	Sensitive for	Does not assess	Utility in liver disease
Whole blood viscoelastic tests	Blood cells, coagulation proteins, fibrinolytic proteins.	VWF levels, protein C system, platelet function defects, role of flow in clot formation.	Better representation of hemostatic capacity compared to routine diagnostic tests, reduces blood product use, likely underestimates hemostatic capacity.
Platelet function tests	Platelet function, most tests also for platelet count.	Interplay between coagulation and platelet activation, other blood cells, role of flow (except for PFA-100/200).	Most platelet function tests are not helpful because they are not only sensitive for platelet function but also for platelet count. Flow cytometry–based analyses may have merit in a research setting.
Thrombin generation tests	All procoagulant and anticoagulant factors, provided thrombomodulin or another protein C activator is added to the reaction mixture. Platelet count when performed in platelet-rich plasma.	Role of blood cells and endothelial cells in supporting thrombin generation, role of flow.	Promising indicator of coagulation capacity—not yet ready for clinical use.
Plasma-based fibrinolysis tests	All fibrinolytic proteins except tissue-type plasminogen activator.	Role of blood cells in fibrinolysis.	

PFA, platelet function analyzer; VWF, von Willebrand factor.

and platelet count. When prophylactic transfusion management of patients with liver disease was guided by viscoelastic tests instead of routine diagnostic tests of hemostasis, a dramatic decrease in blood component transfusions was observed in multiple studies.[37]

Viscoelastic tests have 3 drawbacks that need to be taken into account when interpreting test results in patients with liver diseases. First, the platelet count is a major determinant of clot formation in these assays. However, viscoelastic tests are insensitive for plasma levels of VWF, and the potential compensation of thrombocytopenia by elevated VWF in liver disease patients is not taken into account, leading to an underestimation of hemostatic capacity.[38] Second, viscoelastic tests are insensitive for plasma levels of the protein C system, and the compensation of low levels of procoagulant proteins by low levels of protein C and S is not taken into account, again resulting in an underestimation of true hemostatic potential.[38] Finally, the strength of the initiator of clot formation may alter the interpretation of viscoelastic test results. For example, in patients with liver disease, TEG test results are more often within normal ranges compared to ROTEM test results, which are frequently hypocoagulable.[39,40] This likely relates to the difference in composition of the reagents between tests that lead to slower clot initiation in TEG. This slower clot initiation better allows anticoagulant mechanisms to control coagulation.

Platelet function tests

Platelet function tests are notoriously difficult in patients with liver diseases, as most platelet function tests are strongly influenced by platelet count, which is often decreased in patients with liver disease. In recent reports, researchers have studied ratios of platelet function test results with the platelet count,[30] but it is questionable whether there is a truly linear relation between platelet count and platelet function test results.[41] Flow cytometry–based platelet function tests are independent of platelet count, but such tests as not yet suitable for use in routine diagnostics.

Thrombin generation tests

The use of thrombin generation tests has revolutionized the assessment of hemostatic status in patients with liver disease. Tripodi and coworkers were the first to report preserved thrombin generating capacity in patients with cirrhosis by using thrombomodulin-modified thrombin generation tests.[42] These tests accurately assess the balance between procoagulant and anticoagulant proteins and, in my opinion, these are the best tests currently available to assess plasma coagulation potential. Importantly, thrombin generation tests have been automated, and the Genesia device appears suitable for use in specialized diagnostic laboratories.[43] The obvious limitations of the thrombin generation test are the lack of cellular components and the fact that thrombin generation, not clot formation, is the endpoint of the test. Thus, thrombin generation tests should always be interpreted in the context of tests assessing other aspects of hemostasis, such as platelet function, clot formation, and clot stability. Whole blood thrombin generation tests are in development, and initial results in patients with liver disease have been reported.[44]

Plasma-based fibrinolysis tests

Historically, liver diseases were considered to be associated with a hyperfibrinolytic state. More modern laboratory tests and clinical observations have challenged this dogma. There is a plasma-based fibrinolysis assay in which clot formation is initiated by tissue factor, lysis is induced by tissue-type plasminogen activator, and turbidity measurements are used to monitor clot formation and fibrinolysis.[45] This particular assay has been extensively used to profile the fibrinolytic system in patients with liver diseases, and, importantly, has been extensively validated in the context of thrombotic disease.[45] Fibrinolytic test results measured with this assay vary widely: normal fibrinolytic capacity is seen in compensated cirrhosis; hyperfibrinolysis, in decompensated patients; and hypofibrinolysis, in critically ill patients with acute-on-chronic liver failure and sepsis or acute liver failure.[7] Although a hypofibrinolytic state identified by this test correlates with

risk of venous thrombosis, the test is less well characterized in patients with bleeding. The lack of cellular components in the test needs to be taken into account when interpreting the results.

Back to our case

A variety of clinical and research-type laboratory tests are available to assess hemostatic status in patients with liver diseases. However, for most tests, the interpretation of results is not straightforward. The average patient with liver disease appears in hemostatic balance, but specific hypocoagulable and hypercoagulable features can be identified. Which laboratory characteristics predict hemostasis-related bleeding is uncertain, and, as most spontaneous or procedure-related bleeds in patients with liver disease are unrelated to hemostatic failure, prophylactic hemostatic interventions may not be useful for the majority of patients. Importantly, recent clinical guidance documents argue against routine evaluation of hemostasis using routine diagnostic tests (PT/INR, APTT, platelet count, and fibrinogen) to predict bleeding risk prior to procedural intervention in patients with cirrhosis, even in those who are critically ill.[10,11] Whether prohemostatic therapy is indicated in patients with extreme alterations in their hemostatic system is uncertain. One study identified APTT values >100 sec, a platelet count <30 000/μL, and fibrinogen levels <0.6 g/L as predictors of bleeding in critically ill cirrhosis patients.[24] Whether this association is causal or whether these very abnormal laboratory tests simply signal very severe liver disease is uncertain. Future studies should identify which patients may benefit from hemostatic interventions and which laboratory studies should be used to identify these patients.

Conflict-of-interest disclosure

Ton Lisman: no competing financial interests to declare.

Off-label drug use

Ton Lisman: nothing to disclose.

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How to manage hemostasis in patients with liver disease during interventions

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Patients with advanced chronic liver disease (CLD) often need procedures to both treat and prevent complications of portal hypertension such as ascites or gastrointestinal bleeding. Abnormal results for hemostatic tests, such as prolonged prothrombin time, international normalized ratio, and/or thrombocytopenia, are commonly encountered, raising concerns about increased bleeding risk and leading to transfusion to attempt to correct prior to interventions. However hemostatic markers are poor predictors of bleeding risk in CLD, and routine correction, particularly with fresh frozen plasma and routine platelet transfusions, should be avoided. This narrative review discusses the hemostatic management of patients with CLD using 2 case descriptions.

LEARNING OBJECTIVES

- To evaluate periprocedural bleeding risk in patients with liver disease
- To understand the role and limitations of prophylactic hemostatic interventions in the periprocedural setting

CLINICAL CASE 1

A 38-year-old woman attends a routine hepatology follow-up for known nonalcoholic steatohepatitis with unexplained jaundice. This has developed over the last few weeks and is associated with significant fatigue. Her blood tests reveal bilirubin 182 µmol/L, albumin 32 g/L, sodium 141 mmol/L, creatinine 89 µmol/L, hemoglobin 110 g/L, platelets 57×10^9/L, prothrombin time (PT) 24 s (international normalized ratio [INR] 2.4), activated partial thromboplastin time (APTT) 70 s, and Clauss fibrinogen 0.8 g/dL. Imaging confirms fatty liver disease but without definite features of cirrhosis. There is no evidence of portal hypertension, with spleen measuring 9.8 cm. A diagnostic liver biopsy is planned, and there is concern regarding the bleeding risk in view of her abnormal hemostatic test results.

Procedures are frequently required in patients with liver disease, and concern for bleeding arises both from the perception that chronic liver disease (CLD) constitutes a bleeding diathesis and that hemostatic parameters are commonly abnormal in patients with CLD. Reduced hepatic synthesis of hemostatic proteins along with portal hypertension and consequent hypersplenism commonly leads to prolonged PT, APTT, and thrombocytopenia in patients with CLD. While patients with CLD have a higher baseline risk of bleeding, this risk is largely mediated by portal hypertension. For example, in the PROLIVER study, of 280 patients (53% Child Pugh A) with CLD, 3.6% developed major bleeding per year, with 1.9% per year experiencing clinically relevant nonmajor bleeding.[1] Of note, 91% of major bleeds were secondary to portal hypertension. Patients with acute decompensation or acute-on-chronic liver failure (ACLF) are reported to have higher rates of bleeding, and this remains predominantly mediated by portal hypertension. Drolz et al retrospectively reviewed bleeding in 211 patients with cirrhosis admitted to critical care and reported 17% had bleeding events.[2] Bleeding was most commonly secondary to varices (5.9%), with 10 patients experiencing postprocedural/postoperative bleeding. Ow et al. reported 13% later bleeds (>48 h post admission) in a retrospective cohort of 623 consecutive patients with CLD admitted to critical care.[3] Only 10 bleeds were related to procedures (ie, only 1.6% developed major bleeding related to procedures). This is of note because critically ill patients are frequently subject to multiple procedures over the course of their admission.

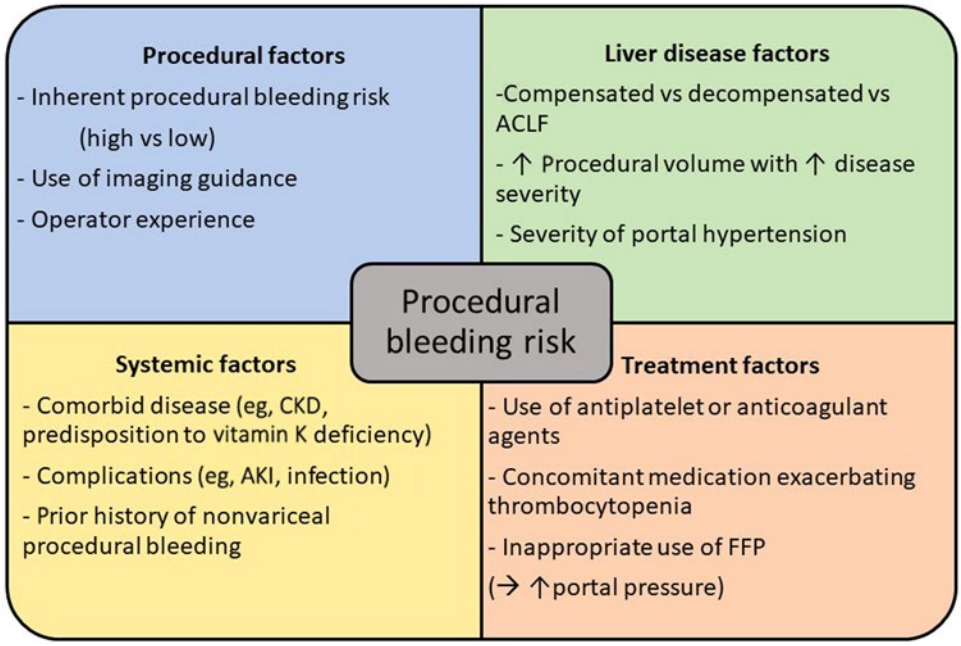

Figure 1. Factors which modulate bleeding risk in patients with chronic liver disease in the periprocedural setting. ACLF, acute-on-chronic liver failure; AKI, acute kidney injury; CKD, chronic kidney disease; FFP, fresh frozen plasma.

There is significant agreement in the recommended approach to periprocedural hemostatic management of patients with liver disease among recently published guidelines.[4-8] All guidelines advocate considering the procedural bleeding risk and patient-specific bleeding risk factors. There is also agreement on avoiding fresh frozen plasma (FFP) and other blood components to correct prolonged PT/INR. The need for correction of thrombocytopenia and/or hypofibrinogenemia is less certain. The rationale for guidance recommendations is further presented in this manuscript. Factors contributing to procedural bleeding risk are summarized in Figure 1.

Procedural bleeding risk

Estimation of procedural bleeding risk for commonly performed procedures in patients with liver disease is largely derived from observational cohorts with variable use of prohemostatic therapy. Recent guidance categorizes procedures as low bleeding risk, for which the major bleeding rate is <1.5%, or high bleeding risk, for which the rate exceeds 1.5% or there is potential for uncontrolled bleeding or severe consequences (eg, organ failure or death).[4-6] There is a need for data from large observational studies without empiric blood component support to accurately evaluate procedural bleeding risk; one such study is the PROCBLeeD, the results of which are awaited (www.clinicaltrials.gov/ct2/show/NCT04076605). The suggested classification of bleeding risk for commonly performed procedures proposed by International Society of Thrombosis and Haemostasis is presented in Table 1. There is some variation in risk stratification between guidance documents; for example, the American Association for the Study of Liver Disease considers both transjugular and percutaneous approaches to liver biopsy to be high bleeding risk procedures, while the European Association of Liver Disease (EASL) considers both approaches to be low bleeding risk procedures, despite comparable definitions for bleeding risk. A recent observational cohort of 302 patients requiring diagnostic liver biopsy reported a low rate of major bleeding (0.6%), with no deaths and only 2 patients requiring vascular embolization to control bleeding.[9] This study supports the EASL classification of both transjugular and percutaneous liver biopsy as low bleeding risk procedures.

Patient-specific factors for periprocedural bleeding risk

In patients with established cirrhosis, the severity of liver synthetic failure and/or portal hypertension may modulate bleeding risk. As highlighted above, the risk of bleeding is higher in critically ill patients, largely due to increased variceal bleeding. Procedural bleeds remain infrequent in these patients. Whilst procedural liver synthetic failure leads to profound changes on routine laboratory testing, with prolonged PT, prolonged INR, and/or thrombocytopenia, as reviewed in detail by Lisman, these parameters poorly predict bleeding tendency.[10] Data demonstrating this lack of association has been available for over 40 years; Ewe reported no association between PT and liver bleeding time for liver biopsies performed under direct laparoscopic vision in 1981.[11] In the more recent prospective observational study, detailed hemostatic profiling took place, including a bleeding questionnaire, evaluation of individual coagulation factors, platelet count, platelet function (measured with PFA-100), thromboelastometry (TEM), thrombin generation, and clot lysis assays; the authors found no association between heemostatic profile and hematoma formation on planned postprocedural ultrasound.[9] A small prospective study of patients with decompensated cirrhosis (n=72) found an association between maximum amplitude <30 mm measured with thromboelastography (TEG) and major bleeding (n=3).[12] Clauss fibrinogen and functional measures of fibrinogen

Table 1. Procedural bleeding risk classification of commonly performed procedures in patients with advanced chronic liver disease from the International Society of Thrombosis and Haemostasis Scientific Subcommittee

	Low bleeding risk	High bleeding risk*
Endoscopic	Diagnostic procedures Endoscopic variceal ligation Transesophageal echocardiogram	Bronchoscopy with biopsy Colonoscopy with polypectomy Endoscopic retrograde cholangiopancreatography with sphincterotomy
Percutaneous	Paracentesis Thoracocentesis	Percutaneous liver biopsy Tunneled ascitic/pleural drain placement Cranial/spinal surgery#
Vascular	Peripheral/central venous catheterization Transjugular liver biopsy	Transjugular intrahepatic portosystemic shunt Transcatheter arterial chemoembolization
Other	Dental procedures including extractions Skin biopsy	Intraocular procedures#

*Classification based on major bleeding >1.5% or on minor bleeding associated with high risk of significant organ damage/death.

#Very high-risk procedures.

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were not measured, but there was no significant difference in platelet count between patients who bled and those who did not. Low fibrinogen has been reported inconsistently as associated with bleeding in observational studies, and it is possible this is simply another marker of severity of liver disease rather than a risk factor for bleeding.[13,14] While there are data to suggest viscoelastic hemostatic assays (eg, TEG/TEM) can reduce inappropriate blood component transfusion,[15] the single study incorporating a no-intervention arm demonstrated that the 'no-intervention' approach was the most cost-effective without compromising safety in low bleeding risk procedures.[16] Further limitations of TEG/TEM include the lack of sensitivity to protein C and von Willebrand Factor, the lack of validated thresholds to guide transfusion, and the lack of clinically meaningful endpoints in published studies to date.[17] Further evaluation of Clauss fibrinogen and TEG/TEM, including functional fibrinogen in larger prospective cohorts, are required to confirm any potential clinical utility as a predictor of bleeding.

Acute kidney injury (AKI) commonly complicates acute decompensation of CLD, affecting up to a third of patients admitted to hospital, and is reported to be associated with an increased risk of procedural bleeding.[18,19] Acute kidney injury is associated with platelet dysfunction, reduced factor XIII, and endothelial dysfunction, which may all contribute to an increased bleeding risk.[20] Active infection/sepsis is thought to increase risk of bleeding based on published reports of a heparin-like effect, platelet dysfunction, and/or hyperfibrinolysis.[20,21] However, some patients exhibit hypofibrinolysis and/or hypercoagulability.[22] In more recent clinical studies of acutely ill patients with cirrhosis, there are conflicting data on the relationship between AKI, sepsis, and bleeding risk.[2,3,12] In acutely ill patients with cirrhosis, particularly those with sepsis, alternate causes for deterioration in hemostatic parameters (thrombocytopenia and hypofibrinogenemia) should be considered.

Just as in patients without cirrhosis, the use of antiplatelet and anticoagulant agents may increase the risk of periprocedural bleeding. Standard approaches to safe interruption of these agents should be adopted and based on the procedural bleeding risk and the individual's thrombotic risk.[23,24]

Prohemostatic therapies to address abnormal hemostatic parameters

Fresh frozen plasma

Fresh frozen plasma is often considered (and used) in the periprocedural setting in attempts to correct the prolonged PT/INR.[25,26] However, as discussed earlier in this article (and in detail by Lisman[10]), the PT/INR does not reflect the complex hemostatic changes in liver disease and does not predict procedural bleeding. Laboratory studies clearly demonstrate that administration of therapeutic doses of FFP (10-20 mL/kg) do not improve the INR (with few patients achieving an INR <1.5), and that FFP administration increases hypercoagulability measured by thrombin generation (Figure 2)[27,28] and markers of *in vivo* activation of coagulation.[28] Additionally, there is evidence that FFP can be harmful, as it associated with significant risks of both transfusion-associated acute lung injury and circulatory overload.[29,30] This risk is further illustrated by the demonstrated association between increased portal pressures and FFP administration, which may paradoxically increase the potential for harm of bleeding (eg, in endoscopic variceal band ligation).[31] This association is supported by retrospective data from a multicenter study of variceal bleeding management that found increased mortality and 5-day rebleed risk in patients receiving FFP.[32]

Prothrombin complex concentrate and recombinant factor VIIa

Both prothrombin complex concentrate (PCC) and recombinant factor VIIa (rFVIIa) may appear to be attractive alternatives to FFP, particularly as smaller administration volumes avoid the risk of circulatory overload. A systematic review identified low-quality data to suggest the efficacy of PCC in correcting PT/INR compared with FFP.[33] However, PCC use did not result in fewer bleeding events than FFP use; furthermore, thromboembolic events were associated only with PCC. *In vitro* spiking experiments in plasma from patients with CLD demonstrate a stronger prothrombotic effect of PCC than of FPP on thrombin generating capacity, and this effect increased in parallel with progressive liver disease severity.[34] A recent small, retrospective multicen-

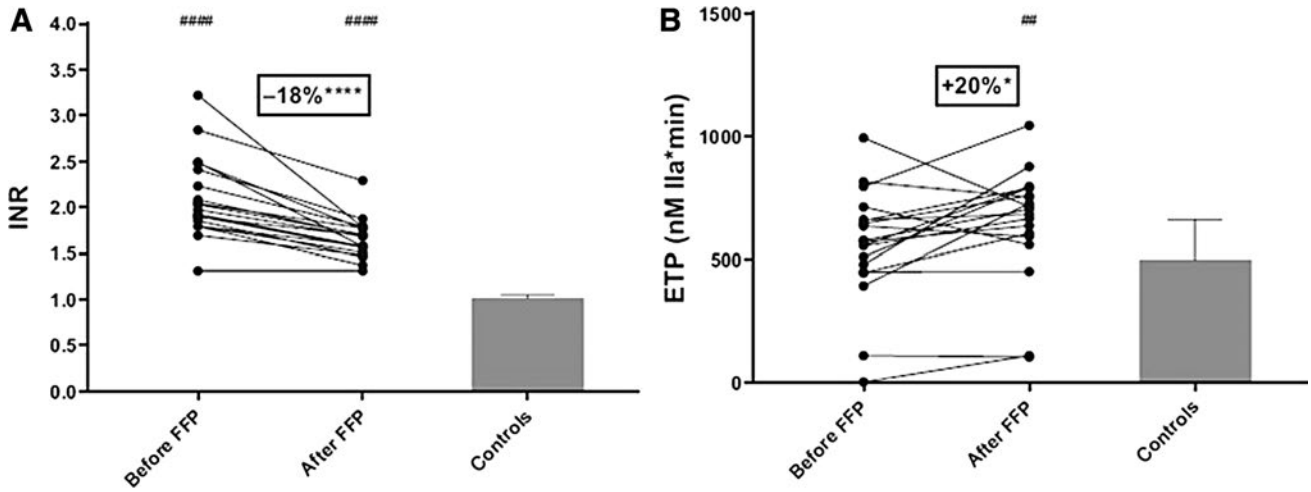

Figure 2. Therapeutic doses of fresh frozen plasma (FFP) on international normalized ratio (INR) and endogenous thrombin potential (ETP). (A) INR and (B) ETP measured in the presence of thrombomodulin in 19 patients with chronic liver disease and a prolonged INR before and after FFP administration, compared with controls (*n*=20). Bars represent median±interquartile ranges. **P*<.05. *****P*<.0001, before vs after FFP transfusion. ##*P*<.01, patients vs controls. ####*P*<.0001, patients vs controls. Adapted from with permission from Elsevier Inc.[28]

ter observational study highlighted significant deterioration in hemostatic markers in a proportion of patients receiving PCC, particularly those with ACLF, raising concerns that its use might precipitate disseminated intravascular coagulation.[35] rFVIIa has been evaluated in randomized controlled trials in patients with CLD and variceal bleeding and had no impact on outcomes.[36,37] Similarly, studies in liver transplantation found that rFVIIa had no impact on clinical outcomes or volume of red cell transfusion.[38,39] Additionally, *in vitro* laboratory data demonstrate only a minor effect on increasing thrombin generating capacity.[34] Neither PCC nor rFVIIa should be used for prevention of bleeding in the periprocedural setting.

Cryoprecipitate/fibrinogen concentrate

There are limited data on the role of fibrinogen supplementation in the periprocedural setting. Laboratory data demonstrate interindividual variation, with some individuals exhibiting increased fibrin clot thrombogenicity despite low fibrinogen levels.[40] In a retrospective cohort of critically ill patients with CLD and hypofibrinogenemia, there was no association between reduced fibrinogen and death, and cryoprecipitate use was not associated with reduced bleeding or improved survival.[41] Retrospective observational cohorts of critically ill patients and liver transplantation suggest an association between cryoprecipitate use and venous thromboembolism.[3,42] There is currently insufficient evidence to support the use of cryoprecitipate/fibrinogen concentrate to prevent periprocedural bleeding.

Vitamin K

Vitamin K may be considered for patients with coexisting risk factors for deficiency, such as advanced malnutrition or cholestasis.[6] However, routine use in patients with CLD and a prolonged PT/INR is not warranted, as high doses of vitamin K are ineffective in reducing INR in patients with cirrhosis.[43]

Tranexamic acid

There are no high-quality data for tranexamic acid as prophylaxis against procedural bleeding in patients with CLD. The HALT-IT randomized controlled trial of tranexamic acid versus placebo in patients with gastrointestinal bleeding found no survival benefit or reduction in transfusion requirements; furthermore, the authors reported an increased risk of venous thromboembolism.[44] Therefore, tranexamic acid should not be routinely used to reduce the risk of bleeding in the periprocedural setting.

CLINICAL CASE 1 (continued)

In our case, aside from the abnormal hemostatic markers and potential severity of CLD (if confirmed), there were no additional patient-related factors modulating procedural bleeding risk. A transjugular approach to liver biopsy was planned with an experienced operator. No prohemostatic therapy was offered prior to procedure, and the biopsy was performed without complication. A diagnosis of cirrhosis was confirmed, and she proceeded to assessment for liver transplantation.

CLINICAL CASE 2

A 70-year-old woman with stable (Child Pugh A) cirrhosis secondary to previous alcohol misuse is under surveillance for a lung nodule. Recent imaging reveals a significant increase in nodule size, with a positron emission tomogram demonstrating moderate fluorodeoxyglucose uptake. There is concern for underlying malignancy, and a computed tomography–guided percutaneous biopsy is planned. Her full blood count reveals hemoglobin 108 g/L, white cell count 3.44×10^9/L, and platelets

32×10⁹/L. Her liver and renal function are normal, as is PT/INR. There is concern about performing the biopsy due to thrombocytopenia (fluctuating from 25–40×10⁹/L over the last year). The proceduralist requests a platelet count of 50×10⁹/L prior to proceeding.

Percutaneous lung biopsy is considered a high bleeding risk procedure, as excess bleeding will be difficult to control with potentially serious consequences. As discussed earlier in this article (and in detail in Lisman[10]), there is a lack of consistent evidence that thrombocytopenia predicts bleeding in patients with CLD and inadequate evidence to inform a specific platelet threshold for procedural interventions. In a prospective observational study of 363 patients undergoing 852 procedures, platelet count was not associated with an increased risk of procedural bleeding.[45] Thrombocytopenia is increasingly reported to reflect severity of portal hypertension rather than bleeding risk. In the aforementioned 211 critically ill patients with CLD, platelet count of <30×10⁹/L predicted an increased risk of (predominantly gastrointestinal) bleeding.[2] It should be noted that thrombocytopenia with a platelet count of <30×10⁹/L is infrequently seen in patients with CLD, and alternate contributing causes should be considered.[6] This might include addressing a potential immune component (particularly in patients with autoimmune or viral hepatitis), medication review for agents that can cause thrombocytopenia, and treating for underlying hematinic deficiency.[6]

The European Association of Liver Disease recommends against routine use of platelet concentrates or thrombopoietin receptor agonists, but with consideration on a case-by-case basis in patients with platelet counts of <50×10⁹/L, in the context of a high bleeding risk procedure in which bleeding would be difficult to control.

Platelet transfusion

Platelet transfusions are commonly used in attempts to increase the platelet count in the periprocedural setting in this patient group.[25,26] There are conflicting data as to whether thrombocytopenia increases bleeding risk in the periprocedural setting[1,2,12,45]; the degree of uncertainty is due to data quality and indiscriminate use of platelet transfusion in earlier observational series. It is important to recognize that in patients with CLD and hypersplenism, platelet transfusion often has minimal impact on raising the platelet count, may have a prothrombotic effect,[28] and exposes the patient to risks of receiving transfusions.[29]

Thrombopoietin receptor agonists

Lusutrombopag and avatrombopag have been evaluated in randomized controlled trials of patients with CLD and thrombocytopenia undergoing procedural intervention and are now licensed for this indication. Recommended dosing schedules are shown in Table 2. Both agents were found to significantly increase platelet count and reduce platelet transfusion compared with placebo. However, there are important limitations to these studies: 1) the importance of the primary endpoint (platelet count) is uncertain and 2) participants predominantly underwent procedures with a low bleeding risk. Therefore, the role of thrombopoietin receptor agonists in reducing clinically relevant bleeding remains uncertain.[46,47] Also of note, an earlier study of eltrombopag in this setting was terminated due to safety concerns with increased portal vein thrombosis in the eltrombopag arm. This finding may have been confounded by lack of pretreatment imaging to exclude preexisting portal vein thrombosis.[48] Portal vein thrombosis was not increased in the studies of lusutrombopag or avatrombopag, in which participants were required to have abdominal imaging confirming the absence of portal vein thrombosis (+/− reduced portal venous flow, <10 cm/s) 2 to 4 weeks prior to randomization.[49,50]

CLINICAL CASE 2 (continued)

The proceduralist did not feel an expectant approach was appropriate. There were no alternate causes/contributors to thrombocytopenia. The patient had previously received a platelet transfusion prior to a high risk bleeding procedure with no improvement in platelet count. She was treated with lusutrombopag (3 mg daily for 1 week) and had a good response; the platelet count was 89×10⁹/L on the day of percutaneous biopsy. There were no bleeding complications.

Clinical practice guidelines and future research

Recent clinical practice guideline recommendations for management of hemostasis in the periprocedural setting are summarized in Table 3. There is agreement across major societal guidance bodies from hematology, hepatology, and interventional radiology that FFP should not be used to correct prolonged PT/INR prior to procedures irrespective of procedural bleeding risk, nor

Table 2. Licensed dosing for thrombopoietin-agonists for patients with cirrhosis and platelet count <50×10⁹/L prior to invasive procedures

TPO-R agonist	Baseline platelet count* (×10⁹/L)	Dose	Procedure date#
Lusutrombopag	<50	3 mg once daily for 7 days	Day 9–14
Avatrombopag	<40	60 mg once daily for 5 days	Day 10–13
Avatrombopag	40–49	40 mg once daily for 5 days	Day 10–13

TPO-R agonist, thrombopoietin-receptor agonist.

*If platelet count <30×10⁹/L, consider and correct other causes of thrombocytopenia.

#Number of days following initiation of TPO-R agonist.

Table 3. Comparison of selected major societal guideline recommendations for correction of hemostatic parameters prior to high bleeding risk procedures

	BSIR 2022[8]	EASL 2022[5]	ISTH 2021[6]	AGA 2021[7]	AASLD 2020[4]	SIR 2019[51]
PT/INR	Do not correct	Do not correct	Do not evaluate	Do not correct	Do not correct	INR >2.5*
Platelets	Consider if <50×10⁹/L	Case by case for <50×10⁹/L	Do not correct†	Do not correct	Do not correct	>30×10⁹/L
Fibrinogen	Consider if <1.2 g/l	Correction discouraged	Do not evaluate	No recommendation	Do not correct	>1 g/L

AASLD, American Association for the Study of Liver Disease; AGA, American Gastroenterology Association; EASL, European Association for the Study of Liver Diseases; BSIR, British Society of Interventional Radiology; INR, international normalized ratio; ISTH, International Society of Thrombosis and Haemostasis; PT, prothrombin time; SIR, Society of Interventional Radiology.

*Give vitamin K if INR >2.5; do not use fresh frozen plasma or prothrombin complex concentrate.

†Consider correction prior to planned elective very-high-risk procedures.

Table 4. Research needs in periprocedural management of hemostasis as proposed by European Association for Study of the Liver[5]

Large prospective observational collaborative studies to • establish the incidence of procedural bleeding • evaluate associations between anemia +/− thrombocytopenia and procedural bleeding • assess relationship between fibrinogen deficiency and procedural bleeding • explore the impact of a hyperfibrinolytic state on procedural bleeding
Evaluate the role of viscoelastic tests in predicting procedural bleeding in patients undergoing high-risk procedures (including adequate numbers of patients with progressive disease severity [ie, compensated, acute decompensation, acute on chronic liver failure])
Randomized, placebo-controlled trials in patients with severe thrombocytopenia undergoing high-risk procedures, to assess the role of 1) platelet transfusions and 2) thrombopoietin-receptor agonists, with clinically significant bleeding as the primary endpoint
Clinical trials to evaluate antifibrinolytics in the periprocedural setting, particularly in patients with known/suspected hyperfibrinolysis

should thrombocytopenia be corrected prior to low bleeding risk procedures. There is less certainty regarding the need to correct thrombocytopenia prior to high bleeding risk procedures, and this is reflected by variation in the guideline recommendations.[4-8,51] Research recommendations proposed by EASL to address knowledge gaps in this domain are summarized in Table 4.

Conclusion

It is important to recognize that the majority of commonly performed procedures in patients with advanced CLD are associated with a low procedural bleeding risk. While prolonged PT/INR and/or thrombocytopenia are commonly encountered, they are not reliable indicators of hemostasis or procedural bleeding risk in this patient group. Accordingly, FFP and/or platelet transfusion should not be used routinely to prevent periprocedural bleeding in patients with ACLD.

Conflict-of-interest disclosure

Lara N. Roberts: no competing financial interests to declare.

Off-label drug use

Lara N. Roberts: Prothrombin complex concentrates, recombinant factor VIIa, and fibrinogen concentrate are not recommended in the periprocedual hemostatic management of patients with liver disease.

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How to manage splanchnic vein thrombosis in patients with liver disease

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Liver cirrhosis and splanchnic vein thrombosis (SVT) are strictly correlated. Portal vein thrombosis, the most common location of SVT, is frequently diagnosed in liver cirrhosis (pooled incidence 4.6 per 100 patient-years), and liver cirrhosis is a common risk factor for SVT (reported in 24%-28% of SVT patients). In cirrhosis-associated SVT, anticoagulant treatment reduces mortality rates, thrombosis extension, and major bleeding, and increases the rates of recanalization, compared to no treatment. Achieving vessel recanalization improves the prognosis of cirrhotic patients by reducing liver-related complications (such as variceal bleeding, ascites, hepatic encephalopathy). Anticoagulation should be therefore routinely prescribed to cirrhotic patients with acute SVT unless contraindicated by active bleeding associated with hemodynamic impairment or by excessively high bleeding risk. Of note, early treatment is associated with higher probability of achieving vessel recanalization. The standard treatment consists of low-molecular-weight heparin, followed by oral anticoagulants (eg, vitamin K antagonists or direct oral anticoagulants), if not contraindicated by severe liver dysfunction. Cirrhotic patients with SVT should be treated long-term (especially if candidate for liver transplantation) since liver cirrhosis is a persistent risk factor for recurrent thrombosis. In this review, we discuss the management of SVT in patients with liver cirrhosis, with a focus on the anticoagulant treatment in terms of indications, timing, drugs, duration, and particular scenarios, such as gastroesophageal varices and thrombocytopenia.

LEARNING OBJECTIVES

- To identify which cirrhotic patients with SVT need anticoagulant treatment
- To choose the most appropriate anticoagulant treatment for cirrhotic SVT

CLINICAL CASE

A 55-year-old man with known liver cirrhosis presented to the emergency department with hematemesis from variceal bleeding. Endoscopic band ligation of esophageal varices was performed, and the patient was started on a beta-blocker. A computed tomogram showed a complete thrombosis of the main trunk of the portal vein. Blood tests showed mild thrombocytopenia (95×10^9/L) and normal kidney function (estimated glomerular filtration rate, 51 mL/min). The patient has Child-Pugh class A. You considered whether to initiate anticoagulation in this patient or not.

Introduction

Splanchnic vein thrombosis (SVT) refers to thrombosis of different veins that drain blood from the abdominal organs and includes portal vein thrombosis (PVT), mesenteric vein thrombosis, splenic vein thrombosis, and obstruction of the hepatic venous outflow (also known as Budd-Chiari syndrome).[1]

Portal vein thrombosis is the most common location in the SVT spectrum, with a pooled incidence in the cirrhotic population without hepatocellular carcinoma or previous abdominal surgery of 4.6 (95% CI, 3.5-5.8) per 100 patient-years.[2] The risk of developing PVT increases in parallel with the progression of liver cirrhosis, showing a pooled prevalence of 13.5% (95% CI, 9.5-18.2) in patients with Child-Pugh A and 23.7% (95%CI, 16.8-31.5) in patients with Child-Pugh B/C.[2]

Liver cirrhosis is a strong risk factor for SVT and is found in approximately 24%-28% of SVT patients.[3,4] In patients with liver cirrhosis, predictive factors for PVT include those related to the severity of portal hypertension (such as low platelet count or previous variceal bleeding), while hemostatic alterations (such as inherited thrombophilia or acquired hypercoagulability) and inflammatory biomarkers do not seem to predict the development of PVT.[5] Thus, peri-

odical assessment of the severity of liver disease and surveillance for hepatocellular carcinoma are recommended, while thrombophilia testing should not be performed in cirrhotic patients.[6]

Compared to other subgroups of SVT patients, cirrhotic patients with SVT exhibit higher rates of major bleeding (10.0 per 100 patient-years) and thrombotic events (11.3 per 100 patient-years) during follow-up.[3] Furthermore, the risk of developing adverse outcomes increases in parallel with the progression of liver cirrhosis.[7] In addition, liver cirrhosis is a known cause of intrahepatic portal hypertension, and the development of portosplenic vein thrombosis can further contribute to prehepatic portal hypertension.[8]

It has been reported that 34%-39% of cirrhotic patients with PVT present with gastrointestinal bleeding at onset.[9] Other clinical manifestations of acute PVT include abdominal pain and nonspecific symptoms, such as anorexia, nausea, vomiting, and diarrhea. Chronic PVT can show signs of portal hypertension, such as ascites, splenomegaly, portal cavernoma, or portal cholangiopathy.[9] In half of cirrhotic patients with SVT, the thrombosis is asymptomatic and diagnosed incidentally at abdominal imaging.[7]

The impact of PVT on the survival of cirrhotic patients is uncertain. Although the mortality associated with PVT has declined in recent years,[10] inpatients with decompensated cirrhosis and PVT showed higher rates of portal hypertension–related complications (such as acute kidney injury and hepatorenal syndrome) and mortality compared to those without PVT.[10] A recent meta-analysis suggested that PVT might worsen the short-term prognosis of cirrhotic patients, being associated with higher risk of hepatic decompensation and higher mortality rate at 1-year follow-up, while it did not seem to influence the long-term prognosis.[11]

Indications to anticoagulant treatment

Most of the management studies involving cirrhotic patients with SVT are small and insufficiently powered to provide definitive conclusions. Previous studies reported that patients with liver cirrhosis are less likely to be treated,[12] showing a variable proportion of cirrhotic patients with SVT receiving anticoagulation (39%-62%).[7,13,14] Results of a systematic review and meta-analysis of 26 studies demonstrated that anticoagulated cirrhotic patients with SVT had lower rates of mortality (9.1% vs 21.0%; relative risk [RR], 0.42 [95% CI, 0.24-0.73]), thrombosis extension (7.1% vs 24.3%; RR, 0.28 [95% CI, 0.15-0.52]) and major bleeding overall (6.4% vs 11.2%; RR, 0.52 [95% CI 0.28-0.97]), compared to nonanticoagulated patients.[15] Regarding the site of bleeding, Loffredo et al reported that variceal bleeding of any severity occurred less frequently in anticoagulated cirrhotic patients (odds ratio [OR], 0.23 [95% CI, 0.06-0.94]).[16] The IMPORTAL individual patient data meta-analysis involving 500 cirrhotic patients with PVT suggested that bleeding not related to portal hypertension might be higher in anticoagulated patients compared to nonanticoagulated patients (9.7% vs 1.7% respectively, $P<0.001$).[17]

In addition, these meta-analyses reported that anticoagulation was associated with recanalization rates approximately 3 time higher than those for no treatment.[15-17] Several studies reported a correlation between vessel recanalization and cirrhosis prognosis, including lower rates of liver-related complications (such as variceal bleeding, ascites, and hepatic encephalopathy)[18]; improvement of the hepatic function, as expressed by the model for end-stage liver disease (MELD) score[19]; and higher transplant-free survival.[20] Conversely, in a retrospective cohort study of 269 untreated cirrhotic patients with SVT, 40% developed the composite outcome of SVT progression, cavernous transformation, intestinal ischemia, portal cholangiopathy, or new arterial or venous thrombotic events.[21]

There is consensus in recent international guidelines (Table 1) that cirrhotic patients with acute SVT should receive anticoagulant treatment[22-25] if it is not contraindicated by active bleeding associated with hemodynamic impairment or by excessively high bleeding risk (for instance, low platelet count, hepatic encephalopathy at risk of falls). However, some guidelines suggest also considering the precise location (main trunk vs branches of the portal vein) and the degree of occlusion (<50% vs >50% of the lumen).[6,22,24]

For patients with chronic SVT (defined by the presence of portal cavernoma or persistent thrombosis), a case-by-case approach has been suggested.[22-24] Since it is difficult to date a chronic SVT and to decide whether anticoagulation is required, other clinical elements might support the choice of anticoagulation in these patients, such as the presence of major inherited thrombophilia, thrombus progression, and history of mesenteric vein thrombosis with bowel ischemia.[22] The benefit of anticoagulation in chronic SVT is less evident.[6] Ai et al enrolled 80 cirrhotic patients with chronic PVT, of whom 40 received oral anticoagulant treatment (with either rivaroxaban or dabigatran) and 40 constituted the nonanticoagulated control group.[26] Anticoagulation resulted in only 12.8% recanalization rates at the 3-month follow-up and 28.2% recanalization rates at the 6-month follow-up.[26] Zhou et al evaluated 84 cirrhotic patients with portal cavernoma, of whom 46 received warfarin.[27] Despite the limited benefit of anticoagulation on recanalization rates (17.4% at a median follow-up of 51 months), anticoagulated patients showed less hepatic decompensation (13.0% vs 34.2%, $P=0.021$) compared to nonanticoagulated patients.[27]

Timing of anticoagulant treatment

Early anticoagulation in SVT increases the chances of achieving vessel recanalization[28] and is recommended by international guidelines[6,23] (Table 1); however, the actual timing of anticoagulant treatment in cirrhotic patients remains unclear. For instance, Delgado et al. enrolled 55 cirrhotic patients with PVT treated with low-molecular-weight heparin (LMWH) or vitamin K antagonist (VKA); of those patients, 35 started anticoagulation within 14 days and 20 started >14 days after diagnosis.[18] Early anticoagulation resulted in higher recanalization rates compared to late anticoagulation (71% vs 40%, $P=0.044$).[18] Rodriguez-Castro et al evaluated 65 cirrhotic patients with PVT on LMWH treatment; 45 of those patients were treated within 6 months; 11, between 7 and 12 months; and 9, after 12 months from the estimated thrombus onset.[29] Recanalization occurred in 76%, 55% and 33%, respectively ($P<0.001$). The time interval between thrombus onset and start of anticoagulant treatment, the severity of liver disease (Child-Pugh class), and the degree of PVT occlusion (partial vs complete) were included by the authors in a model to predict the success of anticoagulation in cirrhotic PVT.[29]

Choice of anticoagulant drug

The standard treatment of SVT in patients with liver cirrhosis consists of LMWH for initial treatment, eventually followed by

Table 1. Recent guidelines on the treatment of splanchnic vein thrombosis in cirrhotic patients

Guideline (year)	Indication	Timing	Drug	Duration
AASLD (2020)[6]	• Anticoagulation is essential in recent PVT and concern for intestinal ischemia • In cirrhotic patients with PVT without ischemic symptoms, consider treatment on a case-by-case basis • Cirrhotic patients with recent thrombosis of small intrahepatic subbranches of PV or minimally occlusive thrombosis of the main PV (<50% lumen): observation with serial imaging is reasonable; anticoagulant treatment if clot progression • Cirrhotic patients with recent occlusive or partially occlusive thrombosis of the main PV (>50% lumen): antithrombotic therapy should be considered	• Anticoagulation should be initiated as soon as possible (not delayed until variceal eradication or adequate beta-blockade is achieved)	• Choice of anticoagulant drug (LMWH, VKA, DOAC) should be individualized, in consultation with a hematologist and/or hepatologist • Limited data on DOAC, use with caution in cirrhotic patients with advanced portal hypertension	Not mentioned
ACG (2020)[22]	• Anticoagulation recommended for: ◦ acute complete main PVT ◦ MVT ◦ PVT extension into MV • Risk of bleeding must be weighted against benefits (eg, low platelet counts <50×10^9/L, or hepatic encephalopathy at risk of falls)	• Initiation of anticoagulation delayed if active bleeding	• Initial treatment with UFH or LMWH • UFH preferred if renal insufficiency • LMWH preferred if thrombocytopenia • Maintenance with oral anticoagulants or LMWH • Limited experience with DOAC	• 6 months for cirrhotic patients with acute PVT or MVT • Continue beyond 6 months in patients on the waiting list for liver transplant
ISTH (2020)[23]	• Anticoagulation recommended for cirrhotic patients with SVT, if no active bleeding or other contraindications	• Early anticoagulant treatment	• Start with therapeutic dose LMWH • Switch to VKA or DOAC, if not contraindicated by severe liver dysfunction • Reduced doses of LMWH or DOAC may be used for longer/indefinite duration	• At least 3–6 months • Longer or indefinite duration if: ◦ thrombosis progression ◦ recurrence after anticoagulant discontinuation ◦ unprovoked SVT ◦ persistent risk factors
AGA (2021)[25]	• Anticoagulation suggested for cirrhotic patients with acute or subacute nontumoral PVT	Not mentioned	• No data to support the use of 1 anticoagulant over another	Not mentioned
Baveno VII (2022)[24]	• Anticoagulation recommended in cirrhotic patients with: ◦ recent complete or partial occlusion (>50%) of the PV trunk ◦ symptomatic PVT ◦ PVT in candidates for liver transplant • Anticoagulation should be considered in cirrhotic patients with minimally occlusive (<50%) thrombosis of the PV trunk if: ◦ thrombus progression at 1–3 months follow-up ◦ involvement of superior MV • Case-by-case basis if low platelet count (<50×10^9/L)	Not mentioned	• Initial treatment with LMWH • Maintenance with LMWH, VKA, DOAC • Monitoring VKA can be challenging in cirrhotic patients • Regarding DOAC: ◦ no major safety concern with Child-Pugh A; ◦ use with caution in Child-Pugh B for possible accumulation; ◦ not recommended in Child-Pugh C	• Anticoagulation should be: ◦ maintained for at least 6 months and until PV recanalization; ◦ continued after recanalization in candidates for liver transplant; ◦ considered after recanalization in all patients, balancing risks and benefits

AASLD, American Association for the Study of Liver Diseases; ACG, American College of Gastroenterology; AGA, American Gastroenterological Association; DOAC, direct oral anticoagulant; INR, international normalized ratio; ISTH, International Society on Thrombosis and Haemostasis; LMWH, low-molecular-weight heparin; MV, mesenteric vein; MVT, mesenteric vein thrombosis; PV, portal vein; PVT, portal vein thrombosis; SVT, splanchnic vein thrombosis; UFH, unfractionated heparin; VKA, vitamin K antagonist; VTE, venous thromboembolism.

oral anticoagulant drugs. LMWH is a versatile drug which allows dose-reductions (eg, in case of severe thrombocytopenia [see paragraph 8] or severe renal insufficiency).[30] However, anti-Xa monitoring is not recommended in liver cirrhosis because the reduced antithrombin levels found in cirrhotic patients are associated with decreased baseline anti-Xa levels.[31] Regarding the dose regimen, Cui et al enrolled 65 cirrhotic patients with PVT and randomized them to enoxaparin 1 mg/kg twice daily (BID) or 1.5 mg/kg once daily (OD).[32] Recanalization (partial or complete) occurred in 80.6% vs 76.5%, respectively ($P = 0.67$); there were no episodes of variceal bleeding, and nonvariceal bleeding was significantly lower in the BID dose group (6.5% vs 23.5%; $P = 0.048$).[32]

Unfractionated heparin (UFH) can be considered in the presence of severe renal failure (estimated glomerular filtration rate <30 mL/min) or in patients who might need invasive procedures. However, it is usually not recommended for cirrhotic patients because of difficulties in monitoring (since the baseline activated partial thromboplastin time can be prolonged in liver cirrhosis) and because of the risk of heparin-induced thrombocytopenia, which might contribute to the cirrhosis-related thrombocytopenia.[24]

Oral anticoagulants, such as VKAs or direct oral anticoagulants (DOACs) can be considered for maintenance and long-term treatment. However, monitoring VKA may be difficult in patients with baseline prolongation of the international normalized ratio (INR).[30] Although DOACs are considered off-label in several countries, there is increasing evidence on their use in noncirrhotic patients, in whom they showed a favorable safety and efficacy profile.[33-35] There are only a few studies specifically evaluating the treatment of cirrhotic SVT,[14,26,36] and they are summarized in Table 2. Nonetheless, cirrhotic patients tend to receive reduced doses of the DOACs in real life clinical practice,[37] and the DOACs are contraindicated in patients with Child-Pugh class C (and rivaroxaban is contraindicated also in Child-Pugh B). A recent systematic review and metanalysis comparing DOACs with VKAs in cirrhotic PVT found that the DOACs had higher recanalization rates (RR, 1.67 [95% CI, 1.02-2.74]) and lower rates of PVT progression (RR 0.14, 95%CI 0.03-0.57).[38] However, these results should be interpreted with caution because most of the included studies were small retrospective observational studies with substandard quality of anticoagulation control in the VKA group.

The safety profile of the DOACs in patients with liver disease has been recently investigated by Lawal et al, who described >10 000 patients with atrial fibrillation and chronic liver disease, including 29% with liver cirrhosis.[39] The incidence rates of hospitalizations for major bleeding were lower in the DOAC than in the VKA group (7.9 vs 15.0 per 100 patient-years; hazard ratio [HR], 0.69 [95% CI, 0.58-0.82]). When analyzing the different DOACs separately, rivaroxaban was associated with higher rates of major bleeding than apixaban (9.1 vs 6.5 per 100 patient-years; HR, 1.59 [95% CI, 1.18-2.14]).[39] In addition, different anticoagulants showed different patterns of bleeding events. In a large study based on the World Health Organization pharmacovigilance database, which includes all reported bleeding in unselected adult patients, Montastruc et al found that when compared to VKAs, DOACs were associated with less cerebral, urological, and nasal bleeding but more gynecological bleeding (adjusted reporting OR, 3.75 [95% CI, 3.41-4.13]).[40] Furthermore, anti-Xa inhibitors were associated with less digestive bleeding (adjusted reporting OR, 0.78 [95% CI, 0.75-0.80]), but more bleeding in other locations compared to direct thrombin inhibitors.[40]

Duration of anticoagulant treatment

Patients with SVT should be treated for at least 3-6 months,[22,23] and a longer treatment duration should be considered for patients with liver cirrhosis, because liver cirrhosis is a persistent risk factor for recurrent thrombosis (Table 1). The Baveno VII consensus suggests to treat cirrhotic patients for at least 6 months or until portal vein recanalization is achieved and to carefully balance risks and benefits when considering prolonged treatment.[24] In a study evaluating 90 patients with SVT after discontinuation of VKA treatment, the rates of thrombotic events in cirrhotic patients were 19.1 per 100 patient-years, and liver cirrhosis emerged as a strong independent risk factor (HR, 7.9 [95% CI, 1.8-35.9]).[41] Furthermore, high rates of PVT extension or recurrence after discontinuing anticoagulation were reported in the study by Pettinari et al, in which 182 cirrhotic patients with PVT were enrolled (36% in patients with partial/complete recanalization, 20% in patients without any sign of recanalization).[13]

Although cirrhotic SVT carries a higher risk of bleeding than noncirrhotic SVT,[3] a recent meta-analysis highlighted that anticoagulant therapy did not further increase the hemorrhagic risk in cirrhotic patients but was actually associated with lower rates of major bleeding events compared to no anticoagulant treatment.[15] This finding can potentially be explained by the higher rates of vessel recanalization,[15] which may prevent portal hypertension–related bleeding. Despite the lack of specific studies on SVT, the guidelines from the International Society on Thrombosis and Haemostasis suggested that reduced doses of LMWH or DOAC be considered for the extended treatment of SVT in order to minimize the risk of bleeding events,[23] as recommended for patients with deep vein thrombosis of the lower extremities or pulmonary embolism.[42] The use of reduced doses of DOACs is also supported by a recently published study that evaluated rivaroxaban 15mg OD vs no anticoagulation for secondary prevention of SVT in noncirrhotic patients.[35]

Long-term anticoagulation is recommended for cirrhotic patients who are candidates for liver transplantation[22,24]; the aim is to prevent SVT progression and to recanalize the portal and superior mesenteric veins, which will simplify vessel anastomosis and improve posttransplant outcomes.[43] In the study by Bert et al, PVT was detected in around 10% of patients on the waiting list for liver transplantation and was associated with more complex surgical procedures and reduced 1-year survival.[44] Of note, despite abdominal imaging screening while patients were on the waiting list, in more than 50% of cases, PVT was diagnosed intraoperatively.[44] The duration of anticoagulation after liver transplantation is still debated. A short course of anticoagulation is recommended to prevent early rethrombosis, which can lead to graft loss, while the need for longer anticoagulation should be evaluated on a case-by-case basis, considering also the type of anastomosis performed and the presence of underlying prothrombotic states.[45]

Anticoagulation and gastroesophageal varices

Gastroesophageal varices do not represent a contraindication to anticoagulant treatment, as long as adequately prophylaxis is provided.[22,23] For primary prophylaxis of variceal bleeding, guidelines recommend nonselective beta-blockers,

Table 2. Studies evaluating the direct oral anticoagulants for the treatment of splanchnic vein thrombosis in patients with liver cirrhosis

Authors (year)	Study design	Patient characteristics	Treatment duration	No. of patients	Treatment	Recanalization (partial or complete)	SVT progression or recurrence	Bleeding events
Nagaoki et al. (2018)[36]	Retrospective	50 cirrhotic patients with PVT: • CP A n=29 • CP B n=16 • CP C n=5	6 months	20	Danaparoid sodium 2500 U/day for 2 weeks ⟶ Edoxaban 30–60 mg	18 (90%)	1 (5%)	Clinically significant GI bleeds: 3 (15%)
				30	Danaparoid sodium 2500 U/day for 2 weeks ⟶ Warfarin (INR target range 1.5–2.0)	9 (30%)	14 (47%)	Clinically significant GI bleeds: 2 (7%)
Ai et al. (2020)[26]	Prospective	80 cirrhotic patients with chronic PVT (all CP classes were enrolled)	6 months	40	DOACs: • Rivaroxaban 20 mg OD • Dabigatran 150 mg BID	11 (28%)	3 (8%)	Any bleed: 3 (8%)
				40	No anticoagulant treatment	1 (3%)	4 (11%)	Any bleed: 1 (3%)
Naymagon et al. (2021)[14]	Retrospective	214 cirrhotic patients with acute PVT: • CP A n=52 • CP B n=99 • CP C n=63	19 months (median)	42	Enoxaparin 1 mg/kg BID	16 (38%)*	8 (19%)	Major bleed: 9 (21%)
				26	Warfarin (INR target range 2.0–3.0)	15 (58%)*	4 (15%)	Major bleed: 5 (19%)
				18	DOACs: • Apixaban 10 mg BID for 7 days ⟶5 mg BID • Rivaroxaban 15 mg BID for 21 days ⟶20 mg OD • Dabigatran 150 mg BID	10 (56%)*	1 (6%)	Major bleed: 3 (17%)
				128	No anticoagulant treatment	34 (27%)*	33 (26%)	Major bleed: 22 (17%)

* Includes complete recanalization only.

BID, twice daily; CP, Child-Pugh class; DOACs, direct oral anticoagulants; GI, gastrointestinal; INR, international normalized ratio; NR, not reported; OD, once daily; PVT, portal vein thrombosis; SVT, splanchnic vein thrombosis; ⟶, then.

such as carvedilol (which has also alpha-adrenergic vasodilatory effect), propranolol, or nadolol. Prevention of a first variceal bleeding is crucial in cirrhotic patients, since variceal bleeding is one of the characteristics that define hepatic decompensation.[24] Endoscopic variceal band ligation (EVL) is recommended for primary prophylaxis if beta-blockers are contraindicated or not tolerated and for the treatment of acute variceal bleeding.[24]

Timing of anticoagulation in patients with gastroesophageal varices is unclear. The 2020 guidelines of the American Association for the Study of Liver Diseases suggest to start anticoagulation early, without the need to wait for varices eradication or complete beta-blockade.[6] In addition, they state that EVL can be performed without the need to stop anticoagulant therapy.[6]

In a randomized controlled trial by Gao et al, 86 cirrhotic patients with PVT and acute variceal bleeding within 48 hours after EVL were randomly allocated to anticoagulation (nadroparin 1 mg/kg for 1 month, followed by warfarin with INR target range 2.0-3.0 for 5 months) or no anticoagulant therapy.[46] Endoscopic variceal band ligation was performed monthly (discontinuing anticoagulation 3 days before) until varices eradication, and all patients received carvedilol. As expected, portal vein recanalization was significantly higher in the treated group (67.4% vs 39.5%, $P = 0.009$). Of note, there were no recurrent variceal bleedings at 5-day and 14-day follow-ups, and the rates were similar in the 2 groups at 4-week (2.3% vs. 4.7%, $P = 0.99$), 6-week (4.7% vs. 9.3%, $P = 0.67$) and 6-month (18.6% vs. 20.9%, $P = 0.78$) follow-ups.[46] Bianchini et al evaluated 265 cirrhotic patients undergoing 553 EVL procedures, of which 169 were performed during treatment with LMWH and 384 were performed without any anticoagulant treatment.[47] Rates of bleeding were similar in the 2 groups (3.8% vs 1.6%, $P = 0.29$).[47]

Anticoagulation in thrombocytopenia

Thrombocytopenia (defined as platelet count $<150 \times 10^9$/L) is found in approximately 76% of cirrhotic patients.[48] Mild thrombocytopenia (>75 to $<150 \times 10^9$/L) is the most common, while moderate (50 to 75×10^9/L) and severe ($<50 \times 10^9$/L) thrombocytopenia were reported in 13% and 1% of patients with liver cirrhosis, respectively.[48] More recently, Basili et al reported the presence of severe thrombocytopenia in ~8% of cirrhotic patients.[49] In patients with liver cirrhosis, low platelet count is not predictive of the overall bleeding risk[49] but is a marker of the severity of portal hypertension. In fact, low platelet count has been reported to predict the development of PVT.[5]

There are no guidelines specifically addressing the management of anticoagulation in cirrhosis-associated thrombocytopenia. In patients with mild to moderate thrombocytopenia, the risk of spontaneous bleeding is low,[50] and full-dose anticoagulation was reported to be safe.[51] Thus, the guidance from International Society on Thrombosis and Haemostasis on the management of cancer-associated thrombosis in patients with thrombocytopenia recommends full-therapeutic dose anticoagulation if platelet count $\geq 50 \times 10^9$/L.[51]

For patients with severe thrombocytopenia, the bleeding risk associated with anticoagulation should be balanced against the risk of thrombus progression (eg, onset and extension of SVT). Intermediate or prophylactic dose of LMWH can be considered if the platelet count is $25\text{-}50 \times 10^9$/L, and temporarily discontinuation if platelet count $<25 \times 10^9$/L.[51] There are limited data on the use of DOACs in severe thrombocytopenia; thus, they are not recommended in this setting.[51]

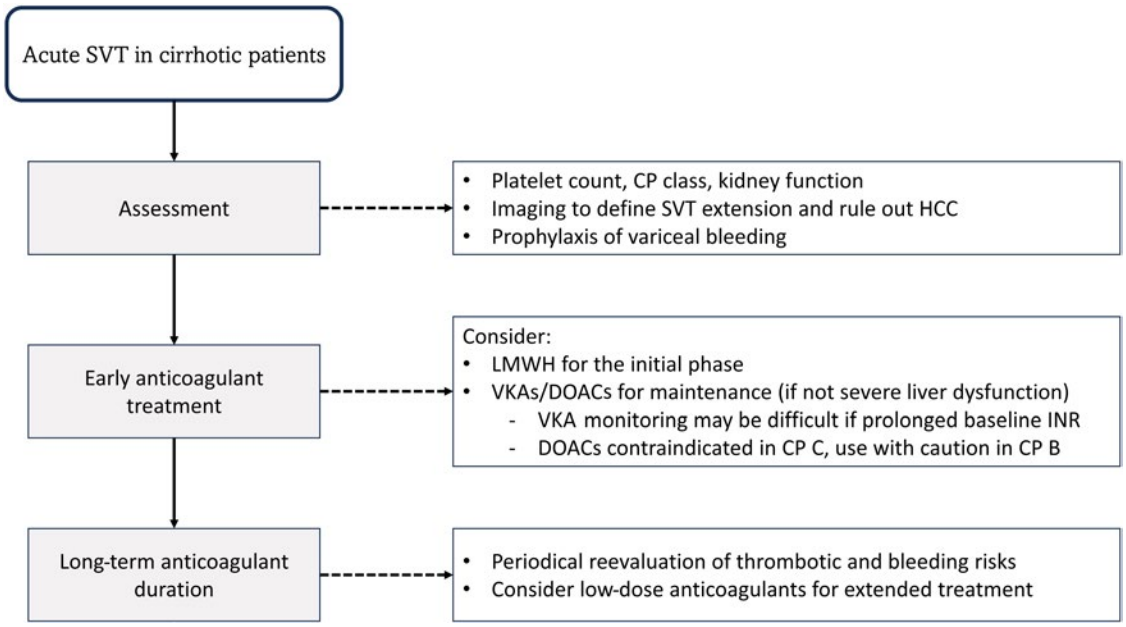

Figure 1. Proposed treatment algorithm for cirrhotic patients with acute splanchnic vein thrombosis. CP, Child-Pugh; DOACs, direct oral anticoagulants; HCC, hepatocellular carcinoma; INR, international normalized ratio; LMWH, low-molecular-weight heparin; SVT, splanchnic vein thrombosis; VKAs, vitamin K antagonists.

CLINICAL CASE (continued)

After a few days of enoxaparin 1 mg/kg twice daily, given clinical stability, the patient was switched to rivaroxaban at standard therapeutic dose (15 mg twice daily for 3 weeks, followed by 20 mg once daily). A computed tomography scan performed at the 6-month follow-up showed a patent portal vein, without any signs of cavernoma. Continuation of long-term anticoagulant treatment was indicated given the underlying presence of liver cirrhosis, and the possibility of a low-dose DOAC (eg, rivaroxaban 10 mg OD or apixaban 2.5 mg BID) was considered.

Conclusion

Cirrhotic patients with acute SVT should receive anticoagulant treatment unless major contraindications exist. Starting anticoagulation early, along with adequate prophylaxis of variceal bleeding, increases the rates of vessel recanalization and improves the prognosis. Anticoagulation should be prescribed long-term; however, it might need to be adjusted based on comorbidities (such as low platelet count and liver impairment). Our proposed management of cirrhotic SVT is summarized in the Figure 1.

Conflict-of-interest disclosure

Nicoletta Riva has no relevant conflicts to declare in relation to this paper.

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Off-label drug use

The use of direct oral anticoagulants for splanchnic vein thrombosis is off-label in several countries.

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EVIDENCE-BASED MINIREVIEW

Direct oral anticoagulants to treat deep venous thrombosis and pulmonary embolism in patients with cirrhosis: are we there yet?

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A 59-year-old female with Child-Pugh class B cirrhosis attributed to nonalcoholic steatohepatitis complicated by hepatic encephalopathy, portal hypertension with esophageal varices, and thrombocytopenia is seen for management of an acute segmental right lower lobe pulmonary embolism in a clinic. She is hemodynamically stable. Complete blood count is notable for hemoglobin 11.6 g/dL and platelets 80 K/μL. Prothrombin time is 12.6 seconds; partial thromboplastin time, 33.7 seconds; and fibrinogen, 221 mg/dL. She was referred to discuss if a direct oral anticoagulant (DOAC) can be used for anticoagulation. What would you suggest?

LEARNING OBJECTIVES

- Review the limited data on the use of the DOACs to treat deep vein thrombosis and/or pulmonary embolism for patients with cirrhosis
- Understand considerations for optimizing bleeding risk for patients with cirrhosis receiving anticoagulation

CLINICAL CASE

A 59-year-old female with Child-Pugh class B cirrhosis attributed to nonalcoholic steatohepatitis complicated by hepatic encephalopathy, portal hypertension with esophageal varices, and thrombocytopenia is seen for management of an acute segmental right lower lobe pulmonary embolism in a clinic. She was referred to discuss if a direct oral anticoagulant (DOAC) can be used for anticoagulation. What would you suggest?

Introduction

Patients with cirrhosis are at a higher risk of deep venous thrombosis (DVT) and pulmonary embolism (PE) than is the general population.[1] Therefore, initiation of anticoagulation and choice of anticoagulant are frequently encountered clinical questions, but limited data are available to guide these clinical decisions in patients with cirrhosis. Most of the oral anticoagulants depend on liver metabolism for excretion; hence, liver disease raises concerns for drug accumulation and toxicity. Traditionally, vitamin K antagonists (VKA) and low molecular weight heparins have been used for outpatient management of DVT and PE in patients with cirrhosis. VKA, although dependent on liver metabolism for excretion, can be dose-adjusted reliably based on international normalized ratio (INR) measurements for patients with an acceptable baseline INR. The direct oral anticoagulants (DOACs), including apixaban, dabigatran, edoxaban, and rivaroxaban undergo some degree of hepatic metabolism and variable degrees of renal clearance (Table 1). The prospective clinical trials that led to the Food and Drug Administration (FDA) approval of the DOACs for treatment of DVT and PE in the United States largely excluded patients with cirrhosis and/or those with modest elevations in liver enzymes or bilirubin (Table 1). Therefore, we have limited data on the pharmacokinetics, pharmacodynamics, safety, and efficacy of DOACs in patients with cirrhosis. Patients with compromised liver function have 3%-10% risk of bleeding per year, which increases as liver disease progresses. Oral anticoagulants can further multiply this risk of bleeding.[2] The anticoagulant effect of DOACs may get enhanced in patients with advanced cirrhosis.[3-5] The risk of spontaneous bleeding

Table 1. Exclusion criteria for liver disease of select randomized clinical trials of the direct oral anticoagulants for VTE

Trial	Population	Exclusion criteria
AMPLIFY (2013)	Acute VTE	ALT or AST >2 x ULN, bilirubin >1.5 x ULN (unless an alternative factor is identified [eg, Gilbert's syndrome]), active and clinically significant liver disease (eg, hepatorenal syndrome)
AMPLIFY-EXT (2013)	Extended VTE	ALT or AST >2×ULN, bilirubin >1.5×ULN (unless an alternative factor is identified [eg, Gilbert's syndrome]), active and clinically significant liver disease (eg, hepatorenal syndrome)
EINSTEIN CHOICE (2017)	Extended VTE	Hepatic disease associated with coagulopathy leading to a clinically relevant bleeding risk
EINSTEIN DVT (2010)	Acute DVT	Significant liver disease (eg, acute hepatitis, chronic active hepatitis, cirrhosis) or ALT >3×ULN
EINSTEIN-EXT (2010)	Extended VTE	Significant liver disease (eg, acute hepatitis, chronic active hepatitis, cirrhosis) or ALT >3×ULN
EINSTEIN PE (2012)	Acute PE	Significant liver disease (eg, acute hepatitis, chronic active hepatitis, cirrhosis) or ALT >3×ULN
Hokusai VTE (2013)	Acute VTE	Significant liver disease (e.g., acute hepatitis, chronic active hepatitis, cirrhosis) or ALT ≥2×ULN, or total bilirubin 1.5×ULN
RE-COVER (2009)	Acute VTE	Liver disease with aminotransferase level that was >2×ULN, known liver disease expected to have an impact on survival
RE-COVER II (2014)	Acute VTE	Liver disease with aminotransferase level that was >3×the ULN, known liver disease expected to have an impact on survival
RE-MEDY (2013)	Extended VTE	AST or ALT >2×ULN, liver disease expected to have any potential impact on survival (eg, acute hepatitis or possibly active hepatitis B, hepatitis C or cirrhosis, but not Gilbert's syndrome or hepatitis A with complete recovery)
RE-SONATE (2013)	Extended VTE	Active liver disease or liver disease decreasing survival (eg, acute hepatitis, chronic active hepatitis, cirrhosis) or ALT >3×ULN
Drug	**CYP metabolism**	**Degree of renal clearance[a]**
Apixaban	Mostly CYP3A4	~27%
Dabigatran	No	~80%
Edoxaban	Minimal	~50%
Rivaroxaban	CYP 3A4/5, CYP2J2	~66%

[a]The exact hepatic portion of DOAC clearance cannot be directly estimated given variable elimination via biliary excretion and direct intestinal excretion.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CYP, cytochrome P; DVT, deep vein thrombosis; ULN, upper limit of normal; VTE, venous thromboembolism.

with DOACs can be of paramount significance for patients with Child-Pugh B and Child-Pugh C cirrhosis.[3] We should, therefore, examine the available data carefully to inform clinical practice regarding treatment of DVT and PE in patients with cirrhosis and to identify areas in need of research.

DOACs for DVT and PE in patients with cirrhosis

Recently, meta-analyses of smaller retrospective studies and prospective pilot studies assessed efficacy and safety of DOACs for treatment of atrial fibrillation and/or splanchnic vein thrombosis in patients with cirrhosis.[6-8] Available data, however, are limited for the use of DOACs to treat extremity DVT and PE in this population. A review of available literature suggests that the efficacy of DOACs is comparable to traditional anticoagulants when measured by rates of thrombus resolution and recurrent thrombosis. Most of these studies report the risk of bleeding with DOACs to be similar to or even lower than the risk with traditional anticoagulation; however, these studies are limited by potential selection bias, sample sizes too small to detect significant differences, short follow-up times, and concerns about publication bias (Table 2). The risk of recurrent thrombosis and bleeding correlate with the severity of liver disease, and patients with Child-Pugh C cirrhosis were unlikely to receive DOACs in these observational studies. Moreover, these studies report pooled outcomes for DOACs when used for any indication (ie, DVT, PE, splanchnic vein thrombosis and/or atrial fibrillation; Table 2) despite the potential for these groups to be clinically dissimilar. When used for splanchnic vein thrombosis, DOACs recanalize splanchnic vasculature, hence reducing portal pressures, risk of variceal bleeding, and progressive liver dysfunction.[9] The outcomes for treatment of splanchnic vein thrombosis may not correlate with outcomes for DVT/PE treatment. Given these differences, caution should be exercised extrapolating data on use of DOACs for splanchnic vein thrombosis or atrial fibrillation to make treatment decisions in patients with cirrhosis presenting with DVT and/or PE until better data are available.

Prediction of anticoagulant-related bleeding and optimizing bleeding risk in patients with cirrhosis

Available risk-prediction models for anticoagulant-related bleeding are unlikely to guide safe selection of cirrhotic patients for initiation of DOACs[10] because they were developed in a general noncirrhotic population and most of these models were for traditional anticoagulants. These risk-prediction models incorporate conventional coagulation abnormalities, including thrombocytopenia, that are suboptimal predictors of hemostasis in cirrhosis. Moreover, these risk-prediction models do not account

Table 2. Retrospective cohort studies comparing direct-acting oral anticoagulants to traditional anticoagulation for patients with cirrhosis including those with deep venous thrombosis and pulmonary embolism

Retrospective study	DOACS (n)	Traditional anticoagulation (n)
Intagliata[12]		
N	20	19
DVT/PE n (% of N)	**4** (20)	**12** (63)
Child Pugh-B (n)	11	10
Child Pugh-C (n)	0	0
Bleeding (n)	4	3
Thrombosis (n)	N/A	N/A
Hum[13]		
N	27	18
DVT/PE n (% of N)	**12** (39)	**8** (44)
Child Pugh-B (n)	12	9
Child Pugh-C (n)	4	2
Bleeding (n)	8	10
Thrombosis (n)	1	1
Jones[14]		
N	42	37
DVT/PE n (% of N)	**9** (21)	**8** (22)
Child Pugh-B (n)	8	19
Child Pugh-C (n)	0	2
Bleeding (n)	7	8
Thrombosis (n)	3	3
Davis[14]		
N	27	82
DVT/PE n (% of N)	**19** (70%)	**62** (76%)
Child Pugh-B (n)	16	49
Child Pugh-C (n)	0	15
Bleeding* (n)	2	11
Thrombosis (n)	3	10
Coons[15]		
N	44	41
DVT/PE n (% of N)	**11** (25)	**8** (20)
Child Pugh-B (n)	24	17
Child Pugh-C (n)	8	9
Bleeding (n)	10	14
Thrombosis (n)	1	2
Aquite[15]		
N	48	52
DVT/PE n[1] (% of N)	**10** (21)	**11** (21)
Child Pugh-B (n)	N/A	N/A
Child Pugh-C (n)	N/A	N/A
Bleeding (n[2])	9.1/100-patient years	14.4/100 patient years
Thrombosis (n)	N/A	N/A

Table 2. Retrospective cohort studies comparing direct-acting oral anticoagulants to traditional anticoagulation for patients with cirrhosis including those with deep venous thrombosis and pulmonary embolism (*Continued*)

Retrospective study	DOACS (n)	Traditional anticoagulation (n)
Oldham[16]		
N	67	32
DVT/PE n[1] (% of N)	**45** (65)	**9** (28)
Child Pugh-B (n)	59	30
Child Pugh-C (n)	0	0
Bleeding (n)	25	7
Thrombosis (n)	3	0

DOACs, direct acting oral anticoagulants; DVT, deep venous thrombosis; PE, pulmonary embolism; traditional anticoagulation refers to low-molecular weight heparin or vitamin K antagonists.
*Only major bleeding was reported.
[1]Number includes splanchnic vein thrombosis.
[2]Only reported patient-years of bleeding.
Child-Pugh class, bleeding, and thrombosis have been reported for the entire study population (N).

for severity of liver disease, class of anticoagulant, or site of prior bleeding, which are relevant considerations. Most of bleeding in patients with cirrhosis originates in the gastrointestinal tract, and this is the very site where some DOACs are associated with more bleeding than are traditional anticoagulants.[11]

Efforts should be made to mitigate the bleeding risk in any patient on anticoagulation. In the setting of cirrhosis, this may entail frequent endoscopies to manage varices and other potential bleeding lesions, use of beta-blockers for portal hypertension, considering proton pump inhibitors or H2 receptor antagonists when appropriate, limiting use of drugs with antiplatelet activity, and close monitoring for changes in liver function over time. This requires a multidisciplinary approach and shared decision-making between patients, gastroenterologists, and hematologists. Selection of the safest anticoagulant should be based on renal function, need for invasive procedures, anticipation of liver transplant, and potential drug interactions. For example, calcineurin inhibitors, frequently used after liver transplants, may interact with most DOACs.

Future research

Considering the complex hemostatic changes of cirrhosis, with higher baseline risks of thrombosis and bleeding, well-designed studies are needed to better define the role of DOACs in management of DVT/PE and understand the hemostatic impact of these drugs. Studies should focus on the indications for DOACs (eg, initial treatment versus extended secondary prevention of venous thromboembolism) to help balance risks and benefits. Dose reduction of DOACs, initiation of DOACs after initial management with parenteral anticoagulation, and use of novel anticoagulants deserve further investigation in this population. A validated tool that predicts DOAC-related bleeding in patients with cirrhosis may facilitate safe selection of patients for DOAC use and prioritize modifiable risk factors that could be addressed to maximize net anticoagulant benefit. Such a tool could also promote clinical trial participation for some cirrhotic patients. In addition, evidence-based strategies for optimizing risk factors for gastrointestinal bleeding, monitoring patients on anticoagulation, and managing anticoagulation around the time of liver transplant need to be developed.

Recommendations

- Discuss the risks and benefits of DOACs compared to traditional anticoagulants (low-molecular weight heparin/VKA) and the limitations of available literature with patients with cirrhosis as part of shared decision-making. The Child-Pugh score should be considered as part of a comprehensive assessment of anticoagulant candidacy and selection. (Strong recommendation, low quality evidence)
- Avoid use of DOACs to treat DVT or PE in patients with Child-Pugh C cirrhosis. (Strong recommendation, low quality evidence)
- Consider DOACs to treat DVT or PE in patients with Child-Pugh A cirrhosis without significant liver enzyme or bilirubin elevation. (Strong recommendation, low quality evidence)
- DOAC use is controversial for Child-Pugh B cirrhosis; FDA package inserts do not recommend use of edoxaban and rivaroxaban for this population. Selective and cautious use of the other DOACs may be reasonable in individual cases after estimation of risk versus benefit. (Weak recommendation, low quality evidence).

Conflict-of-interest disclosure

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Off-label drug use

The prescribing information for apixaban does not provide dose adjustment recommendations for Child-Pugh class B or moderate hepatic impairment but notes limited experience of clinical use in this population. It suggests avoiding use in Child-Pugh class C. Edoxaban advises against use in moderate to severe hepatic impairment (Child-Pugh B and C). Rivaroxaban suggests avoiding use in Child-Pugh B and C and in hepatic disease associated with coagulopathy.

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MSM and blood donation: shifting to individualized risk assessment

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Deferring donors at higher risk for transfusion transmissible infections is an important part of ensuring blood safety. The deferral for gay, bisexual, and other men who have sex with men (gbMSM) was implemented in the 1980s in many countries, since they were identified as a high-risk group for AIDS/HIV. With the introduction of increasingly sensitive HIV antibody testing, augmented by nucleic acid testing, the window period for HIV infection—when a donor may be infectious but have negative test results—has shrunk dramatically. In Canada, this has led to progressively shorter deferral periods for gbMSM, decreasing from a permanent deferral for sex with another male since 1977 to a 5-year, 12-month, and eventually 3-month deferral period. These time-based deferrals maintained safety; however, they are seen as stigmatizing by many and still result in the deferral of sexually active gbMSM. More recently, several countries have moved to a donor screening approach based on assessing sexual risk behaviors in all donors. This article outlines research supporting changes in policy, current eligibility screening policies in several countries, and preliminary results postimplementation of new eligibility policies in Canada in September 2022.

LEARNING OBJECTIVES

- Explain the evolution of deferral policies for high-risk sexual behaviors over time
- Compare time-based deferrals for gay, bisexual, and other men who have sex with men with sexual risk–based policies for all donors
- Identify methods of evaluating the safety of policy changes postimplementation

CLINICAL CASE

Walter, a married gay man, and his husband, Bill, who have been in a mutually exclusive sexual relationship for many years, hear an urgent appeal for blood donors on the radio and decide to overcome their fear of needles and make an appointment to donate blood. While waiting to be seen by the screener at the Toronto fixed site, they see their neighbor, Casanova, who is always boasting about his success finding new lady partners on Tinder.

Who do you think will be eligible to donate?

Introduction

Blood safety depends on donor selection, testing, and for some components, pathogen reduction. In many countries, including the US, Canada, the UK, and Australia, blood establishments performing collection, testing, and distribution are highly regulated, and a standardized donor health questionnaire (DHQ) and criteria manual are used. The DHQ questions divide donors into standard and higher risk groups, with the higher risk group undergoing additional testing or, more often, deferring from donation for a period of time or indefinitely. If testing is unavailable for an infectious agent in a nonpathogen-reduced component, donor selection is vital to reduce risk. Once testing is developed, the importance of donor selection decreases.

Introduction of criteria for gay, bisexual, and other men who have sex with men

Donor criteria for gay, bisexual, and other men who have sex with men (gbMSM) were introduced in the 1980s, when it was recognized that AIDS could be transmitted by blood.[1,2] Initially, donors were provided written materials instructing them to self-defer if they fell into risk categories, including male homosexuals with multiple partners. These policies are thought to have resulted in an approximately 90% reduction in the risk of AIDS/HIV transfusion

Table 1. Postimplementation monitoring over shorter time-based deferral periods, Canadian Blood Services

• HIV rates remained low (0.2–0.4 per 100,000 donations)
• No HIV NAT-only positive cases
• HIV-positive donors were noncompliant or denied risk factors
• No positive lookback or traceback cases for HIV
• Noncompliance rate remained low (<1%) as assessed by anonymous donor surveys
• Residual risk of HIV calculated as 1 in 12.9 million units transfused

transmission in high-risk areas, such as San Francisco.[2,3] Once antibody testing began, interviews with HIV-positive donors indicated that many did not necessarily identify as gay or have multiple partners. In 1985, the deferral for a man who has had sex with another man even once since 1977 was mandated by the Food and Drug Administration (FDA); 1977 was thought to be the time AIDS appeared in North America.[4] Many other countries introduced similar criteria. Many other criteria were also put in place to reduce the risk of HIV and/or hepatitis B and C transmission, including deferrals for having an HIV-positive sexual partner, for paying or receiving money or drugs for sex, or for using injection drugs.[4]

The epidemiology of HIV differs in different geographic areas. This discussion is focused on deferral policies in countries where gbMSM have a higher prevalence and incidence of HIV compared with the general population.

Evolution of criteria over time: shorter time-based deferrals for gbMSM

The relative importance of donor screening questions for HIV risk decreased with the introduction of increasingly sensitive tests for HIV 1/2 antibodies, antigen testing (p24), and eventually nucleic acid testing (NAT). Improved automation, process control, and quality systems have virtually eliminated errors in testing and blood center quarantine procedures. The window period, when donors may be infectious but have negative test results, is estimated at 9 days for NAT tests performed in minipools in North America. Additionally, individuals are more aware of risk factors for HIV transmission, and rapid, confidential testing is easily available.

However, criteria for gbMSM were slow to change, in part because of a highly precautionary system born out of the tragedy of transmission of HIV and hepatitis C (known as non-A, non-B hepatitis at the time) to thousands of blood- and plasma-derived clotting factor recipients in the 1980s and early 1990s.[5]

In 2000, Australia implemented a 12-month deferral policy for gbMSM, with no increase in HIV positivity rates.[6] Risk modeling done in several countries suggested that moving from a permanent to a 5-year or 12-month deferral would lead to a negligible increase in HIV risk of under 1 in 5 million transfusions.[7] The US REDS-III Blood Donation Rules Opinion Study (BloodDROPS), in surveys of the gbMSM community and noncompliant gbMSM who donated blood, demonstrated that the HIV prevalence in gbMSM donors was 0.25%, considerably lower than the estimated rate in the general US gbMSM population.[8]

Based on the above, in addition to advocacy group pressure, many countries moved from a permanent deferral for gbMSM to progressively shorter time-based deferrals.[9] In Canada, the permanent deferral was replaced by a 5-year, 12-month, and 3-month deferral in 2013, 2016, and 2019, respectively.[10] In the US, the FDA issued revised recommendations for reducing the risk of HIV transmission in 2015, including a 12-month deferral for gbMSM, and again in 2020, including a 3-month deferral.[11]

Postimplementation monitoring studies (Table 1) showed that changing to shorter deferral periods maintained safety.[12]

Change to sexual risk behavior policies

Although shorter time-based deferrals allow some men who had remote experiences with male-to-male sex to donate, they still result in the deferral of most sexually active gbMSM. Additionally, asking male donors about sex with another male can be seen as stigmatizing and the deferrals seen as discriminatory, generating "ban the ban" campaigns and cancelled blood drives on university campuses, and legal challenges in several countries.[1,13] Under these criteria, our possible donors, Walter and Bill, would be deferred, while Casanova may likely be eligible.

Italy and Spain

Several countries, including Spain and Italy, implemented policies based on sexual behaviors considered to be at higher risk, regardless of whether the partner is same sex or opposite sex; these are sometimes referred to as gender-neutral or risk-based behavior policies. For example, in Spain, donors were deferred for 12 months after sex with more than 1 concurrent partner, or sex with an occasional partner. In Italy, donors were deferred for 4 months after sexual contact with a new or occasional partner whose risk behavior is unknown, and indefinitely deferred for usual/recurrent sex with more than 1 partner whose risk behaviors are unknown or multiple new partners. Published HIV rates, including NAT-only positives, were higher compared with countries using time-based deferrals; it is unclear if this was related to the criteria, methods of questionnaire administration (use of medical doctors), and/or poor donor understanding and compliance. Both differences in blood center functioning and higher donor HIV rates impeded adoption on these approaches in other countries.[14-16]

The UK FAIR approach

UK policies evolved from a permanent deferral for male-to-male sex to a 12- and then 3-month deferral. The UK FAIR (For the Assessment of Individual Risk) steering group, comprising the UK blood services, Public Health England, Nottingham University researchers, and stakeholders including 2SLGBTQI+ (two-spirit, lesbian, gay, bisexual, transgender, queer, intersex, and other people who identify as part of sexual and gender diverse communities) groups, reviewed the literature on individual risk sexual

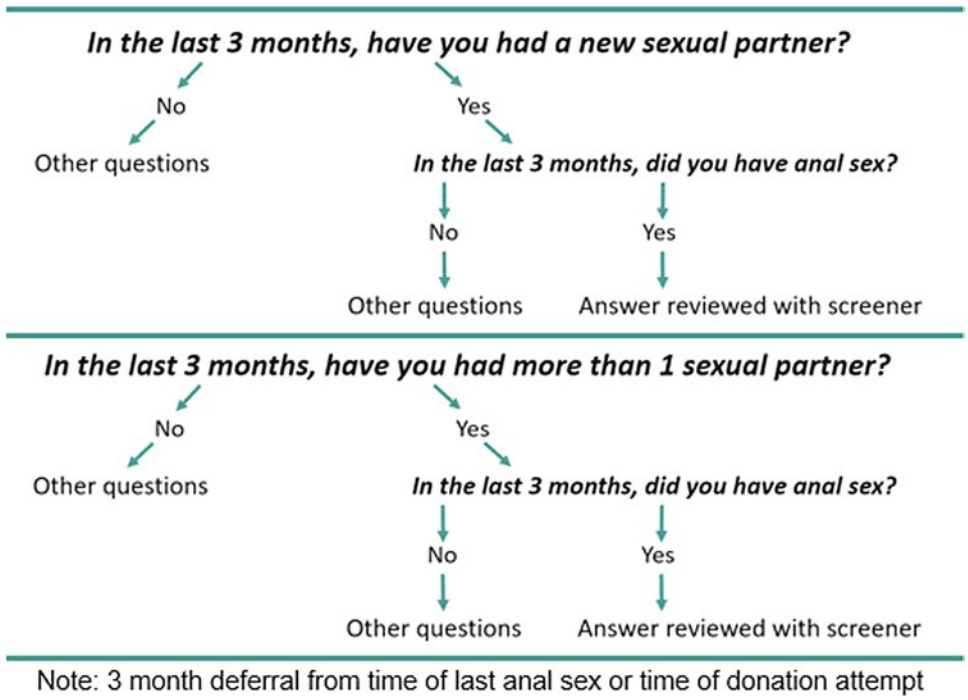

Figure 1. Current sexual risk behavior questions, Canadian Blood Services. Identical criteria are included in the FDA Guidance for Industry and the AABB DHQ_, version 4.[26,27]

behaviors and markers of risk. Focus groups and surveys of stakeholders were undertaken to assess the feasibility and acceptability of potential questions. FAIR recommended deferring donors with a new partner or multiple sexual partners in the last 3 months only if they had anal sex, since anal sex carries the highest rate of HIV transmission and deferral of all donors with a new partner or multiple partners would have too great an impact on the adequacy of the blood supply.[17] Donors who participated in chemsex (use of drugs such as amyl nitrite to enhance sexual experience) in the past 3 months are also deferred since this was identified as a marker of HIV risk. These recommendations were implemented by the blood services in the UK in 2021.

Canadian research programs and policy changes

In 2017, the Canadian federal government funded research programs, administered by Canadian Blood Services and Héma-Québec, the two Canadian blood suppliers, to develop the evidence for a sexual risk behavior approach for whole blood, plateletpheresis, and source plasma donation. Research themes, developed at a kickoff meeting, included safety, operational feasibility, acceptability to donors and gbMSM community members, and the impact of pathogen reduction. Nineteen projects at 11 different research sites were funded, resulting in over 20 publications to date.[18] Many studies, such as risk modeling of possible changes, risks for HIV infection identified in a prospective cohort of gbMSM, operational feasibility of various policies, and acceptability of various approaches to donors and high-interest stakeholder groups, proved critical to supporting a successful regulatory submission to move to an approach very similar to FAIR.[19-23]

Criteria implemented at Canadian Blood Services for all donors and all donation types (whole blood, plasma and plateletpheresis, source plasma) in September 2022 are shown in Figure 1. Implementation was preceded by an extensive staff training program. Héma-Québec implemented identical criteria, first for source plasma and then for all other donation types, slightly later in 2022. Results in the first 6 months postimplementation have shown no increase in HIV rates, no HIV NAT-only positive donors, few complaints from donors, and lower than expected donor deferral rates. Walter and Bill may now be eligible, while Casanova may find himself deferred, depending on specific sexual behaviors. Table 2 shows actual compared with predicted deferral rates.[19,24] A similar discordance between expected and observed rates was seen at Héma-Québec, the blood supplier for the province of Québec. Reasons for this discordance are unclear but may relate to how thoughtful people are about answering survey questions compared with the actual donor questionnaire. As expected, younger donors are more likely to be deferred by the new criteria. These observations would be strengthened by a longer observation period. Additionally, an anonymous donor survey will be performed in late 2023 to access compliance and provide some data on the

Table 2. Observed vs expected answers to new donor questions, % of all donation attempts

In the last 3 months:			Deferrals
Sex with a new partner	2.1% **(5.2%)**	+ anal sex →	0.06% **(0.4%)**
Sex with >1 partner	0.94% **(2.7%)**	+ anal sex →	0.03% **(0.4%)**
One or both	2.3% **(6.3%)**	+ anal sex →	0.085% **(0.6%)**

number of currently eligible male donors who previously would have been deferred. It is possible that noncompliance has been reduced as some gbMSM may not have answered questions truthfully that they considered discriminatory.

US ADVANCE Study and upcoming policy changes in the US and other countries

The US ADVANCE (Assessing Donor Variability and New Concepts in Eligibility) Study, funded by the FDA, enrolled close to 1600 sexually active gbMSM, from 18 to 39 years old in 8 participating communities. Study participants completed a questionnaire and were tested for HIV antiretroviral drugs (medications that can be used as pre-exposure prophylaxis for HIV, known as PrEP). The goal of the study was to evaluate whether sexual behavior risk questions could identify participants who recently became infected with HIV.[25] The FDA used study data, as well as implementation data and risk modeling studies performed internationally, to issue recommendations for evaluating donor eligibility using individual risk-based questions to reduce the risk of HIV transmission by blood and blood products (in draft guidance in January 2023, final guidance in May 2023). The questions and criteria proposed are similar to what has been implemented in the UK and Canada, shown in Figure 1.[26] The Association for the Advancement of Blood & Biotherapies has incorporated these changes into the DHQ version 4.0 and prepared resources to assist in training and implementation.[27]

Many other countries, such as Israel, France, the Netherlands, and Germany, have implemented or will soon implement criteria based on sexual risk behaviors and remove time-based deferrals for gbMSM.

Future considerations

Some future considerations are outlined in Table 3. PrEP therapy with antiretroviral medications is highly effective at preventing sexual transmission of HIV. However, if donors get infected by HIV on or shortly after discontinuing PrEP, viral kinetics and the host immune response may be altered, possibly prolonging testing window periods. Many blood centers have added PrEP-related criteria, deferring some otherwise eligible donors.[28]

Treatment of HIV with antiretroviral medications is highly effective at reducing sexual transmission risk, leading to public health messaging that undetectable equals untransmissible (U=U). However, this is not necessarily the case for transfusion of a large volume intravenously. Communicating this message is challenging.

Pathogen reduction technologies are used in manufacturing plasma protein derivatives and more recently have been introduced for platelets and plasma for transfusion. These technologies result in several log reduction of many pathogens, such as HIV, hepatitis B virus, and hepatitis C virus. Theoretically, criteria could be less stringent when pathogen reduction technology is used. A good place to start may be PrEP/post-exposure prophylaxis criteria.

Trans or *transgender* is an umbrella term that refers to people whose current gender identity differs from their biological sex assigned at birth. Trans people may identify as trans males (assigned female at birth, gender identity male), as trans females (assigned male at birth, gender identity female), or as nonbinary. Blood centers have grappled with several complex issues in attempting to respectfully screen trans donors while ensuring donor and recipient safety.[29] Screening is simplified by the use of a sexual risk behavior approach, since all donors are asked the same questions, regardless of their sex or gender, and the questions focus on behavior, regardless of the sex or gender of the donor's sexual partner(s) (gender-neutral questions). However, most blood centers do not have a nonbinary option, partly due to software limitations.[30]

Table 3. Future considerations

Issue	Comments
PrEP	PrEP use is increasing ↓ Will otherwise eligible gbMSM donors be deferred for PrEP use?
Pathogen reduction	Methods in use for source plasma, platelets, and transfusible plasma are highly effective against HIV ↓ Can eligibility criteria be modified if pathogen reduction is being performed? HIV-infected donors on PrEP or who have recently taken PrEP and have negative test results have very low viremia, so are deferrals for PrEP needed for pathogen-reduced components?
Trans donors	A binary donor registration system does not adequately cover the gender identity spectrum ↓ Can we modify our computer systems to be more inclusive of all potential donors?

Conclusions

After many decades of using male-to-male sex as a surrogate marker for high-risk sexual behavior, blood services are moving to criteria based on sexual risk behaviors in all donors. Initial implementation in the UK and Canada have shown promising results but would be strengthened by further observation. These changes are an important step toward a more inclusive approach to blood donor selection.

Conflict-of-interest disclosure

Mindy Goldman is on the biomedical advisory board of ITL. She has given a presentation at a Roche corporate event about donor diversity.

Off-label drug use

Mindy Goldman: There is nothing to disclose.

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Clonal hematopoiesis in frequent whole blood donors

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Healthy volunteer donors are committed to contributing key medical resources. Repeated, regular donation of whole blood represents a specific trigger of hematopoietic stress. Hematopoietic stem cells (HSCs) are known to respond to environmental triggers by altering their differentiation and/or proliferative behavior. This can manifest in long-term changes in the clonal dynamics of HSCs, such as the age-associated expansion of HSCs carrying somatic mutations in genes associated with hematologic cancers—that is, clonal hematopoiesis (CH). A recent study revealed a higher prevalence of CH in frequent donors driven by low-risk mutations in genes encoding for epigenetic modifiers, with *DNMT3A* and *TET2* being the most common. No difference in the prevalence of known preleukemic driver mutations was detected between the cohorts, underscoring the safety of repetitive blood donations. Functional analyses suggest a link between the presence of selected *DNMT3A* mutations found in the frequent donor group and the responsiveness of the cells to the molecular mediator of bleeding stress, erythropoietin (EPO), but not inflammation. These findings define EPO as one of the environmental factors that provide a fitness advantage to specific mutant HSCs. Analyzing CH prevalence and characteristics in other donor cohorts will be important to comprehensively assess the health risks associated with the different types of donation.

LEARNING OBJECTIVES

- Discuss the long-term effects and safety of large-volume phlebotomy in healthy individuals
- Review adaptive clonal hematopoiesis in the context of erythropoietic stress

CLINICAL CASE

A 57-year-old man who has been a volunteer blood donor (ID:101X) at the German Red Cross Blood Donor Service for the past 35 years and given a total of 116 donations came in for his third whole-blood donation in 2022. His hemoglobin (Hb) was 15.8 g/dL, and his standard donor questionnaire did not raise any concerns. Donor 101X has no history of cardiovascular disease. Before proceeding with the donation, he asked to speak to a physician to discuss his family's medical condition. His older brother (aged 64) had been diagnosed with acute myeloid leukemia (AML) a month earlier. Donor 101X and his younger brother (aged 55) had undergone screening as potential stem cell donors for a matched related allogeneic hematopoietic stem cell transplant (alloHSCT). HLA typing revealed both healthy brothers to be a full match. Donor 101X was in an overall better health condition compared to the younger brother based on the general assessment. Interestingly, the transplant center at which donor 101X's brother was scheduled for the procedure had implemented mutational analysis as part of the stem cell donor workup a few years prior. A targeted sequencing panel covering 49 genes recurrently mutated in myeloid neoplasms was used. Donor 101X was found to carry 2 mutations in the gene encoding for the enzyme DNA methyltransferase 3A (*DNMT3A*), corresponding to 2 hematopoietic clones, a small population representing 2% of the cells and a larger one comprising 6% of the cells. This finding prompted the treating transplant physician to defer the blood donor and choose the younger brother instead as the stem cell donor. Donor 101X learned about the concerns associated with the engraftment of (his) mutant HSCs, such as a higher risk for the cells to expand after the transplant and to develop into donor-derived leukemia, as well as their hyperinflammatory profile and therefore higher risk of causing graft-versus-host disease. However, he was given no information about the potential personal consequences of being a *DNMT3A* mutation carrier. He was wondering about the origin of the mutations and their

pathogenic potential, whether external factors including his extensive history of whole-blood donation may have contributed to the emergence of the clones, whether blood donation was safe for him as well as the recipients, and whether he should take any measures in the future to avoid the acquisition of additional clones as well as the current ones developing into leukemia.

Clonal hematopoiesis and HSC turnover

As we age, hematopoietic stem cells accumulate mutations.[1] The older an individual, the more cycling an HSC has undergone and hence the higher the likelihood of having acquired a driver mutation that changes the cellular phenotype.[1] The bone marrow is an inherently competitive environment, as the space is limited. If a mutant HSC turns out to be "fitter" than the others, it can clonally expand. Clonal hematopoiesis (CH) refers to an age-associated overrepresentation of HSCs carrying certain genetic aberrations within the healthy blood system.[2-4] That is, instead of 50 000 HSCs contributing to healthy hematopoiesis, selected clones of mutated HSCs and their progeny contribute disproportionally and thus become detectable by sequencing.[2-4] Originally, the sequencing techniques used required a variant allele fraction of at least 2% for reliable detection of a clone. Technical advances have lowered the detection limit down to 0.01%, although the biological significance of hematopoietic clones that small remains to be determined.[5]

The competitive advantage of a mutation can be apparent at a steady state, for example, due to improved self-renewal and/or become more pronounced under specific conditions, such as exposure to cytotoxic signals, growth factors, proinflammatory stimuli, and so on. Depending on the type of selection pressure, the advantages of particular types of mutations have been demonstrated; mutations in genes involved in DNA damage response show superior fitness in the context of cytotoxic therapy.[6-8] Mutations in *DNMT3A* are acquired very early in life and are by far the most common in older healthy individuals.[2-4] They typically have a slow rate of expansion[9,10]; however, an altered responsiveness of *DNMT3A*-mutant cells has been demonstrated under inflammatory conditions.[11] On the molecular level, aging-induced tumor necrosis factor-α as well as inflammation-associated interferon and IL-6 have been demonstrated to selectively promote the growth of *DNMT3A* mutant vs wild-type cells.[11,12]

Clonal hematopoiesis and disease risks

CH clones driven by certain mutations have been demonstrated to have a high likelihood of undergoing subsequent leukemic transformation.[13-15] Hence, individuals with CH show an increased risk of developing hematologic malignancies.[2,4] Moreover, the pathologic conditions associated with CH are not limited to primarily hematopoietic diseases. Thus, CH has been linked to an increased risk and severity of cardiovascular and chronic obstructive pulmonary disease,[16,17] gout,[18] chronic liver disease,[19] and many more. Additionally, as the presence of CH is increasingly recognized as a likely rather than aberrant path of HSC development, conditions in which it can benefit patients' outcomes have been identified, such as Alzheimer's disease and in fact matched related alloHSCT.[20-22]

The acquisition of CH mutations is not associated with immediate changes in the differentiation potential of the HSCs and is very difficult to identify by standard laboratory analysis until close to the diagnosis of malignancy.[13,14] Significant effort has been dedicated to devising a scoring system to help predict the risk of malignant transformation in CH-positive individuals and allow for an early intervention.[13-15,23] Genes encoding for splicing factors (*SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*) and the AML-associated genes *IDH1/2* and *RUNX1* as well as *JAK2* and *TP53* have been identified as high-risk genes as opposed to low-risk genes, which include the epigenetic modifiers *DNMT3A* and *TET2*. Association with the different malignant progression risk groups, defined based on genetic and laboratory parameters, was shown to also correlate with nonhematologic CH comorbidities—in particular, cardiovascular events.[23] This is remarkable since the pathophysiology of the latter has been best described for *TET2*-mutant CH, whereas *TET2* mutations were not included in the risk-stratification as a separate variable.

Whole-blood donation

Fewer than 5% of all eligible donors contribute over 90% of all blood products worldwide. As a result, instead of donating once every 10 years, committed healthy individuals donate several times per year to meet the steadily increasing demand for these lifesaving resources. Most of the studies of long-term outcomes in frequent blood donors have focused on the consequences of iron depletion for donor safety.[24,25] Only one recent study assessed the incidence of hematologic malignancies in frequent blood donors and reported a subtle decrease in AML risk.[26]

Whole-blood donation of 500 mL corresponds to a loss of approximately 10% to 15% of the total blood volume. This represents a considerable erythropoietic stress that manifests in short term as well as more protracted changes in the blood parameters.[24,27] Thus, about 2-fold elevated levels of the hematopoietic cytokine erythropoietin (EPO), produced by the kidneys following blood donation to replenish the lost cells, have been detected up to 56 days after a whole-blood donation and could conceivably have an impact on HSC dynamics.[28]

Frequent blood donation as a model of erythropoietic stress

Repeated large-volume phlebotomy represents a unique model to study the impact of erythropoietic stress on the clonal dynamics of the hematopoietic system. By contrast, investigations performed in pathologic erythropoietic stress conditions, in particular anemia (sickle cell disease,[29,30] acquired aplastic anemia[31]), are confounded by multiple systemic characteristics, including increased inflammation, autoimmune selection, multisystem organ damage, and so on, as well as long-term, systemic medications.

In the first—and so far, only—comprehensive assessment of the long-term effects of frequent whole-blood donations,[32] a cohort of healthy male blood donors (median age 66 years, 120 lifetime donations, 3.2 donations per year every 114.5 days over the past 3 or 4 decades; n = 105) was compared to age-matched male sporadic blood donors (median age 63 years, 5 lifetime donations; n = 103). The prevalence and spectrum of CH was assessed with error-corrected sequencing using a broad panel (141 genes) of genes associated with hematologic malignancies (Figure 1).

The prevalence of CH was not significantly different between frequent and control donors (54.2% vs 39.8% and 32.4% vs 20.3%

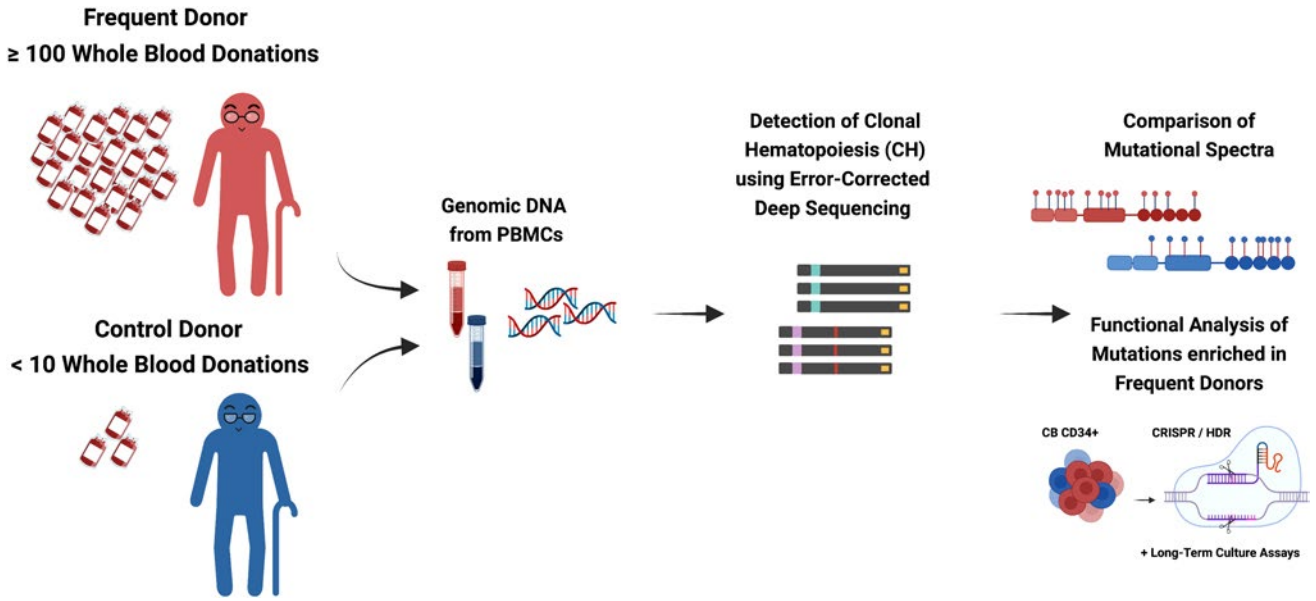

Figure 1. Effects of large-volume phlebotomy on clonal hematopoiesis. Study setup and analysis performed in blood donors. CRISPR, clustered regularly interspaced short palindromic repeats; HDR, homology directed repair; PBMC, peripheral blood mononuclear cells. Figure created using BioRender.[32]

in frequent vs control donors using a detection limit of 0.5% and 2%, respectively) and well within the range of mathematical modeling–based predictions for a given age and sequencing detection range.[33]

Furthermore, the spectrum of drivers was similar in that, in agreement with all CH studies in individuals without hematologic malignancies published to date,[2-4,13,14] *DNMT3A* and *TET2* were by far the 2 most commonly mutated genes in both cohorts (Table 1). However, frequent donors showed an overall significant increase in CH driven by mutations in genes encoding for epigenetic modifiers (44.7% vs 22.3%), including *DNMT3A* and *TET2*.

In the context of malignant transformation, mutations in epigenetic modifiers represent the so-called very early events that do not cause immediate, stark changes in the proliferative behavior or in the differentiation potential of the cells. Accordingly, while many CH mutations have been directly linked to hematologic malignancies, with the exception of *IDH1/2*, epigenetic modifier mutations were associated with a low hazard ratio when the risk of malignant transformation vs persistence in the form of a CH clone was assessed in large cohorts.[13,14,23] Moreover, as mentioned above, mutations in *DNMT3A* alone display a markedly benign profile—that is, a low risk of myeloid neoplasms compared to other CH genotypes.[13,14,23]

Table 1. Spectrum of mutations detected in blood donors

Cohort	Frequent donor	Control donor
Characteristics	n = 105, median age, 66y Median # donations, 120	n = 103, median age, 63y Median # donations, 5
Low-risk mutations[13]	*DNMT3A* (34)[a] *TET2* (14) *KRAS* (2) *CBL* (1) *ASXL1* (1)	*DNMT3A* (23)[a] *TET2* (7) *CBL* (1) *ASXL1* (1)
High-risk mutations[23]	*RUNX1* (1) *SRSF2* (1)	*RUNX1* (1) *U2AF1* (1)
Unclear risk mutations[34]	*KMT2C* (5), *MPL* (3), *SH2B3* (2), *SF3B1* (2)[b], *BCR* (2), *EED* (2), and 1 each of *SMC1A*, *TERT*, *WAS*, *HNRNPK*, *JAK3*, *ATM*, *ASXL2*, *STAT3*, *LRRC4*, *KAT6A*, *KDM6A*, *IKZF1*, *FAM154B*, *EP300*, *ELANE*, *DNMT1*, *CTCF*, *CRLF2*, and *BRINP3*	*SMC1A* (2), *HNRNPK* (2), *FAM47A* (2), *DNM2* (2), *BRCA2* (2), and 1 each of *KMT2C*, *SH2B3*, *SF3B1*[b], *BCR*, *WAS*, *TERT*, *JAK3*, *ATM*, *ASXL2*, *TAL1*, *SETBP1*, *NPAT*, *NOTCH1*, *IL7R*, *GJB3*, *FIP1L1*, *DDX41*, *CUX1*, *CHEK2*, *BRCA1*, *ANKRD26*, and *ABL1*

[a]Including 3× and 2×*R882* mutations in frequent and control donors, respectively.

[b]Assigned as unclear risk mutation due to controversial predictions in the literature.

Data compiled from Karpova et al.[32]

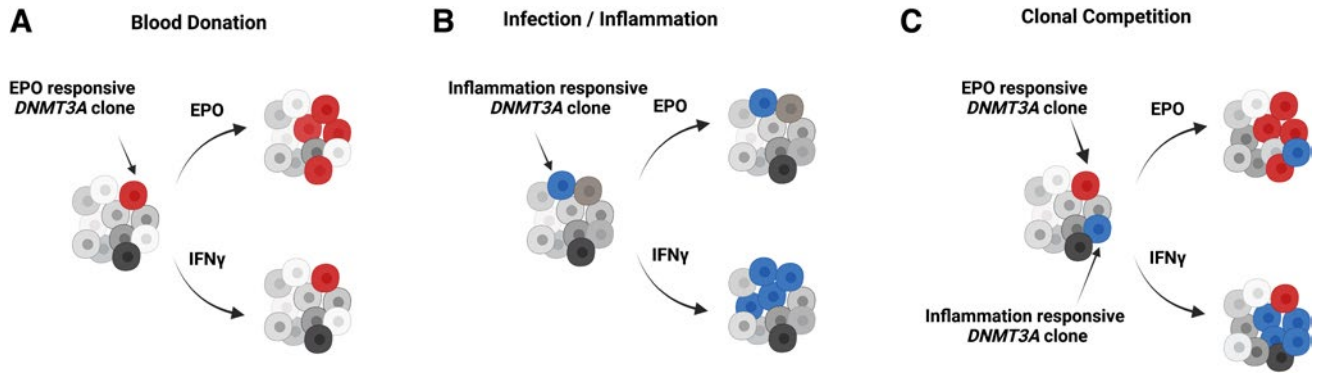

Figure 2. Clonal dynamics of human hematopoiesis under different conditions. Figure created using BioRender.

Adaptive hematopoiesis in frequent blood donors

In addition to the differences in the overall characteristics of CH between frequent donors and controls, the type and distribution of *DNMT3A* mutations also differed between the groups (Figure 1).[32] When selected mutations were analyzed functionally (Figure 1), 3 out of 3 *DNMT3A* mutations chosen from the frequent donors showed responsiveness toward EPO but not to inflammatory stimuli. This is in striking contrast to the proliferative behavior of the well-known preleukemic *DNMT3A* mutations, R882H and R882C, that expand under inflammatory conditions but not with EPO (Figure 2).[11,32] Importantly, there was no difference in the incidence of R882 *DNMT3A* mutations between frequent and control donors.

This observation implies several new and important insights. First, EPO is identified as a novel factor shaping the clonal dynamics of the human hematopoietic system at the level of the HSCs. Second, it defines a new class of *DNMT3A* mutations that promote a proliferative advantage to HSCs in EPO-rich as opposed to inflammatory environments known to favor preleukemic R882 hits. And third, it suggests that in frequent blood donors, in addition to the spectrum of age-associated mutations, CH is likely driven by mutations with a competitive advantage in EPO-rich environments. Given that the acquisition and accumulation of mutations in HSCs is an inevitable process, it is tempting to speculate that repeated large-volume phlebotomy favors the benign, lower-risk mutations such as the EPO-responsive *DNMT3A* variants.

Blood donation is safe

Overall, the assessment of clonal dynamics in individuals with a lifelong blood donation history confirmed that blood donation is safe. Even decades of donating whole blood several times per year do not result in a significant overall transformation of the clonal hematopoietic composition. An increase in CH driven by mutations in genes encoding for epigenetic modifiers was observed in frequent compared to control donors. Given the mild pathophysiological profile of these mutations, it is unlikely these outgrowths will lead to myeloid malignancy over a lifetime. Moreover, no increase in CH driven by established preleukemic hits is found in frequent blood donors. With regard to nonhematologic comorbidities, detailed risk-assessment studies assigning CH-positive individuals into high- vs low-risk categories have not yet been performed. Despite the overall CH prevalence and mutational spectrum being well in agreement with mathematical models and previously published cohorts,[2,23,33] the link between CH and cardiovascular pathologies remains to be verified in frequent blood donors, a cohort preselected for exclusively healthy individuals.

In the specific case of donor 101X, both the presence of 2 (most likely independent) *DNMT3A* clones as well as the fact that neither of the clones is driven by an R882 mutation is in line with what one would expect for a blood donor with a decades-long history of regular whole-blood donations in general and given the low frequency of R882 mutations (only 10%) found in blood donors in particular.[32] As a relative of a patient with a myeloid malignancy, donor 101X is more likely to be a myeloid CH carrier,[21] yet the acquisition of *DNMT3A* mutations per se must have occurred in his 30s and was not triggered by regular blood donation.[9,10]

Naturally, the presence of *DNMT3A*-mutant CH does not preclude donor 101X from further donation since (as expected) no blood count abnormalities (signs of cytopenia, aberrant red blood cell parameters, low Hb) have ever been detected in the donor. Moreover, if he continues to donate, his blood parameters can and should be monitored closely as changes in Hb, hematocrit, mean corpuscular volume, and red-cell distribution width represent very early phenotypic abnormalities that occur in individuals with CH years prior to developing myeloid neoplasms.[13] Additionally, clonal composition can be assessed via sequencing at intervals of 2 to 3 years. Preleukemic clones have been shown to have markedly augmented growth kinetics; that is, they grow quickly and continuously as compared to benign CH.[13-15,33] By contrast, *DNMT3A*-driven clones show very slow growth kinetics compared to other CH drivers,[13,14] consistent with their benign profile.[23] If the hypothesis is true and the donor's EPO-responsive clones indeed emerge and persist at the expense of preleukemic ones, one would predict the 2 *DNMT3A* clones to remain at a stable size within the next decades. Lastly, the decision of the treating physician to exclude donor 101X as a stem cell donor, primarily due to the concern that the CH clones would undergo disproportional expansion in the recipient (as has been shown for *DNMT3A*-mutant CH[21,22]) was debatable. In the HLA-matched related alloHSCT setting, AML/myelodysplastic syndrome patients who received *DNMT3A* CH-positive grafts have been demonstrated to have a lower risk of relapse/disease progression due to higher rates of chronic graft-versus-host disease, in particular when transplantation was performed in a noncomplete remission state.[21] Moreover, low rates of donor-derived

myelodysplastic syndrome/AML in recipients of *DNMT3A* mutant grafts have been reported.[23]

Outlook

Healthy volunteer donors, including whole-blood, platelet, and stem cell donors, selflessly provide lifesaving resources. A recent study of whole-blood donors with a lifelong history of regular donations is a first comprehensive sequencing-based analysis of the clonal dynamics in this group of donors. The marks of continued significant erythropoietic stress were apparent in the form of the increased prevalence of CH driven by epigenetic modifier mutations. However, this finding does not suggest an elevated risk of developing myeloid neoplasms in frequent blood donors as the detected CH clones, especially those driven by *DNMT3A* mutations, are likely benign. While the suggested direct effect of EPO on HSPC dynamics is novel, the role of another hematopoietic cytokine, thrombopoietin, in HSC biology is well documented.[36] Furthermore, platelet donation is associated with a significant increase in thrombopoietin levels,[37] whereas the maximum total number of platelet donations (up to 24) is 6 times higher compared to whole-blood donations.[38] Frequent platelet donors therefore represent the next obvious donor cohort to be analyzed with regard to CH prevalence and characteristics. Though clinical actionability in the context of CH detection remains a controversial topic, recent analysis of big cohorts, including the invaluable UK biobank resource, have further advanced our interpretation of risks linked to mutations in specific genes. The assessment of CH is critical for donor safety and helps provide insight into the long-term effects of certain types of selection pressure in humans without any confounding pathologic conditions.

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Conflict-of-interest disclosure

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T-cell lymphopenia in frequent volunteer platelet donors

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In the United States, more than 2 000 000 apheresis platelet units are collected annually from volunteer donors. Platelet donors in the United States and elsewhere are permitted to donate up to 24 times per year. Recently, frequent apheresis platelet donation has been associated with severe T-cell lymphopenia. Several frequent platelet donors have been found to have peripheral blood CD4⁺ T-cell counts below 200 cells/μL, the threshold for AIDS in HIV-positive individuals. Independent risk factors for plateletpheresis-associated lymphopenia include lifetime donations, age, and donations on the Trima Accel instrument (Terumo BCT), which uses a leukoreduction system (LRS) chamber to trap white blood cells. Less often, severe lymphopenia can occur in donors collected on the Fenwal Amicus instrument (Fresenius Kabi), which has no LRS. For Trima Accel donors, lymphopenia can be partially mitigated by performing a plasma rinseback step at the end of collection. To date, there is no definitive evidence that plateletpheresis-associated lymphopenia is harmful. In a study of frequent platelet donors with lymphopenia who were administered COVID-19 messenger RNA vaccines, immune responses were normal. The homeostatic mechanisms responsible for maintaining a normal peripheral blood T-cell count remain obscure, as do the causal mechanisms underlying plateletpheresis-associated lymphopenia.

LEARNING OBJECTIVES

- Examine risk factors for plateletpheresis-associated lymphopenia
- Evaluate clinical consequences and mitigation approaches for plateletpheresis-associated lymphopenia

CLINICAL CASE

On routine screening, a 58-year-old man in his usual state of good health was discovered to be lymphopenic (lymphocyte count 512 cells/μL; reference range, 720–4100 cells/μL.) The patient's hemoglobin level, white blood cell count, and platelet count were within normal limits. Follow-up studies were remarkable for a CD4⁺ T-cell count of 106 cells/μL (441–2156 cells/μL) and a CD8⁺ T-cell count of 92 cells/μL (125–1312 cells/μL). The patient's IgG level was normal, and he tested negative for HIV by immunoassay and nucleic acid testing. The patient was referred by his primary care physician to a hematologist, who performed a bone marrow biopsy. No abnormalities were found. The patient reported donating platelets every 2 weeks for the past 10 years.

Introduction

By the 1970s, apheresis technology had advanced to the point where it became feasible to collect high numbers of platelets from individual volunteer donors during a single donation. Concerns were soon raised about whether frequent apheresis platelet donations might render donors thrombocytopenic, potentially putting them at risk for bleeding. Investigators also noticed that frequent platelet donors' lymphocyte counts tended to decline, and worries were raised about possibly making donors immunodeficient. In 1981, Koepke et al[1] reported a prospective study of lymphopenia in plateletpheresis donors. Ten healthy male volunteers, aged 21 to 33 years, donated platelets by apheresis 10 times over 12 weeks. None had donated whole blood or platelets previously. The volunteers' total lymphocyte counts decreased by approximately 20% during the study period (Figure 1). The investigators concluded that this was a statistically significant change but not one that was clinically meaningful.

Apheresis technology continued to evolve, and with the introduction of better automated instruments, the question of whether plateletpheresis could cause lymphopenia was set aside. In 2007, the US Food and Drug Administration dropped its requirement that donor consent forms include

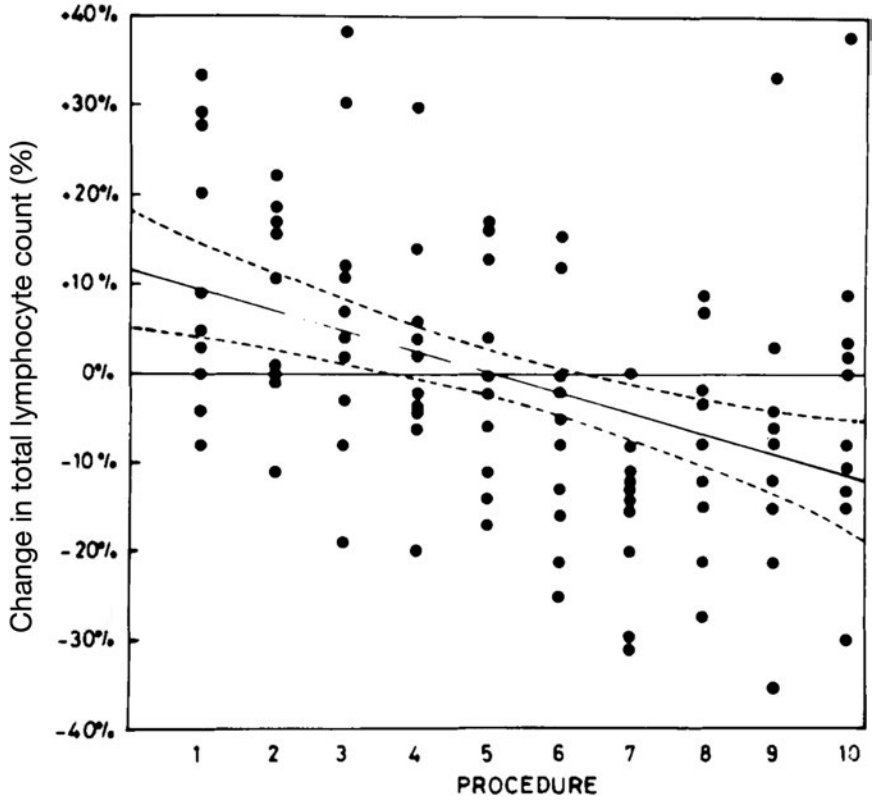

Figure 1. Peripheral blood lymphocyte trends in naive volunteers undergoing frequent plateletpheresis. Ten healthy male volunteers donated apheresis platelets 10 times in 12 weeks on the Haemonetics Model 30 blood processor. Blood samples were obtained at baseline and at each of the plateletpheresis procedures. The plotted points show, for each volunteer, the percentage change in total peripheral blood lymphocytes relative to that volunteer's mean lymphocyte count for the entire study period. The solid regression line shows the average change for all participants; ±2 SD confidence intervals are indicated by the dashed lines.

the potential risk of lymphocyte depletion.[2] A 2008 editorial by Ronald Strauss[3] asserted, "Donor lymphocytes collected or lost during plateletpheresis are so few that the risk of significant lymphocytopenia and/or immune dysfunction is nil, and no additional measures are needed." That is where things remained until 2017, when a frequent platelet donor in Boston was incidentally discovered to have a $CD4^+$ T-cell count below 200 cells/μL. The observation of severe T-cell lymphopenia in this donor led to subsequent investigations in frequent plateletpheresis donors, summarized below.

Risk factors for acquiring plateletpheresis-associated lymphopenia

In 2019, Gansner et al[4] reported a single-center, cross-sectional study of 60 apheresis platelet donors. The donors were divided into 3 groups based on the number of platelet donations in the past 365 days: 1 or 2, 3 to 19, or 20 to 24. All donors had donated on the Trima Accel (Terumo BCT) instrument exclusively. $CD4^+$ T-cell counts below 200 cells/μL were found, respectively, in 0 of 20, 2 of 20 (10%), and 6 of 20 (30%) donors in the 3 groups ($P = .019$). Similarly, $CD8^+$ T-cell counts in the 3 groups were below the lower limit of normal in 0 of 20, 4 of 20, and 11 of 20, respectively ($P < .001$). There appeared to be a cumulative donation effect: a lifetime history of donating platelets 50 or more times was associated with a significant decrease in $CD4^+$ and $CD8^+$ T-cell counts (Figure 2). In multivariable analyses, both donor age and platelet donations were independently associated with reduced $CD4^+/CD8^+$ counts. All the donors in this study reported being in good health.[4]

The Trima Accel instrument's circuit incorporates a leukoreduction system (LRS) chamber, which is a small plastic cone that traps approximately 15% to 20% of circulating lymphocytes and monocytes. Approximately 1 to 2×10^9 of mononuclear cells are retained in the LRS following a platelet donation.[4,5] Donor centers often provide postplateletpheresis LRS chambers to researchers, as they provide a convenient source of mononuclear cells.[5] It was hypothesized that bulk removal of T cells by the LRS could contribute to the development of plateletpheresis-associated lymphopenia in donors collected on the Trima Accel. On that basis, a cross-sectional study was performed of frequent platelet donors collected on the Fenwal Amicus (Fresenius Kabi) apheresis instrument, which does not have an LRS. Among 30 frequent platelet donors collected on the Fenwal Amicus, none had a $CD4^+$ T-cell count below 200 cells/μL. In contrast, a large single-center retrospective study conducted in Germany found that frequent donors collected mainly using the Amicus instrument had a median lymphocyte count of 1530 cells/μL, compared with 1960 cells/μL among new donors. The authors concluded that plateletpheresis-associated lymphopenia can occur without an LRS, only to a lesser degree than when an LRS is used.[6] In a recent international study conducted by the Biomedical Excellence for Safer Transfusion Collaborative, $CD4^+$

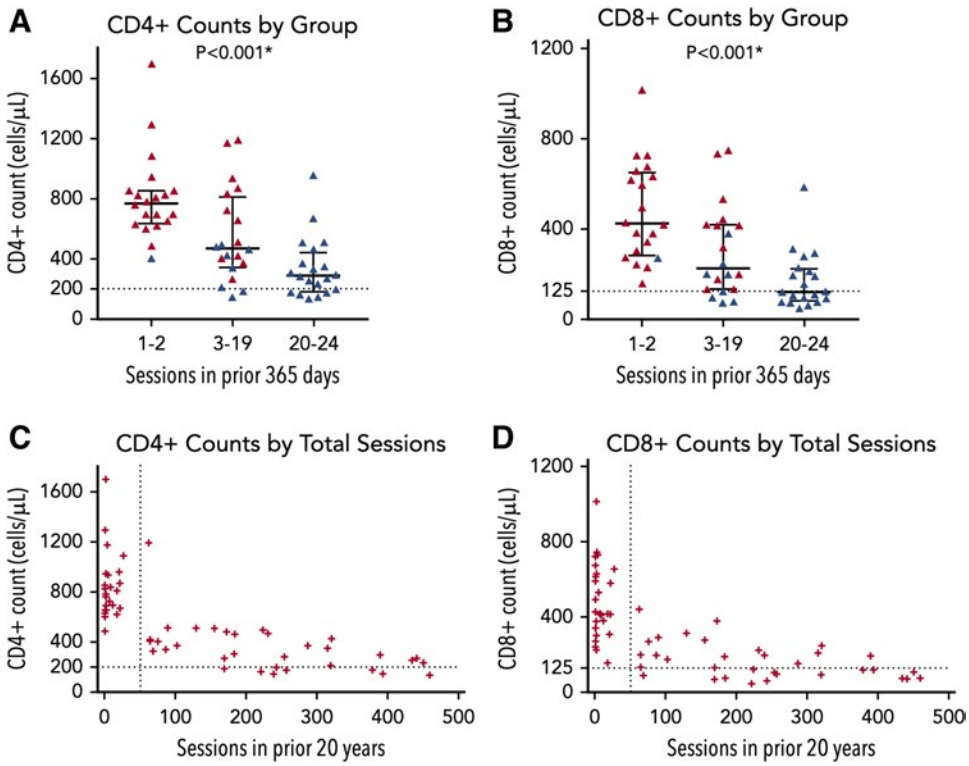

Figure 2. Peripheral blood T-cell counts in frequent plateletpheresis donors. Cell counts are plotted for the 3 donor groups. (A) $CD4^+$ T-cell counts. The horizontal dotted line indicates 200 cells/μL. (B) $CD8^+$ T-cell counts. The horizontal dotted line indicates the lower limit of normal. Blue symbols indicate volunteers who donated 20 to 24 times in a 365-day period during the past 20 years. (C) $CD4^+$ T-cell counts by total plateletpheresis sessions. (D) $CD8^+$ T-cell counts by total sessions. The vertical dotted line indicates 50 donations.

T-cell counts below 200 cells/μL were observed in 10% of frequent platelet donors collected on the Trima Accel and 4% of frequent platelet donors collected on the Fenwal Amicus (Richard M. Kaufman, unpublished data). Fenwal Amicus donors had lymphocyte counts and $CD4^+/CD8^+$ counts that were intermediate between those of age-matched whole-blood donor controls, who had higher counts, and frequent Trima Accel donors, who had lower counts. Donation on the Trima Accel vs the Fenwal Amicus was thus determined to be an independent risk factor for plateletpheresis-associated lymphopenia. However, severe lymphopenia can develop following frequent platelet donation on the Fenwal Amicus instrument, in the absence of an LRS.

Are platelet donors with severe T-cell lymphopenia immunodeficient?

An intrinsic problem with studying volunteer platelet donors is that they are, by definition, healthy. (To qualify for blood donation, donors must answer "yes" to the first question on the Donor History Questionnaire: "Are you feeling healthy and well today?"[7]) Rahmani et al[8] attempted to contact individuals who used to donate platelets frequently but had stopped donating for at least 1 year for any reason. Of 15 former donors, 2 were found to have $CD4^+$ T-cell counts below 200 cells/μL despite not donating for 1 to 2 years. Thus, plateletpheresis-associated lymphopenia can persist long after donors stop donating. But does severe T-cell lymphopenia in platelet donors reflect an acquired immunodeficiency state, or is it simply an abnormal laboratory result without meaningful clinical consequences?

In a nationwide cohort study, Zhao et al[9] attempted to look for signs of clinical risk in frequent platelet donors using the Swedish Scandinavian donations and transfusions (SCANDAT3-S) database. SCANDAT3-S pulls together data from national registers in Sweden and Denmark on blood donors, blood products, transfusions, and transfusion recipients. The data span from 1968 through 2017 and encompass over 26 million person-years of donor follow-up.[10] At the time the SCANDAT3-S study was performed, lymphopenia had been observed in platelet donors collected on the Trima Accel instrument but not yet among donors collected on the Fenwal Amicus.[8] Zhao and colleagues[9] also sought to avoid potential confounding by donor self-selection—the so-called healthy donor effect—whereby donors who feel unwell donate less often. Therefore, the investigators chose to compare apheresis platelet donors collected using an LRS chamber (COBE Spectra and Trima Accel) with platelet and plasma donors who donated the same number of times but without an LRS (Spectra Optia; all 3 instruments from Terumo BCT). A second maneuver to mitigate against the healthy donor effect involved using a time-dependent analysis. The investigators defined a 10-year exposure window that excluded donations in the most recent 1 year, to try to prevent imbalances in the numbers of donations analyzed due to donors becoming ill and donating less frequently as a result.

A total of 74 048 plateletpheresis and plasmapheresis donors were included in the SCANDAT3-S analysis. Among donors with the same number of donations, there were significantly more immunosuppression-related infections (Figure 3A) and common bacterial infections (Figure 3B) among donors collected using an

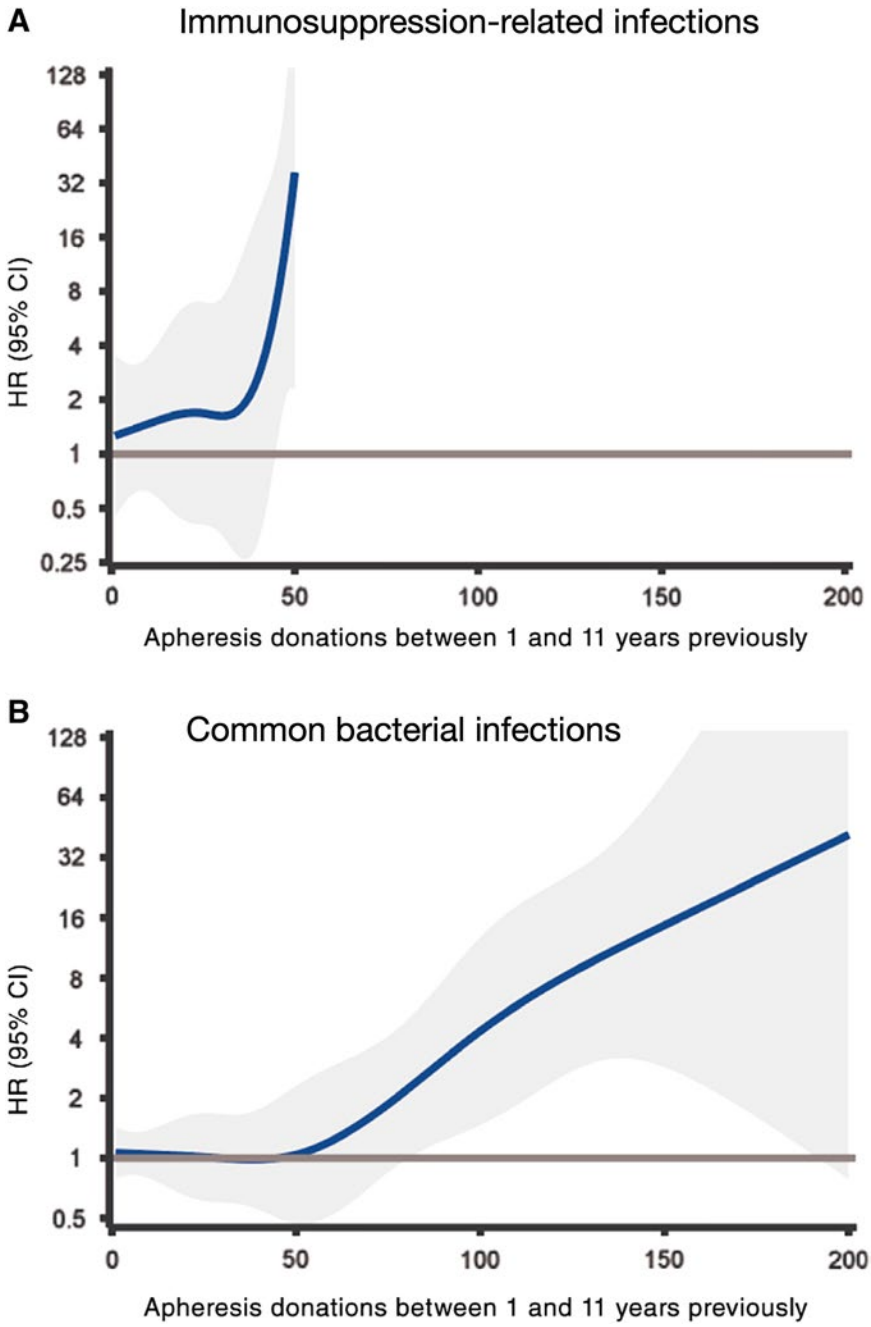

Figure 3. Risk of infections with LRS+ donations vs LRS− apheresis platelet donations, in relation to number of donations between 1 and 11 years in the past, modeled as a restricted cubic spline. (A) Immunosuppression-related infections. (B) Common bacterial infections. Confidence intervals are shown in gray. HR, hazard ratio.

LRS compared to those collected without an LRS. Notably, no immunosuppression-related infections were observed among the highest-risk group, LRS donors who had donated more than 50 times. In all, only 11 immunosuppression-related infections were found among LRS donors, mainly herpes zoster. No donor was found to have had a severe illness (eg, disseminated mycobacterial infection or aspergillus). While provocative, there were important limitations to the Zhao et al[9] study. This was a retrospective, observational study with potential for residual confounding. There were relatively few frequent plateletpheresis donors: just 95 donors in the data set (1.4%) had donated 20 or more times in a single year. Finally, as noted above, the number of infection events was small.[11]

Laumaea et al[12] evaluated the ability of frequent apheresis platelet donors to respond to COVID-19 vaccination. The investigators recruited a cohort of 43 COVID-19 infection-naive platelet donors who had donated more than 5 times per year on the Trima Accel instrument. The donors were administered 2 doses of a COVID-19 messenger RNA vaccine. Blood samples were collected at baseline and approximately 5 or 6 weeks after the first dose and

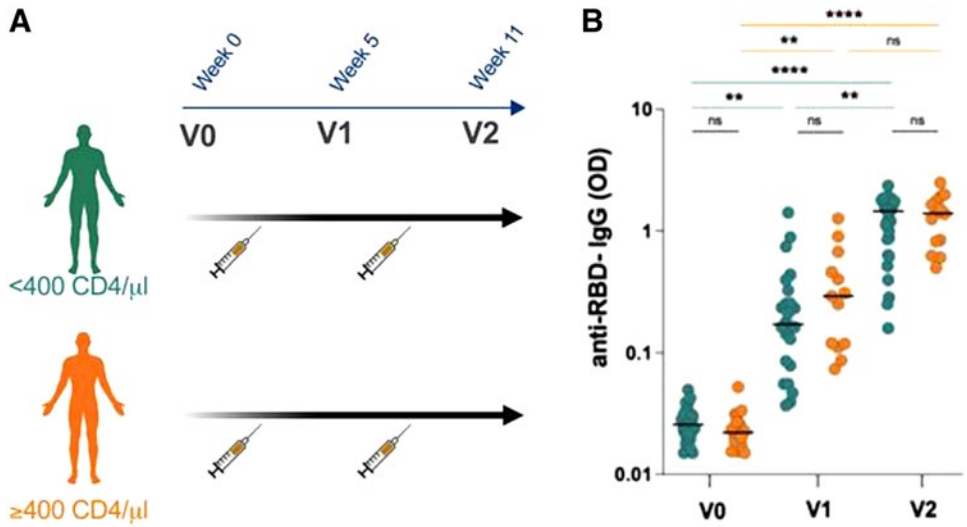

Figure 4. Anti–receptor binding domain (RBD) antibody responses in plateletpheresis donors receiving COVID-19 messenger RNA (mRNA) vaccines. (A) Study schema. COVID-19 infection-naive CD4⁺-low (teal) and CD4⁺-high (orange) platelet donors were administered 2 mRNA vaccine doses per the schedule shown. (B) Anti-RBD IgG levels at baseline (V0) and after (V1, V2) each dose of vaccine. ***P* < .01; *****P* < .0001.

second doses, respectively (Figure 4A). The donors were divided into 2 groups: those with CD4⁺ T-cell counts below 400 cells/μL (n = 27) and those with CD4⁺ T-cell counts at or above 400 cells/μL (n = 16). Consistent with earlier studies, donors in the CD4⁺-low group had a median of 166 lifetime donations vs a median of 24 lifetime donations in the CD4⁺-high group (*P* < .0001). Antibody to SARS-CoV-2 receptor binding domain was low at baseline (V0) in both groups and increased significantly in both groups following vaccination. The IgG response in the CD4⁺-high group was slightly higher than in the CD4⁺-low group, but the difference was not statistically significant (Figure 4B). The CD4⁺-low and CD4⁺-high groups were also similar in their IgM and IgA anti–receptor binding domain responses, in spike-specific antibody formation, in pseudovirus neutralization assays, and in an anti–SARS-CoV-2 antibody-dependent cellular cytoxicity assay. In aggregate, these data provide reassuring evidence of preserved immune function in frequent plateletpheresis donors.

Mitigation

T-cell lymphopenia tends to be more frequent and severe when donating on the Trima Accel instrument vs the Fenwal Amicus instrument, consistent with mononuclear cell capture by the LRS contributing to the development of lymphopenia. Although not routinely used by most blood donor centers, the Trima Accel provides a "plasma rinseback" option, which returns to the donor 22%[13] to 74% (A. Razatos, personal communication, 2022) of white blood cells remaining in the disposable tubing of the apheresis circuit. The rinseback procedure was designed to minimize red blood cell loss in the disposable tubing and does not flush cells out of the Trima Accel's LRS chamber. In 2013, Canadian Blood Services (CBS) instituted rinseback for all platelet collections using the Trima Accel instrument. Multiple CBS blood centers contributed data to the Biomedical Excellence for Safer Transfusion Collaborative study of plateletpheresis-associated lymphopenia. The CBS blood centers were directly compared to other centers using the Trima Accel that do not perform rinseback. The results suggest that Trima Accel plasma rinseback partially mitigates against the development of plateletpheresis-associated lymphopenia. Among 40 frequent platelet donors collected on the Trima Accel using rinseback, the mean CD4⁺ T-cell count was significantly lower than that of matched whole-blood donor controls. However, 0 of 40 Trima Accel donors receiving rinseback had a CD4⁺ T-cell count below 200 cells/μL, compared with 13 of 91 Trima Accel donors (14%) collected without rinseback.

T-cell homeostasis and other open questions

In adult humans, approximately 10^{11} naive T cells circulate in the peripheral blood and through lymphoid organs.[14] T-cell progenitors originate in the bone marrow, then migrate to the thymus, where they undergo maturation and selection. Some of these cells are eventually released from the thymus as naive T cells, ready to respond should they encounter specific antigen. In mice, the thymus continually produces large numbers of naive T cells throughout the life of the organism. In contrast, thymic export of naive T cells is sharply curtailed in childhood in humans. A small number of new thymic emigrants can be found in middle-aged adults, but the bulk of the naive T-cell population in adult humans is sustained by cell proliferation in other lymphoid organs.[15-18] T cells circulating in the blood are easy to access and can provide critical prognostic information, as in patients infected with HIV. But only about 2% to 3% of the body's total T cells circulate in the peripheral blood at any given time.[16,19] Most naive T cells reside in lymph nodes.[18] Mouse experiments suggest that within lymph nodes, IL-7 and IL-15 provide key survival and proliferation signals to resident T cells.[20] More recently, the cysteine-rich with EGF-like domains 1 (*CRELD1*) gene has been implicated as a regulator of T-cell homeostasis in humans.[21]

Overall, however, how the peripheral blood T-cell count is normally maintained remains poorly understood. In frequent platelet donors with low blood T cells, nothing is known at this point about T-cell numbers and homeostatic proliferative

activity within their lymphoid tissues. The vaccine study[12] summarized above provides reassuring evidence of preserved immune function in donors with plateletpheresis-associated lymphopenia. As illustrated by the Clinical Case, hematologists should be aware of this entity to avoid unnecessary medical workups in otherwise healthy individuals. Donor centers using the Trima Accel instrument are advised to perform plasma rinseback routinely to reduce the risk of donor lymphopenia. The mononuclear cell content of LRS chambers is largely preserved following plasma rinseback,[13] so the LRS chambers remain useful for research. Finally, during the donation consent process, frequent platelet donors should be made aware of the possibility of developing plateletpheresis-associated lymphopenia.

Conflict-of-interest disclosure

Richard M. Kaufman: no competing financial interests to declare.

Off-label drug use

Richard M. Kaufman: Nothing to disclose.

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Acute leukemias and complicated lymphomas: pearls to optimize management when patients stay local

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Hematologic malignancies often present acutely with a constellation of infectious complications, pancytopenia, tumor lysis, and renal dysfunction. Acute leukemias and aggressive lymphomas often require hospitalization for rapid diagnostic evaluation, urgent management of complicating presentations, and timely management of intensive systemic therapies. There is an emerging paradigm whereby complex cancer care can be safely and effectively provided in the community, where the majority of cancer is treated. A substantive and effective network between local oncologists and their academic counterparts will enhance care for the patient, advance research, and help bring complicated therapies to local centers, thereby improving access. Here we present several cases that highlight a collaborative approach to complicated hematologic malignancies in the community.

LEARNING OBJECTIVES

- Identify aggressive hematologic malignancies and initiate appropriate diagnostic and supportive measures rapidly
- Understand the timing of involving an academic center or disease expert
- Explore the role and use of sophisticated therapies along with toxicities in the community

Introduction

Acute leukemias (acute myeloid leukemia/acute lymphoblastic leukemia) and non-Hodgkin lymphoma (NHL) represent about 1.3% and 4.1%, respectively, of all new cancer cases annually, with the aggressive NHL subgroup approximation likely to be in the 2% to 3% range.[1,2] The combination of rarity, the need for intensive and urgent therapies, protracted transfusion support, and significant complications have traditionally left these challenges to be managed largely in the academic arena with disease experts.

While the historical precedent was tertiary center referral if options were locally unavailable, geographic and socioeconomic considerations limit this option for most patients. Fortunately, runaway advances in immune engager therapy and the expansive infrastructure of community-based clinical trials that has developed over the past 2 decades have helped bridge this gap considerably. We present cases that crystalize the democratized access to sophisticated therapy in patients with hematologic malignancies in the community.

CLINICAL CASE 1

An 18-year-old woman with heavy menses/gingival bleeding presents with the following: white blood cell (WBC) count, 28; hemoglobin, 10; platelets, 15; coagulopathy; and a peripheral smear showing 75% blasts. She was empirically initiated on all *trans*-retinoic acid (ATRA) for possible acute promyelocytic leukemia (APL) vs M4 acute myeloid leukemia. She was aggressively transfused to maintain platelets >50 000, fibrinogen >150, and fresh frozen plasma to normalize prothrombin time/partial thromboplastin time. Prophylactic prednisone (0.5 mg/kg/d) was instituted with ATRA to preemptively address the possibility of high-risk APL presenting with a WBC >10.

APL has historically had the highest incidence of early mortality rates mostly due to hemorrhagic complications leading to death. Early intervention with ATRA, along with transfusion support to remediate coagulopathies,

has yielded cure rates of >80%. In conjunction with empiric therapy, minimizing invasive procedures decreases complications related to disseminated intravascular coagulation (DIC). ATRA should be started before confirmatory molecular studies if there is a high index of suspicion based on presentation, peripheral smear, and coagulopathy. Delays in starting ATRA are associated with potentially fatal hemorrhagic complications while initiating ATRA in a non-APL diagnosis has little associated toxicity and can readily be discontinued once a diagnosis has been formally made. While it is not unreasonable to reach out to an academic center for a potential transfer of care, the diagnostic and supportive measures instituted locally within the first 24 to 48 hours can be life-saving and need to be recognized by all.

CLINICAL CASE 1 (continued)

Flow cytometry on the peripheral blood/bone marrow aspiration revealed HLA-DR$^-$ and CD33$^+$ with fluorescence in situ hybridization ultimately showing t(15;17) 48 hours later consistent with APL.

The patient developed pulmonary decompensation with ATRA syndrome, requiring intubation despite initiating high-dose dexamethasone. ATRA was held and arsenic trioxide (ATO) and idarubicin were initiated since differentiation syndrome rates are similar with ATRA/ATO or ATRA/chemotherapy, despite arsenic having a risk of DS.[3] With aggressive management of the DIC, tumor lysis syndrome prophylaxis, and differentiation syndrome, oxygenation improved, permitting the reintroduction of ATRA. The remainder of her course was notable for an upper-extremity deep venous thrombosis managed with serial Dopplers in place of anticoagulation due to thrombocytopenia and ongoing DIC, along with an asymptomatic 4-mm central nervous system (CNS) bleed.

In high-risk APL, it is reasonable to use an ATRA/ATO backbone, which has established data in the low and intermediate subgroups but remains an outstanding question for use in all risk groups. The addition of gemtuzumab ozogamicin or idarubicin to ATRA/ATO has been shown to yield overall survival >85% in high-risk patients.[4-6] In the APL 15 trial, Wang et al[7] investigated ATRA/ATO with and without chemotherapy for all risk groups. The high-risk group represented about one-third of the patients, and the 2-year disease-free survival was 94% and 87% for the ATRA/ATO and ATRA/ATO/chemotherapy, respectively. This study was designed to be a noninferiority study, which opens the way for further investigation before holding all gemtuzumab ozogamicin or idarubicin.

CLINICAL CASE 1 (continued)

The patient was polymerase chain reaction negative about 36 days from the start of induction and facilitated the transition to outpatient ATO/ATRA consolidation along with continued CNS prophylaxis.

ATO and ATRA consolidation was initiated with ATO 0.15 mg/kg 5 d/wk for 1 to 4 weeks and then repeated every 8 weeks × 4 cycles with ATRA 45 mg/m^2 for 7 days on and 7 days off for 28 weeks. Our group has a dedicated team approach to continue consolidation with a nurse navigator, advanced practice provider (APP), and a malignant hematology physician that leads the care team (see Table 1).[8] We set up thrice-weekly APP visits with electrocardiograms, as well as supportive labs/infusions on site to replete electrolytes in the setting of arsenic. Our supportive ancillary team with nutrition, social work, and palliative care buttressed the clinical management. Our clinic is open 7 days a week to effectively manage these complicated patients and their needs as well as 24-hour/7-day availability of our hematologic malignancy team to any of the surrounding clinics and smaller hospitals to help manage or transfer patients to a central site of care.

The role of maintenance therapy in the era of ATRA/ATO remains controversial if molecular remission is achieved by the end of consolidation.[9] The high-risk population who has had an ATRA/ATO backbone as part of the induction warrants further investigation with regards to the benefits vs toxicity of a maintenance regimen and a corollary of the optimal drugs, whether it be ATRA alone or in combination with ATO.[7]

Table 1. Infrastructure required to support delivery of supportive care after intensive chemotherapy in the outpatient setting

Inpatient management	• Nursing education on treatment, roadmap, expected complications • CVC education and training • Clear written discharge instructions with information for nonurgent and emergent situations • Clear communication with outpatient team
Outpatient management	• SOP for CVC care, antimicrobial prophylaxis, transfusion thresholds, management of neutropenic fever • 24-hour phone access to experienced provider in AML for emergencies • Regular care team available for 3 times per week visits and nonscheduled evaluations of symptoms • Infusion center with extended daily and weekend/holiday hours for frequent monitoring and transfusion • Blood bank with large transfusion capability and rapid delivery of blood products to clinic setting • Ability to rapidly evaluate and initiate treatment of neutropenic fever in clinic (eg, antimicrobial cocktail available for rapid administration before hospital transfer) • Multidisciplinary expertise (infectious disease, pulmonary) in management of AML and therapy complications • Ancillary support staff with expertise in AML management; nursing, social worker, pharmacists, physical therapists, nutritionists

AML, acute myeloid leukemia; CVC, central venous catheter; SOP, standard operating policy.

Ref. Halpern and Walter.[8]

While arsenic does have CNS penetration, there remains a small risk of CNS relapse in patients with a CNS bleed or presenting with a high white count. Many of the large pinnacle APL trials did not offer CNS prophylaxis despite a number of them including high-risk patients, and despite that, very low rates of CNS relapse were noted.[10–12] Since the patient presented with a high WBC and a small CNS bleed, a comprehensive discussion was had with the patient and her family regarding the current data. Due to her age and the negative factors noted, CNS prophylaxis was delivered via the intrathecal route once her coagulopathy and circulating blasts had cleared. She remains in complete remission 3 years out.

CLINICAL CASE 2

A 19-year-old man presented with stage IV diffuse large B-cell lymphoma (DLBCL) with a T-cell–rich component, a 30-cm spleen, a 20-cm abdominal mass, significant pancytopenia, and ascites. Fluorescence in situ hybridization testing revealed rearrangements of C-MYC but failed to show abnormalities in BCL-2 and BCL-6. His bone marrow biopsy specimen was negative. National Institutes of Health pathology review confirmed the initial diagnosis.

Given the presentation and C-MYC rearrangement, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin (R-EPOCH) was chosen for induction therapy with the hope of intensifying doses based on the dose-adjusted algorithm, although cytoxan, doxorubicin, vincristine, prednisone, rituximab (R-CHOP) would be a completely viable and comparable option.[13,14] From a practical standpoint, this critically ill young man needed his first cycle of therapy in the hospital setting due to tumor lysis syndrome, extensive cytopenias, and borderline performance status. This case predated the approval of polatuzamab-cyclophosphamide, doxorubicin, prednisone (CHP), which could also have been a first-line option,[15] but polatuzamab may not be readily available in all hospital settings.

After 3 cycles of dose-adjusted R-EPOCH, he had an improvement in his hematologic parameters, spleen size, and tumor masses, but his disease progressed within weeks of completing his sixth R-EPOCH. Transfusion-dependent cytopenias precluded him from participating in clinical trials, and he underwent splenic external beam radiotherapy to improve his counts followed by second-line therapy with rituximab, ifosfamide, carboplatin, etoposide (R-ICE). Colleagues at an academic center were consulted for stem cell evaluation vs chimeric antigen receptor T cells (CAR-T) depending on response.

An early referral to an academic center at first relapse allows for greater coordination of care since the ultimate path would lead to CAR-T or stem cell transplant (see Figure 1).[16] Having strong relationships with academic centers and disease experts can help facilitate the rapid evaluation of patients in need of advanced therapies, especially since primary refractory DLBCL has broad resistance to cytotoxic therapy.

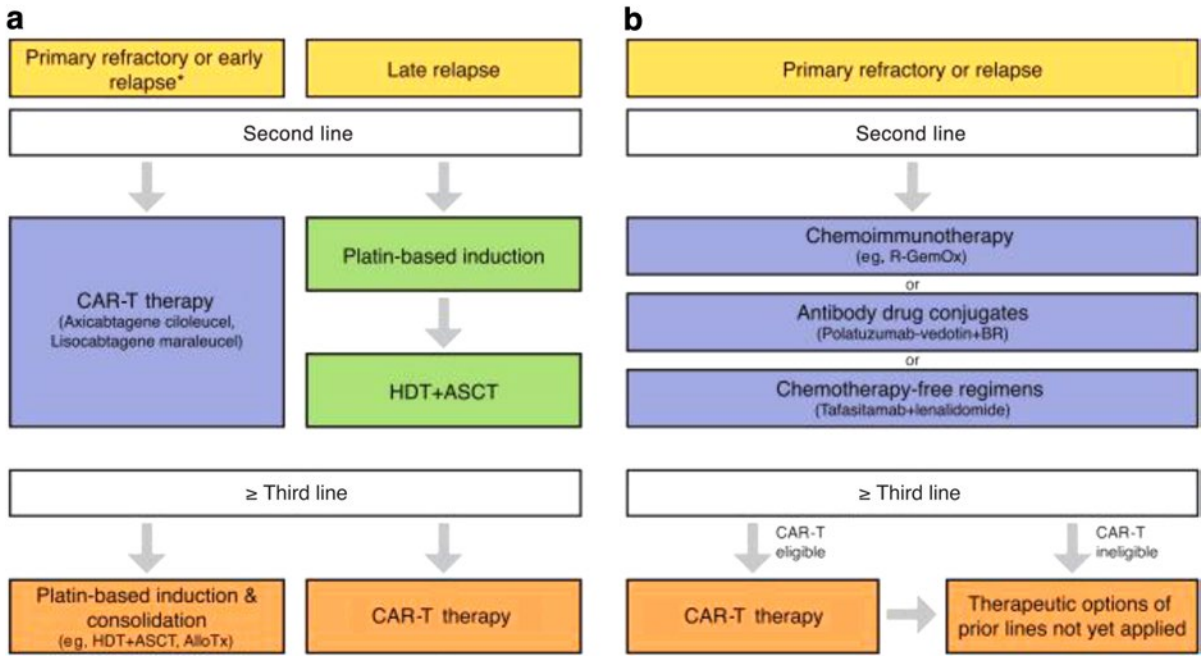

Figure 1. Therapeutic algorithm for patients with relapsed/refractory DLBCL. (a) For transplant-eligible patients, depending on the time point of relapse, either an anti-CD19 CAR-T therapy (using axicabtagene ciloleucel or lisocabtagene maraleucel) or platin-based induction followed by high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) represents the standard approach (*within 12 months after completion of first-line therapy). (b) For transplant-ineligible patients, chemoimmunotherapy, antibody drug conjugates, and chemotherapy-free regimens represent potential therapeutic options in second line. Third-line therapy using anti-CD19–directed CAR-T represents a potentially curative option for eligible patients.

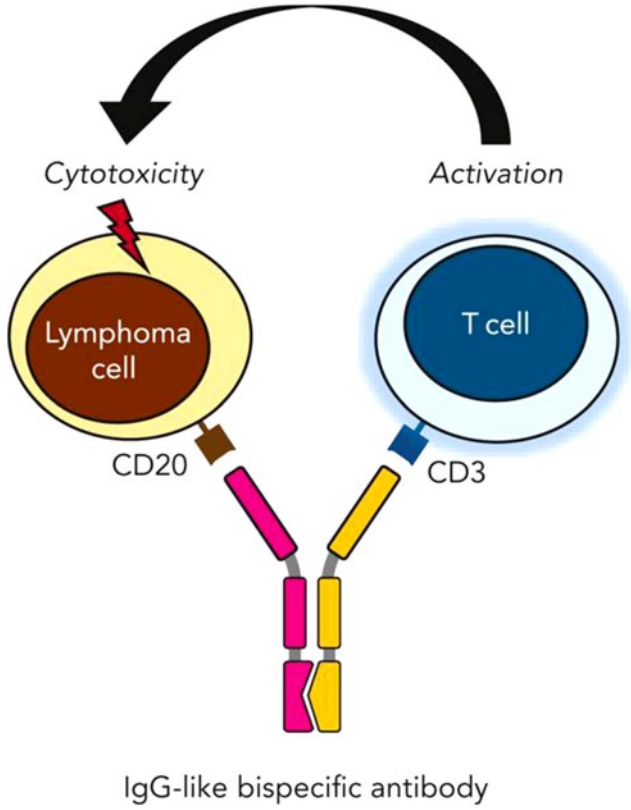

Figure 2. Possessing a Fab specific to an epitope on CD20 of malignant lymphoma cells along with a separate Fab segment for CD3 binding and subsequent T-cell activation, bispecific antibodies facilitate the creation of an immune synapse, resulting in T-cell–mediated cytotoxicity. Falchi et al.[26]

CAR-T has revolutionized treatment for patients with relapsed/refractory DLBCL who cannot attain a complete remission to move to allogeneic hematopoietic cell transplantation (AHCT) or have relapsed after AHCT. While response rates are high, durable remission is achieved in approximately 30% to 40% of patients. Axicabtagene ciloleucel, tisagenlecleucel, and lisocabtagene maraleucel are the 3 anti-CD19 CAR-T therapies that are approved by the US Food and Drug Administration for relapsed DLBCL after ≥2 prior lines of systemic therapy or for primary refractory disease. Significant toxicities such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) can impact up to 20% and 30% of patients, respectively (see Figure 2).[17-20] Accessibility and cost remain barriers to broader utilization as well.

Phase 3 trials are exploring the benefit of second-line CAR-T over standard of care (SOC) followed by AHCT.[21,22] Westin et al[22] show in the ZUMA 7 trial an overall survival benefit with axicabtagene ciloleucel in the second-line setting compared to SOC. However, Bishop et al[21] show in the BELINDA trial that tisagenlecleucel did not show a higher response rate or event-free survival in the AHCT group. Comparison is hindered by the differing number of salvage regimens, bridging therapy, organ-compromising disease, and percentage of patients receiving CAR-T. The PILOT study also offers an evolving CAR-T landscape for transplant-ineligible patients in the second line as well.[23] Our patient's cytopenias precluded pheresis, and rapid disease progression necessitated cytotoxic salvage over moving to CAR-T at this point.

CLINICAL CASE 2 (continued)

In this clinical case, 2 cycles of R-ICE yielded a good partial response but residual hypermetabolic disease on positron emission tomography. However, count improvement allowed T-cell collection for CAR-T and ultimately administration. Unfortunately, his disease relapsed within 30 days. Repeat biopsy showed the absence of CD19, the presence of CD20, and no actionable mutations on next-generation sequencing.

Progression after CAR-T is associated with dismal outcomes,[24] and the loss of surface CD-19 expression may render treatments approved by the Food and Drug Administration, such as tafasitamab-based[25] therapy or loncastuximab tesirine,[25] less effective, emphasizing the need for biopsy-informed therapy. For this reason, polatuzumab-bendamustine, rituximab was given locally. The local team and colleagues at academic centers remained engaged throughout the entirety of his clinical course.

He enrolled in a protocol at a quaternary academic center with an epicortimab/gemcitabine/oxaliplatin trial in place of an epicortimab monotherapy trial locally. He continued to be seen locally to manage complications and transfusions and remain with family.

Durable remissions can be challenging after CAR-T therapy, but bispecific monoclonal antibodies (BsAbs) are offering encouraging results (see Figure 3).[26] With antibodies targeted at the CD20 on DLBCL and the CD3 on T cells, glofitamab has shown complete response in about 39% of heavily treated patients for a median of 12.6 months. However, much like CAR-T, these BsAb therapies have significant toxicities, including CRS and ICANS, which need to be carefully considered and managed.[27]

CLINICAL CASE 2 (continued)

Unfortunately, his disease proved to be resistant to epcortimab and a further salvage with tafasitamab/lenalidomide as a bridge to a trial with allogeneic CAR-T cell therapy. He succumbed to his progressive disease with hepatic failure after an 18-month struggle.

While the outcome in this young adult with primary refractory NHL was quite devastating, his parents and family took solace in the fact that he received novel options and were deeply grateful for our guidance and quaternary care collaboration toward a common goal.

CLINICAL CASE 3

An 84-year-old man with excellent health and an Eastern Cooperative Oncology Group Performance Status of 0 developed mantle cell lymphoma 6 years before presentation to

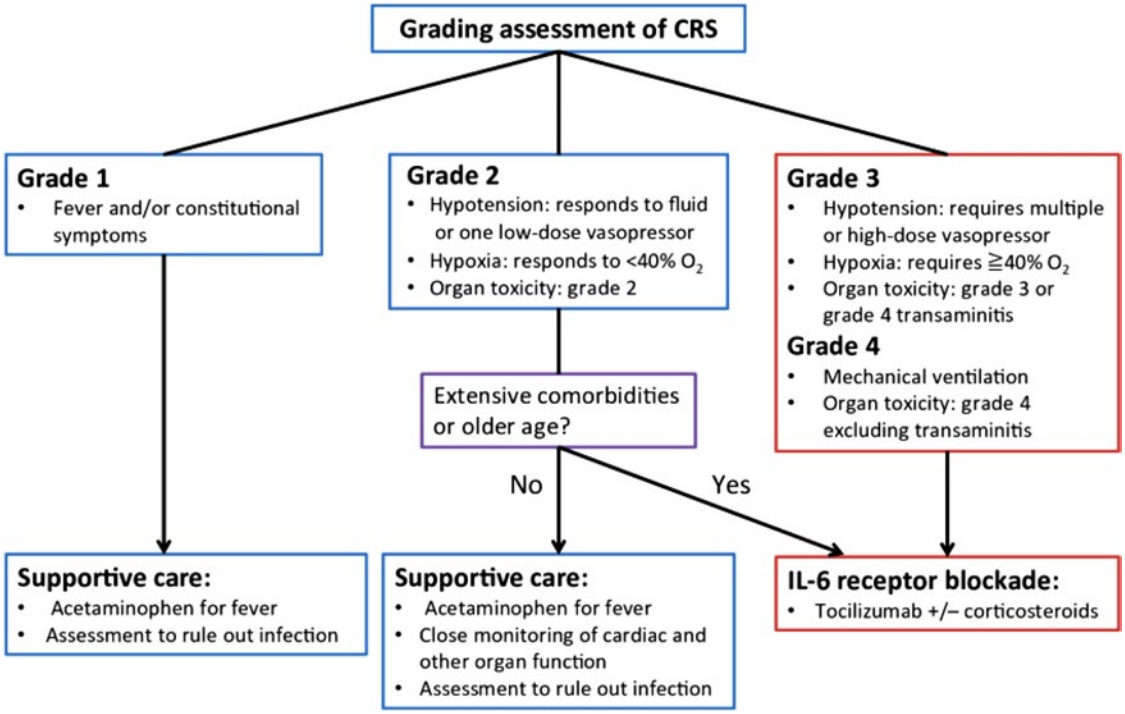

Figure 3. CRS is a known complication of T-cell redirecting therapy, observed in patients receiving CAR-T and/or bispecific antibodies, with clinical manifestations a result of an exuberant immune activation and concomitant proinflammatory cytokine production. Makita et al.[20] Grading is predicated on progressive symptoms from the hyperinflammatory state ranging from fevers to the development of hypoxia and vasodilatory mediated hypotension. Stepwise but rapid escalation of therapy is needed according to the severity of symptoms and is critical to avoid adverse clinical outcomes.

our clinic. He achieved a durable response to rituximab bendamustine followed by rituximab maintenance. Approximately 2 years after completion of maintenance, he developed rapidly progressive abdominal adenopathy with compression of the renal vasculature, resulting in acute kidney injury. Successive trials of acalabrutinib and rituximab lenalidomide were met with an attenuated response, a lymphoma mass effect upon the inferior vena cava resulting in volume retention, and electrolyte derangements.

Relapsed mantle cell lymphoma has been a notoriously challenging disease, with the historical literature reporting 20% to 30% response rates on various combinations of salvage chemoimmunotherapy, discouragingly brief progression-free survival intervals,[28] and the absence of a coalescent approach. Consequently, the efficacy of Bruton tyrosine kinase inhibitors (BTKis) was particularly welcomed. First reported by Wang and colleagues,[29] ibrutinib resulted in unprecedented response rates of 68% and a progression-free survival of 17.5 months. Acalabrutinib[30] and zanubrutinib[31] have similarly improved upon this benchmark and are regularly used by community oncologists, familiar with this class of agents after almost a decade of experience with chronic lymphocytic leukemia. Nonetheless, they are not curative, and at the inexorable progression, there is a dearth of choices. While cellular therapy with brexucaptagene autocel has regulatory approval, the geographic logistics precluded our ability to realistically consider this as an option.[32]

Fortunately, our center was participating in a clinical trial evaluating outpatient administration of a BsAb therapy. The study is pivotally tasked with assessing both efficacy and safety, with emphasis on community-based outpatient management of CRS and ICANS. The patient received the investigational agent on a weekly step-up dosing regimen before commencing the maintenance phase. His course was complicated by grade 1 CRS, managed entirely as an outpatient with supportive care medications, close monitoring with daily phone calls and home vital signs, and immediate office evaluation for concerning symptoms. After the first 3 weeks of therapy, he had complete resolution of his significant lower extremity edema, orthopnea, and acute kidney injury. Surveillance imaging corroborated the clinical improvement with a complete metabolic response on functional imaging, which remains ongoing. It is not difficult to envision a scenario where this patient would have been recommended entirely supportive measures with a transition to end-of-life care in the recent past. The availability of an investigational bispecific antibody at an experienced nonacademic research center, however, was able to meaningfully extend a life of excellent quality in a previously dire scenario.

Discussion

Hematologic malignancies can present in a clinical crisis with a high disease burden, transfusion needs, complicated infections, and rapid proliferation all requiring a rapid activation of medical, diagnostic, and therapeutic interventions. Traditionally, this has been the domain of disease experts in academic centers.

However, greater than 80% of cancer care is provided in the community setting. With the advent of complex, targeted regimens, older and even medically unfit patients may have more options. However, these are the very patients who want to manage therapy in their communities where support is the strongest and quality of life is optimized.[33]

Oncology has had a seismic shift toward personalized care with a better understanding of the molecular drivers of the disease informing treatment choices. In this evolving environment, even within community oncology practices, there is a subspecialization that aligns with therapeutic options similar to academic centers.[34] Outcome data for patients treated at academic centers vs community centers along with clinical trial engagement are confounded by socioeconomic, racial, and financial biases. These same barriers also impact the access of patients to clinical trial enrollment and are now a focus of regulatory authorities and trial sponsors (https://www.fda.gov/consumers/minority-health-and-health-equity/clinical-trial-diversity).

Community-hybrid practices combine medical oncologists, radiation and surgical oncologists, palliative care MD/APPs, genetic counselors, and ancillary supportive care such as nutritionists and social workers to provide comprehensive care locally. With the increase in subspecialization, the number of clinical trials and participation in these trials also rise.[35] Our common goal is to provide patients with excellent cancer care comprehensively with access to clinical trials closer to home, which may lower some barriers to enrollment. Breakthrough therapies may originate in the academic setting, but a closely coordinated clinical and educational paradigm between the community and academic oncologists can help operationalize new therapies quickly and safely locally as well as define options for the academic setting.

The simplest method to achieve this is through direct communication, strong ties, and transparency.

The cost of cancer care is rising exponentially with cellular therapies, targeted drugs, and management of associated therapies in hematologic malignancies, but also in all aspects of oncology. In the era of value-based oncology care, treatments in community-based clinics are more cost-efficient than the same care offered in hospital-based clinics.[36] As the cost of our complicated therapeutics increases, further investigation is certainly warranted for the operationalization of novel therapies quickly and effectively into the community for equity and impact.

Jillella et al[37] provide an effective, collaborative model of early education with their community practices on the identification of APL and access to experts, along with defined treatment algorithms and a comanagement strategy that allows community oncologists to maintain early mortality rates similar to academic institutions. Engagement with community oncologists and their academic peers more intricately can lead to gains toward a common goal with the possibility of lowering costs and increasing clinical trial enrollment locally while ultimately benefiting patients.

Conflict-of-interest disclosure

Dipti Patel-Donnelly: ABIM medical oncology Longitudinal Knowledge Assessment Committee.
Mitul Gandhi: Advisory boards: Janssen Oncology 11/2021, Sanofi 12/2022, Janssen 5/2023.

Off-label drug use

Dipti Patel-Donnelly: none.
Mitul Gandhi: none.

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Multiple myeloma: a paradigm for blending community and academic care

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The care of the multiple myeloma (MM) patient is complex, with most patients requiring multiple lines of therapy over a span of many years to decades. Since the days when autologous stem cell transplantation became the standard of care for a large subset of patients, it was imperative that community practices and specialized academic centers work together to optimize the initial care of patients. Now, with the unprecedented number of treatment options and the introduction of chimeric antigen receptor T-cell therapies and bispecific T-cell engagers, that collaboration has become even more important and stretches from the upfront treatment to the relapsed and refractory disease setting. I will discuss the unique safety profile and logistical aspects that pose challenges and opportunities for the safe and successful delivery of these therapies. Close interaction, communication, and established partnerships between the primary oncologist, the myeloma specialist, and the transplant or immune effector cell provider will be required to provide the optimal care longitudinally for each patient. This multidisciplinary approach to treating MM can serve as a paradigm for blending community and academic care.

LEARNING OBJECTIVES

- Identify best practices for safe and successful delivery of therapies for multiple myeloma
- Identify opportunities to anticipate and address acute phase and late phase toxicity
- Prepare and plan for a clinical model that promotes a good rapport between academic and community practices

CLINICAL CASE

A 66-year-old man is diagnosed with revised international staging system II IgGk multiple myeloma with standard risk cytogenetics after presenting with back and left hip pain. He undergoes induction with bortezomib, lenalidomide, and dexamethasone and achieves a very good partial response (VGPR) after 4 cycles. He is referred to the local transplant center, where he undergoes autologous stem cell transplantation (ASCT). He does well and is maintained on single-agent lenalidomide. Three years later, he has progression indicated by rising paraprotein and begins salvage therapy with daratumumab, carfilzomib, and dexamethasone. He achieves VGPR, but 18 months later, he has elevated calcium and rising paraprotein. He then moves on to elotuzumab, pomalidomide, and dexamethasone. He achieves partial response (PR) but, 12 months later, has progression indicated by rising paraprotein. You begin fourth-line selinexor and dexamethasone. He has a minor response and has progression 4 months later. He is felt to be an excellent candidate for bispecific or chimeric antigen receptor (CAR) T-cell therapy. Are you and your institution ready to proceed with this type of therapy? Will you need to refer to an academic/transplant center? What are the next steps in shepherding this patient safely and successfully through this process?

Introduction

Multiple myeloma (MM) is the second-most common hematologic malignancy and is characterized by clonal expansion of malignant plasma cells.[1] Although largely incurable, the incorporation of ASCT, proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), and anti-CD38 monoclonal antibodies have resulted in continued improvement in overall survival.[2] Unfortunately, despite these advances, the median survival of patients who progress after a PI, IMiD, and anti-CD38 antibody is quite poor.[3] B-cell maturation antigen targeting treatments have emerged as effective antimyeloma therapies in triple class refractory (TCR) MM patients. Two CAR T-cell therapies, idecabtagene vicleucel (ide-cel)[4] and ciltacabtagene autoleucel (cilta-cel),[5] and

one bispecific T-cell engager, teclistamab,[6] are now approved by the US Food and Drug Administration (FDA) for patients in this setting. Several others are being investigated in clinical trials.

ASCT and use of CAR T-cells, and bispecific T-cell engagers may require referral from the community to an academic or transplant center. Although these therapies are not unique to MM, the inability for any of these therapies to be curative on their own will likely mean incorporation of more than 1 of these types of therapies along the treatment course for a patient. Thus, to provide optimal care for a myeloma patient, a good strategy between the community practitioner and the specialist will be of utmost importance at several times along the care of an MM patient. These unique nuances may pose both a challenge and opportunity for delivery and access of care, but MM treatment is poised to serve as a paradigm for the treatment other diseases.

Autologous stem cell transplantation

The initial treatment of a transplant-eligible patient with myeloma currently is likely to require induction chemotherapy followed by ASCT consolidation and finally maintenance therapy.[7,8] This initial line of therapy already requires and establishes the relationship between the treating oncologist and a transplant physician who often is located in a separate practice at an academic center or specialized transplant center. Depending on when the initial referral is made, establishing close communication is key and includes discussion about the specific induction regimen used, the number of cycles needed, and coordination of transfer for leukapheresis and stem cell collection (Figure 1). Sometimes further or alternate induction is needed prior to ASCT, and this is usually delivered at the primary oncologist practice in close coordination with the transplant center. Following ASCT, the close contact continues and includes choice of consolidation and maintenance therapy and posttransplant interventions, such as prophylactic medications and revaccination. On occasion, the decision may be made to collect stem cells to store for future use and not proceed with the transplant at that time. In that scenario, the patient will need to continue further induction and maintenance per the nontransplant standard of care. This paradigm is well established and highlights the close communication between the treating oncologist and the transplant physician for the optimal delivery of care for this first line of therapy already in place.

Autologous CAR T-cell therapy

Without a doubt, the resource-heavy processing and delivery of autologous CAR T products provide unique challenges. A pictorial representation of the basic autologous CAR T process is shown in Figure 2. The initial process best mirrored what had already been established by transplant programs. But, even then, the processes in place required adjustments, and specific immune effector cell programs/paradigms were created. Figure 3 depicts some of the practical and logistic differences between the standard autologous hematopoietic cell transplantation process and the autologous CAR T process. The current indication for use of CAR T-cells in myeloma is in the relapsed and refractory space. Thus, patients often are being evaluated in the setting of relapsing or high burden disease that may make it difficult for the patient to withstand the inherent treatment delays. Furthermore, having undergone multiple lines of therapy over the course of many years, the patient may have developed residual cytopenias and organ dysfunction that may preclude eligibility.

Table 1 depicts some considerations to determine if a patient is appropriate for CAR T therapy. Close attention should be paid to the immediate therapy given prior to planned T-cell collection in order to maximize a functional and efficacious CAR T product.[9-12] After T cells are secured for CAR T manufacturing, there will likely be a 4- to 6-week window of time before CAR T-cells are infused. Many patients will need to undergo bridging therapy to control or debulk their disease to both minimize toxicity and maximize efficacy of therapy. Figure 4 outlines some consideration for selection of the most appropriate bridging therapy, which may be different for each patient. These various time points require very specific decisions and treatments delivered

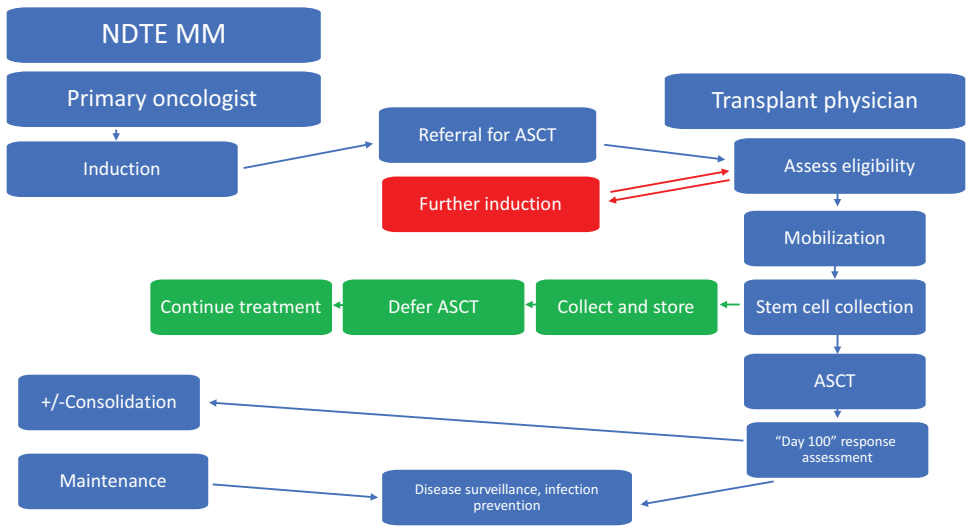

Figure 1. Management of a newly diagnosed transplant-eligible (NDTE) patient with multiple myeloma (MM): key roles of the primary oncologist and transplant physician. ASCT, autologous stem cell transplantation.

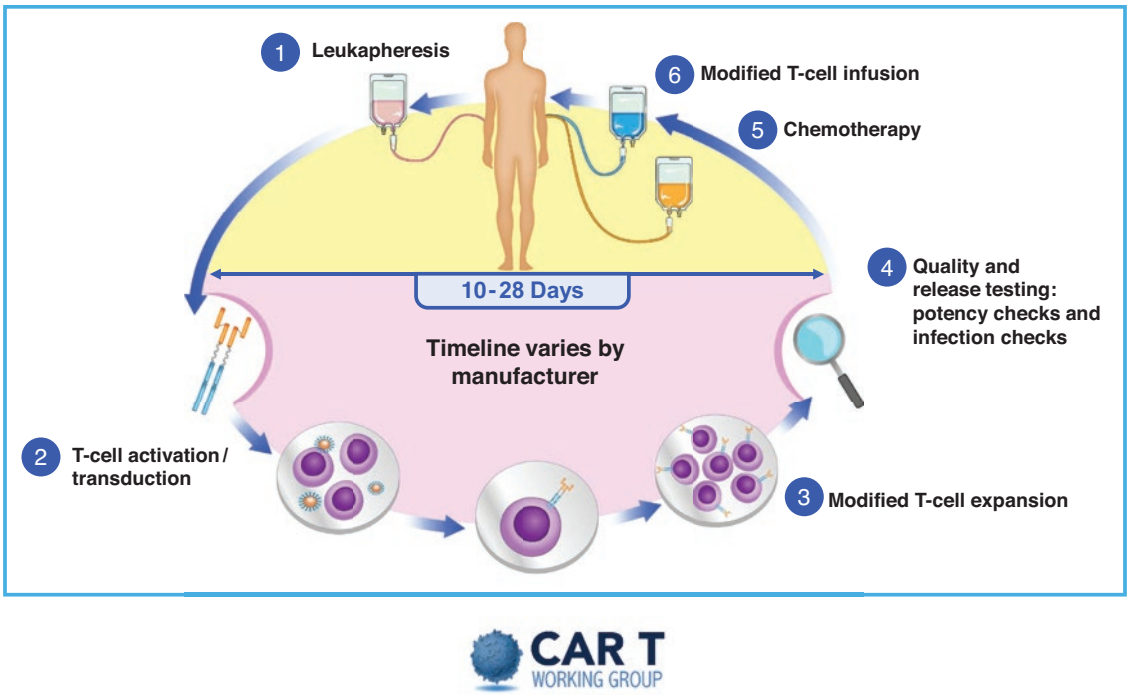

Figure 2. Overview of CAR T therapy. Reproduced with permission from the slide library of the chimeric antigen receptor (CAR) T Working Group (v2 9.4.2019).

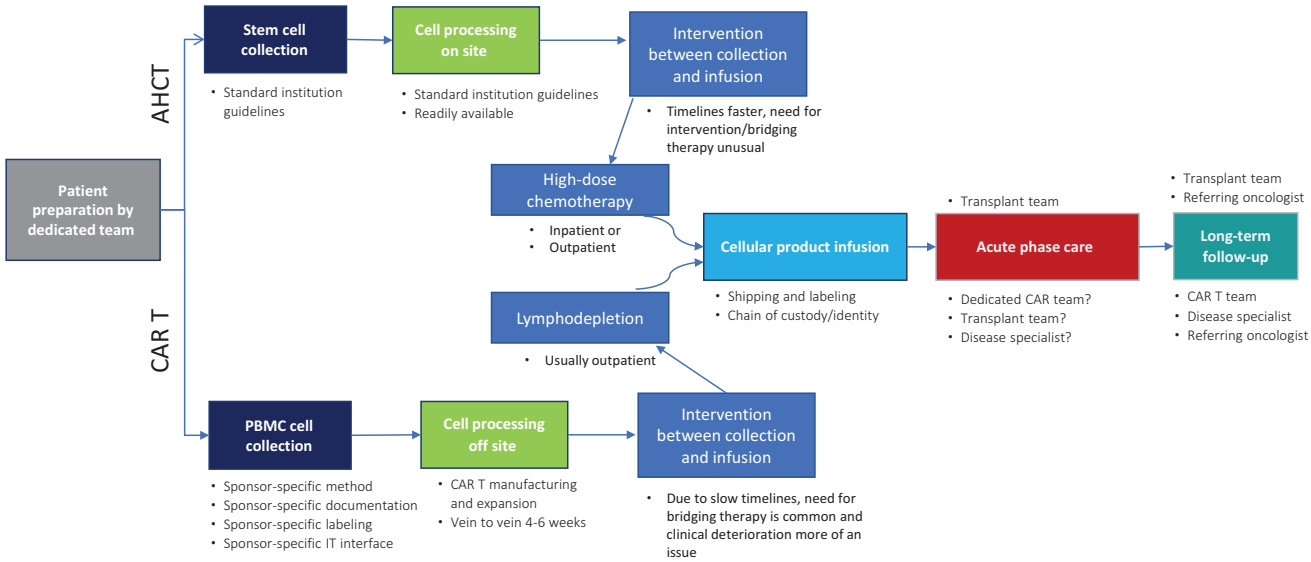

Figure 3. AHCT vs CAR T therapy: similarities and differences. AHCT, autologous hematopoietic cell transplantation; CAR, chimeric antigen receptor; IT, information technology; PBMC, peripheral blood mononuclear cells.

by both the primary oncologist and the immune effector cell provider, which again highlights the importance of close communication and collaboration.

Toxicity profile

The toxicity profile associated with cellular redirecting therapies have been extensively described elsewhere and are summarized in Table 2.[13-16] In general, toxicity can be divided into an acute phase (30 days) and a late phase (beyond 30 days). In the acute phase, cytokine release syndrome, neurotoxicity, and cytopenias predominate. The rapidity and onset of these potential toxicities require expert, multidisciplinary vigilance, availability, and management. Currently, these toxicities are treated mostly in the inpatient setting, but they are slowly starting to be managed as in outpatient settings as well.

For the most part, late-phase toxicity can be dominated by persistent or recurrent cytopenias and increased infection risk. Issues pertaining to infectious prophylaxis, revaccination,

Table 1. Key considerations to determine if CAR T-cell therapy is appropriate for a patient with multiple myeloma

Consideration	Comments
Indication	• Does the patient meet the label indication or clinical trial eligibility?
Kinetics of disease progression	• Is the patent able to come off all therapy, including a 2-week washout, prior to leukapheresis? • Does the patient need alternative therapy prior to CAR T-cell therapy consideration?
Immediate therapy prior to leukapheresis	• How would this affect the ability to successfully manufacture CAR T-cells (ie, obtain sufficient numbers of T-cells and expand)? • Recent alkylators, prior BCMA therapies may impact effectiveness of CAR T-cells.
Need for bridging	• Does patient need bridging therapy while waiting for CAR T-cell manufacturing?
Contraindicated medications	• Immunosuppressants, anticoagulants. • Can these be safely stopped prior to collection and acute treatment phase?
No active infection	• Higher risk of complications if patient experiences CRS.
Adequate organ function (renal, cardiac, pulmonary, BM)	• Is the patient healthy enough to receive LD chemotherapy? • Does the patient have organ function reserve to tolerate toxicities of CR T-cell therapy, namely CRS and ICANS?

BM, bone marrow; BCMA, B-cell maturation antigen; CART, chimeric antigen receptor T-cell; CR, complete response; CRS, cytokine release syndrome; ICANS, immune effector cell–associated neurotoxicity syndrome; LD, lymphodepletion.

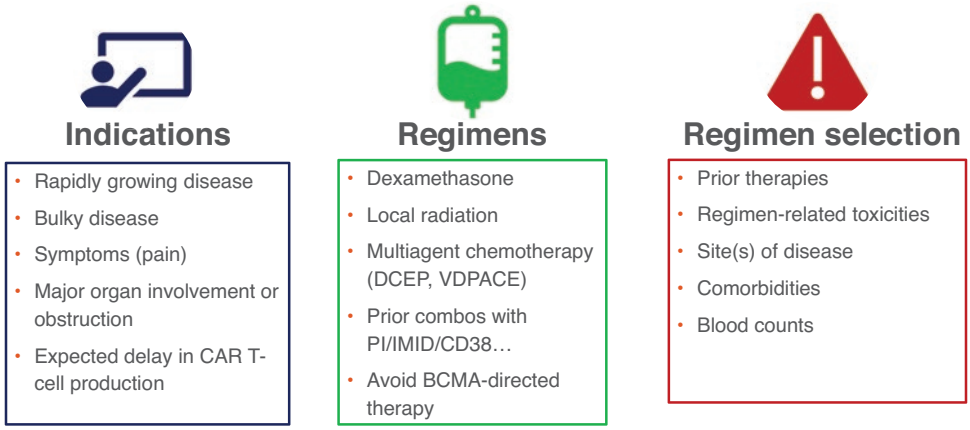

Figure 4. Bridging therapy considerations for CAR T therapy. BCMA, B-cell maturation antigen; CAR, chimeric antigen receptor; CD, clusters of differentiation; DCEP, dexamethasone, cyclophosphamide, etoposide, and cisplatin; PI, proteasome inhibitor; IMID, immunomodulatory drugs; VDPACE, bortezomib, dexamethasone, cisplatin, adriamycin, cyclophosphamide, and etoposide.

immunoglobulin deficiency, and need for replacement predominate. More and more, longer-term sequelae, such as fatigue, memory loss, and lapses in concentration, are starting to become better described.[16-18] Although the optimal management of patients continues to evolve and be defined, there are clear processes and resources required for the safe delivery of these therapies.

Bispecific T-cell engagers

Unlike ASCT and autologous CAR T-cell therapy, which are usually done only once, the bispecific T-cell engagers require continuous therapy, much like other standard myeloma therapies. Furthermore, the off-the-shelf nature of these products significantly simplifies the overall process and allows for prompt initiation of therapy (Table 3) Thus, this therapy has the most potential for direct management and treatment by the community oncologist. However, although acute and late-phase toxicities are similar, both differ qualitatively and quantitatively from CAR T-cells and require careful attention to determine how to proceed next. In fact, the FDA recommends 48-hour hospitalization following each of the first 3 doses (first full dose and 2 step-up doses) of teclistamab. Some practices may continue the paradigm of referring patients to a specialized center for the entire treatment or at least the initial cycle, when the risks of cytokine release syndrome and neurotoxicity and the need for hospitalization may be high. Others practices will develop a spoke and wheel setup, with certain clinics but not others having the expertise to deliver the treatment. It should be noted that long-term data are not available. As studies mature, there is an emerging infection signal that does not abate but continues and may increase for as long as patient is on therapy. Thus, continuous surveillance and monitoring is imperative. The degree of collaboration between the primary oncologist and the academic center will depend on the treatment setting in place to deliver this therapy.

The patient in this case was given the option of enrolling in a clinical trial or proceeding with FDA-approved therapy. The patient opted for teclistamab as a standard of care. In our large

Table 2. CAR T-associated toxicities

Acute phase (Days 0–30)	Late phase (After first 30 days)
• CRS • ICANS • Cytopenias o MAS or HLH is a very rare and severe form o DIC • B-cell aplasia and hypogammaglobulinemia • Life threatening if not managed by expert multidisciplinary team • Tumor lysis is rare and likely varies by disease and disease burden	• Persistent cytopenias • B-cell aplasia and hypogammaglobulinemia o IVIG replacement? • T-cell deficiency o PJP and VZV prophylaxis, other? • Infection prophylaxis • Residual effects of acute toxicity • Delayed CRS and neurotoxicity is rare but can occur • Impairment to QOL—fatigue, memory issues, not yet well described

CRS, cytokine release syndrome; DIC, disseminated intravascular coagulopathy; HLH, hemophagocytic lymphohistiocytosis; ICANS, immune effector cell–associated neurotoxicity syndrome; IVIG, intravenous immunoglobulin; MAS, macrophage activation syndrome; PJP, pneumocystis jirovecii pneumonia; QOL, quality of life; VZV, varicella zoster virus.

Table 3. Bispecific antibodies vs CAR T-cell therapy

	Bispecific antibody	CAR T-cell
Approved product	Teclistimab	Ide-cel and Cilta-cel
Efficacy	+++	++++
CRS	++	+++
Neurotoxicity	+	++
Infections	+++	+++
How given	IC/SC every 1–4 weeks continuous	Only once
Availability	Off-the-shelf	Limited
Need for bridging	No	Often
Where given	Specialized center, Community possible	Specialized center
Unfit patient	Possible	Challenging

CAR, chimeric antigen receptor; CRS, cytokine release syndrome; IV, intravenous; SQ, subcutaneous.

community practice, we currently follow the spoke and wheel model. The patient was able to receive therapy in a clinic only 20 minutes away from his home; although this was not his home clinic, he still did not have to travel 3 hours to get to the nearest academic center.

Conclusion

Since the adoption of ASCT as a standard of care for a subset of patients with MM, primary oncologists have established close relationships with myeloma specialists and/or transplant physicians in order to deliver safe and optimal care for their patients. Now, with the unprecedented number of treatment options and the introduction of CAR T-cell therapies and bispecific T-cell engagers, which have unique logistics and toxicities that span the entire treatment journey of a patient, the need for multidisciplinary collaboration has only strengthened. Close interaction, communication and established partnerships will be required to provide the optimal care longitudinally for any patient. This multidisciplinary approach to treating MM can serve as a paradigm for blending community and academic care.

Conflict-of-interest disclosure

Jesús G. Berdeja's company received research funding in his name from 2 Seventy Bio, Acetylon, BMS, CARsgen, Celgene, CRISPR Therapeutics, Fate Therapeutics, GSK, Incyte, Karyopharm, Novartis, Sanofi, and Teva. His company received consultation funding in his name from BMS, Celgene, CRISPR Therapeutics, Janssen, Kite Pharma, Legend Biotech, Roche, and Takeda.

Off-label drug use

Jesús G. Berdeja: Nothing to disclose.

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Clinical research in the community

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Most patients with high-risk hematologic malignancies are treated in community oncology practices near their residence. This is partly due to patients' ardent desire to be closer to home and trust in local caregivers. Treatments are increasingly complex, even as initial therapy, and more so upon relapse. Improved outcomes in the past decade are largely available through clinical trials primarily offered through academic medical centers. Limited availability of clinical trials at community oncology practices is a major contributor to outcome disparities among minorities, rural, and elderly patients, all of whom are underrepresented in clinical trials. Between 2003 and 2023, the National Cancer Institute (NCI) established programs to address these challenges: the Community Clinical Oncology Program, Minority-Based Community Clinical Oncology Program, NCI Community Cancer Centers Program, and NCI Community Oncology Research Program. However, disparities have persisted, particularly for pharmaceutical-directed clinical research. Lack of representation in clinical research results in data absenteeism, data chauvinism and hallucination, and a delay in treatment availability for high-risk hematologic malignancies in community practice. To address this, the US Congress enacted the Food and Drug Administration Omnibus Act in 2022 to help establish diversity plans that would broaden clinical trial patient enrollment in the United States. We recommend using these initiatives in community oncology practices, including the adoption of the DRIVE strategy in collaboration with pharmaceutical companies, as well as using the NCI-established programs to promote clinical trial availability for patients with high-risk malignancies treated in community oncology practices.

LEARNING OBJECTIVES

- Evaluate opportunities and challenges in clinical trials for patients with hematologic malignancies treated in community oncology practices
- Identify, use, and apply effective strategies to improve availability and enrollment in clinical trials in community oncology practices
- Apply governmental and nongovernmental processes to improve access to clinical trials, health care outcomes and research disparities

CLINICAL CASE

A 54-year-old African American man who lives with and cares for his elderly mother presents with generalized lymphadenopathy, weight loss, and night sweats. Diagnostic lymph node biopsy is pathologically consistent with germinal center diffuse large-cell lymphoma (DLBCL) without mutations or gene rearrangements of BCL-2, BCL-6, or MYC. Upon staging, he was determined to be stage IVB. His Eastern Cooperative Oncology Group performance status was 1, and his age-adjusted International Prognostic Index (IPI) was 3, with an estimated poor survival of 30 months.[1] He was offered participation in an ongoing phase 3 randomized clinical trial of polatuzumab, rituximab, cyclophosphamide, doxorubicin, and prednisone (Pola-R-CHP) versus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) by his community oncologist but declined due to a required 30-mile travel to an academic center, his caregiver responsibilities, and a desire to be treated by his well-trusted local care team. He then received 6 cycles of R-CHOP and achieved complete remission. Nine months later, he relapsed. He is interested in aggressive therapies but wants to remain in the care of his community oncologist.

Opportunities and challenges to clinical research for high-risk hematologic malignancies in community oncology

Availability of clinical trials in community oncology practice

The most common high-risk hematologic malignancies typically requiring intense and complicated therapies are acute leukemias, multiple myeloma (MM), and lymphomas, with approximately 150 000 patients diagnosed annually in the United States,[2,3] most of whom are treated at centers within 5 miles of their residence through their community medical oncologist.[4] However, clinical cancer trials have traditionally been conducted at academic medical centers, while 80% to 85% of cancer patients are treated at community-based clinical practices.[5,6] The current workforce of community medical oncology comprises highly trained physicians with substantial experience and expertise in clinical research, but most do not have access to clinical trials of new and emerging therapies for high-risk hematologic malignancies. Therefore, community-based cancer research is critical in advancing cancer care for the large, diverse patient population receiving treatment in various local health care delivery settings. In addition, the participation of community oncologists and primary care physicians in cancer prevention, control, and treatment trials significantly facilitates the translation of research advances into practice.[7] Most clinical trial enrollees receive their treatment at academic centers, but community oncologists enroll more patients into cooperative trials sponsored by the National Cancer Institute (NCI), with about 65 percent of these patients entering from community-based practices.[8]

Challenges to the availability of cancer therapeutic trials in community oncology practices include inappropriateness of trial designs and significant system-, patient-, and physician-level barriers resulting in less than 5% enrollment.[9]

History of NCI programs designed to facilitate community oncology research

Since 1982, NCI has initiated several programs to improve access to NCI-sponsored oncology clinical research, aiming to engage community physicians and academic medical centers and to facilitate the incorporation of research findings into practice. Two such networks, the Community Clinical Oncology Program (CCOP) and the Minority-Based Community Clinical Oncology Program (MB-CCOP),[10] were created to increase participation in clinical trials. MB-CCOP was developed to provide infrastructure for clinical trials in the institutions that serve communities with large minority and underserved populations. In 2007, NCI expanded its efforts by creating the NCI Community Cancer Centers Program (NCCCP). NCCCP was a pilot, public-private program emphasizing new research and care delivery partnerships with organized patient communities, community-based health care providers, and academic researchers.[11] Self-reported data from NCCCP sites showed an increase of NCI-supported phase 3 studies by 16% compared with 8% nationally and an improvement of patient accrual by 66% compared with 30% nationally. Additionally, enrollment of racial and ethnic minorities in oncology trials increased by 82% (Figure 1). The accrual of patients aged 65 years or older also rose by 221%.[12] In 2014, NCI created the NCI Community Oncology Research Program (NCORP) to further community oncology clinical research. The goal of NCORP is aligning the 2 existing programs, CCOP, MBCCOP, their research bases, and NCCCP. NCORP is intended to build on the strengths of the CCOPs/MBCCOPs and NCCCPs and expand the scope of research to include cancer care

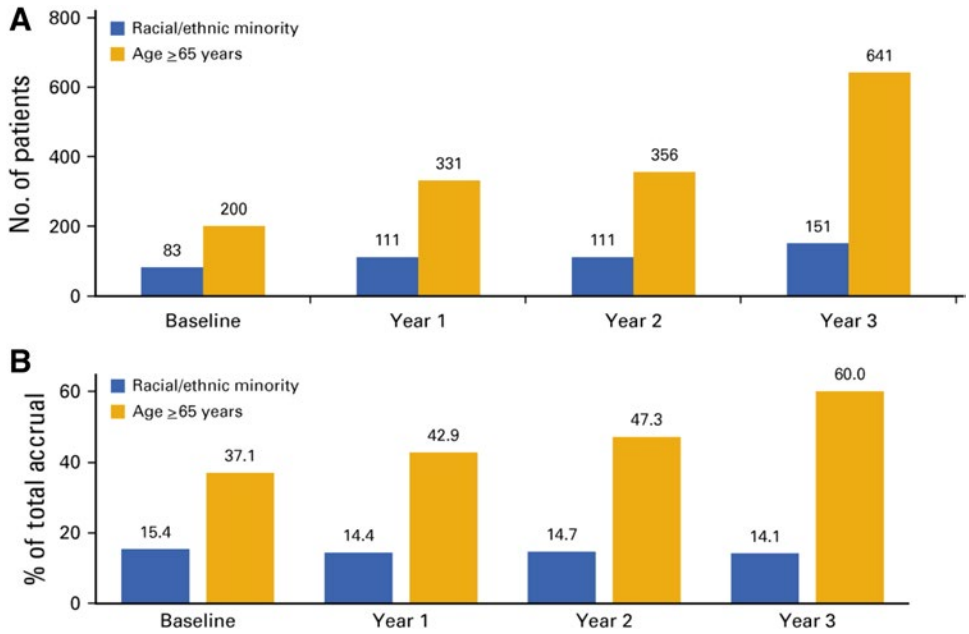

Figure 1. Experience of the National Cancer Institute Community Cancer Centers Program on community-based cancer clinical trials activity. Change in the accrual of underserved patients by (A) number and (B) percentage of total accrual. Reproduced with permission from Hirsch BR, Locke SC, Abernethy AP. Experience of the National Cancer Institute Community Cancer Centers Program on community-based cancer clinical trials activity. *J Oncol Pract*. 2016;12(4):e350–8.[12]

delivery. NCORP is intended to serve as a network to support clinical trials, cancer care delivery, and cancer disparities research. Core principles of NCORP are "including community-based organizations with a variety of research capacities linked to the NCI's Clinical Trials Network; providing support to oncology practices with varied organizational settings as a collaborative network; engaging patients within and outside of clinical trials, organizations, and clinicians as research subjects; encouraging commitment of management within organizations to support the research agenda; and integrating cancer care disparities, care delivery research, and clinical trials."[13]

Disparities in clinical trials

Despite lofty efforts by the NCI, however, clinical trial disparities persist, particularly with novel therapeutics through pharmaceutical industry–sponsored trials and not NCI. A study of 358 trials (pharmaceutical company–sponsored trials, 85; Southwest Oncology Group [SWOG] Cancer Research Network trials, 273; comprising 93,825 patients [pharmaceutical company-sponsored trials, 46,313; SWOG trials, 47,512]) for 15 cancer types from 2008-2018 also found significant underrepresentation of Blacks in pharmaceutical company–sponsored trials compared with SWOG trials (2.9% vs 9.0%), which was consistent across individual cancer types.[14] Race reporting is frequently omitted in clinical trials resulting in regulatory approval but is worse in studies outside regulatory purview. Between 2008 and 2018, only 7.8% of 230 trials (recruiting 112,293 patients) documented the 4 major races in the US. The representation of trial participants was 76.3% White, 18.3% Asian, 3.1% Black, and 6.1% Hispanic. In perspective, compared with their proportion of US cancer incidence, this underrepresents the proportion of Blacks and Hispanics (22% and 44% of expected, respectively) compared with Whites and Asians (98% and 43.8% of expected, respectively).[15,16] This gap in representation is worse for specific tumor types, particularly in prevalence-adjusted participation for cancers that are more common in African Americans.[17] Pooled data from 9 large cooperative group clinical trials in newly diagnosed MM over 2 decades showed only 18% of participants were non-White,[18] shocking for a disease with incidence rates in Blacks more than double those seen in Whites (15.9 vs 7.5 cases per 100,000). This trend also extends to mortality (5.6 vs 2.4 MM deaths per 100 000 for African Americans compared with Whites.[19,20] Additionally, in pivotal trials leading to US regulatory approval of immune checkpoint inhibitors, Black patients constituted less than 4% of enrollees. This is particularly problematic because clinical responses to immunotherapeutic agents depend on unique, individual, frequently racially determined, genetically mediated host and tumor biological interactions.[21]

NCI has also mandated NCI-designated comprehensive cancer centers to create catchment areas that promote increased community participation in clinical research, but recent data in patients with acute myelogenous leukemia showed that over a 15-year study, there were 3041 trial enrollees at US sites; national incidence adjusted enrollment odds by race-ethnicity showed that non-Hispanic Black, non-Hispanic Asian, and Hispanic persons were enrolled at significantly lower rates than non-Hispanic Whites (NHW). Non-Hispanic Native American enrollment was significantly higher. Enrollment odds were lower for non-Hispanic Black, non-Hispanic Asian, and Hispanic enrollees at comprehensive cancer center sites when adjusted by catchment area incidence (Figure 2). Among trial enrollees, there were no univariable predictors of biobank participation; however, NHW race-ethnicity (OR 1.33; 95% CI 1.12, 1.57; $P<0.001$) was associated with correlative study participation. Multivariable models of correlative study participation, with predictors selected based on univariable significance, are for all trial enrollees and when restricted to biobank enrollees; in both cases, the NHW race predicted participation.[22]

The American Cancer Society estimates that 89 380 new cases of lymphomas will be diagnosed in the US in 2023, with 21 080 estimated deaths.[23] Despite improvements in treatments for hematologic malignancies and specifically in lymphomas, disparities in outcomes remain. In non-Hodgkin's lymphomas, recent data showed that compared with White patients at diagnosis, Black patients were younger and more likely to have ≥1 comorbidity, be HIV positive, and have both B-symptoms and stage IV disease (all $p<0.001$). Compared with age-matched White patients, Black patients age ≤60 had worse median overall survival (OS) (46 vs 76 months) along with 5- and 10-year OS (65% vs 69%) (all $p<0.001$). Comparable results were seen for Black and White patients between the ages of 61 and 79 years, but these differences were not demonstrated for patients ≥80 years. On multivariate analysis, Black race was independently associated with worse OS (HR 1.06; CI 1.01-1.10; $p = 0.02$). Interestingly, the propensity-matched analysis demonstrated no significant OS difference between Black and White patients (median 127 vs 117 months; HR 1.0; CI 0.94-1.06; $p = 0.90$).[24] Comparable results were also seen in patients with chronic lymphocytic leukemia (CLL). Results from the National Cancer Database of CLL patients diagnosed from 2004-2018 showed that White patients compared with Black patients had a shorter median OS of 7.0 years (CI 6.7-7.3 years) versus White patients' (9.14 years [CI 9.0-9.3]); $p<0.001$), as well as inferior OS at 5 years (61% vs 69%) and 10 years (36% vs 46%), $p<0.001$. On a multivariate analysis adjusted for age and Charlson-Deyo score, Black race was independently associated with shorter OS (HR 1.51 [CI 1.46-1.57]; $p<0.001$). Referenced to the White population, Black patients diagnosed between 2004 and 2006 had an HR of 1.64 (CI 1.52-1.76) for mortality, and those diagnosed between 2016 and 2018 had an HR of 1.64 (CI 1.44-1.85).[25] This was a surprising finding, given the availability of Bruton's tyrosine kinase and BCL-2 targeted inhibitors in treating CLL over the same period. Pivotal studies of targeted agents currently approved for CLL in the US enrolled a limited number of Black patients.[26]

Racial disparities are due to several factors, chiefly the lack of access to high-quality care across the cancer continuum, including participation in clinical research. Due to emerging complex treatment protocols, this issue is particularly important in high-risk hematologic malignancies. Thus, strategies to decrease these disparities must include an attempt to close the research participation gap. However, increasing access alone is insufficient to close these gaps. For example, even among individuals with a median annual household income of ≥$75,000, 5-year relative cancer survival is lower among Black people (67%) than among White people (72%[27]).

Clinical trial disparities also result in the creation of 3 main problems of health care big data disparities: data absenteeism (lack of representation from underprivileged groups), data chauvinism (faith in the size of data without considering quality and contexts),[28] and, in its most extreme form, what we now term

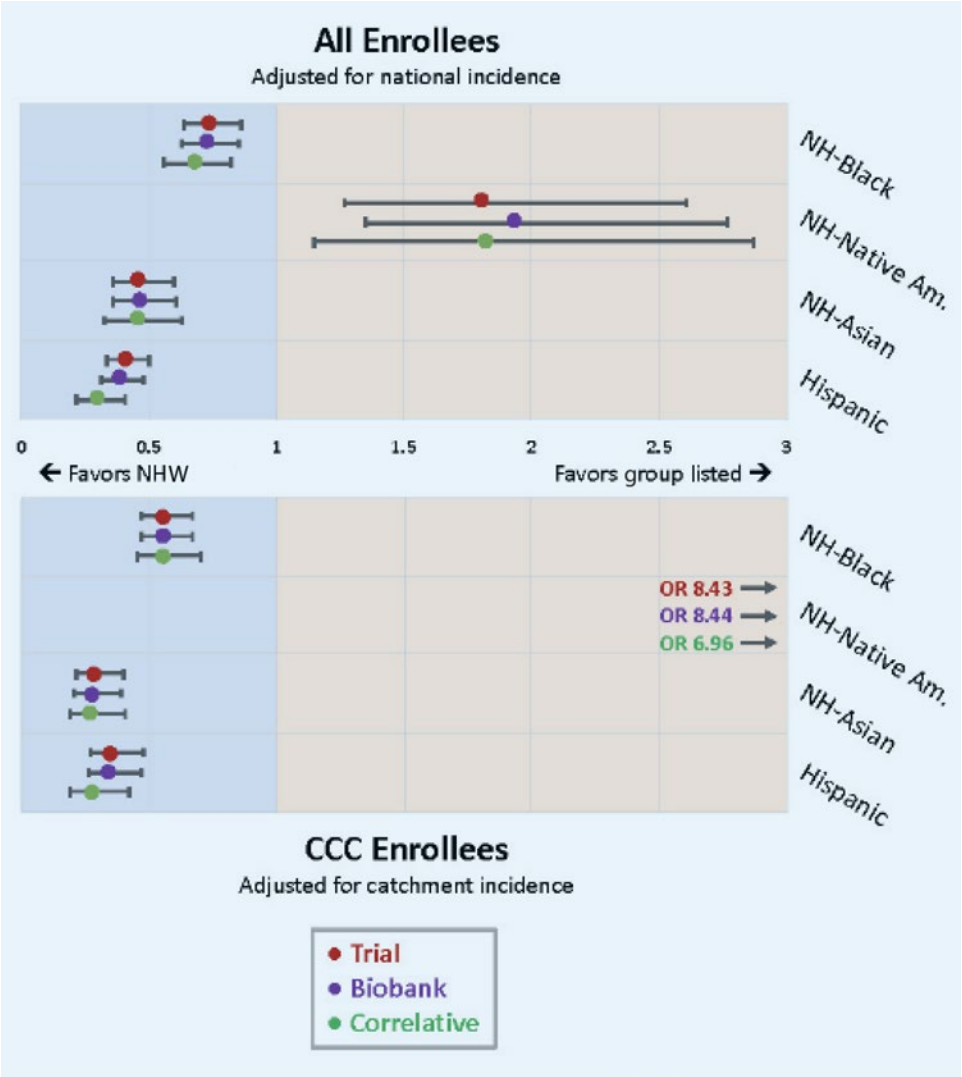

Figure 2. Enrollment odds versus non-Hispanic Whites. Reproduced with permission from Hantel et al.[22]

data hallucination (the assumption and use of nonexistent data to generalize treatment recommendations, outcomes, and results). For illustration and relevant to our clinical case, examples of these problems are seen in the clinical studies of polatuzumab[29] in the initial treatment of DLBCL (7% Black and Hispanic enrollment), chimeric antibody receptor T-cell therapy in relapsed DLBCL (5% Black and Hispanic enrollment),[30] and the generation of the widely used IPI[31] respectively (no racial or ethnicity data reported).

Barriers to participation in clinical trials[32]

Barriers to participation in clinical trials (Figure 3) typically disproportionally affect minority patients and ultimately results in delayed accrual, delayed generation of clinical data, and the generalizability of such data to all persons, thus promoting outcome disparities. Therefore, addressing such barriers may also improve cancer population outcomes. Improving clinical trial availability in community oncology practices where most cancer patients are treated can uniquely improve and address these disparities.

Structural barriers

1. Access to a clinic can be influenced by many structural factors, such as transportation, travel costs, access to insurance, and availability of childcare
2. Availability of a clinical trial for the patient's histology and stage

Clinical barriers

1. Narrow eligibility criteria

Physician attitudes

1. Physician decision or preference, a primary reason for nonparticipation in half the patients for whom a protocol was available and the patient was eligible
2. Lack of appropriate incentives to participate in clinical research
3. Time-consuming trial paperwork
4. Obtaining informed consent

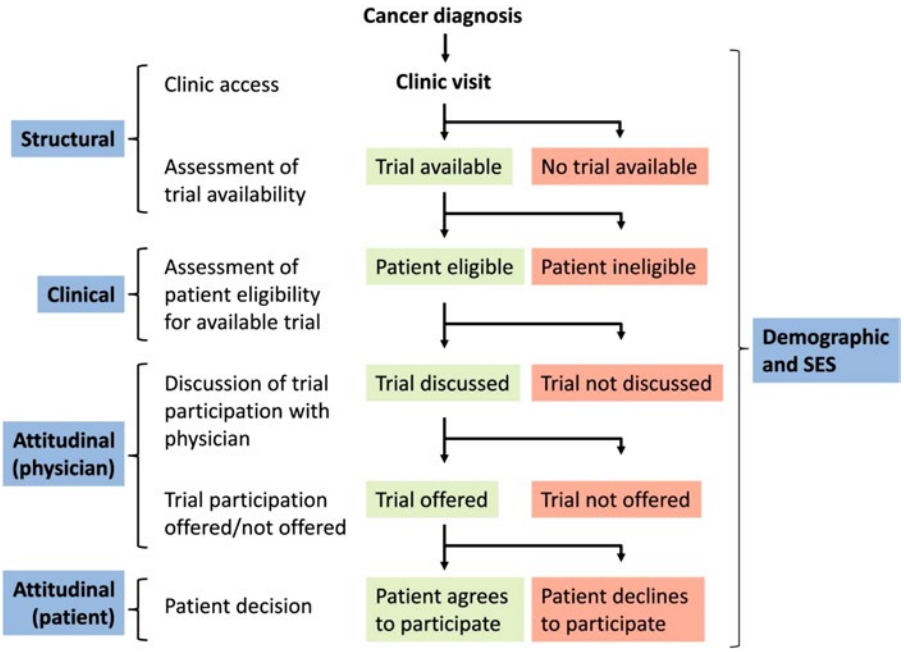

Figure 3. Model pathway of trial enrollment process. SES, socioeconomic status. Reproduced with permission from Unger JM, Cook E, Tai E, Bleyer A. The role of clinical trial participation in cancer research: barriers, evidence, and strategies. *Am Soc Clin Oncol Educ Book*. 2016;35:185–198.[32]

Patient attitudes

1. Patient unease or fear about the prospect of participating in clinical trials, including residual mistrust of medical science due to past abuses such as the infamous Tuskegee Syphilis Study or the history of human experimentation with radiation following World War II
2. Complicated consent forms
3. Patient dislike of randomization
4. Patient's unease about potentially toxic effects of chemotherapy in trials, especially for experimental therapies
5. Patient's ardent desire for a particular treatment they wish to receive after discussion with their physicians
6. More frequent monitoring than nontrial care, for example, traveling to and from the clinic
7. Concern about how to pay for trials

Demographic and socioeconomic disparities

1. Older age
2. Race
3. Low socioeconomic status

Overcoming barriers to participation in community clinical research for high-risk hematologic malignancies

Our patient illustrates the challenges of clinical trial participation for minority high-risk lymphoma patients treated in the community. First, the lack of minority enrollment in major clinical trials prevents us from truly estimating his prognosis using the IPI, potentially resulting in us underestimating his clinical risk (data hallucination). Second, significant structural and personal barriers precluded his participation in clinical studies that could have improved his survival. Additionally, upon relapse, his access to more complicated but potentially curative therapy was impacted by the lack of this therapy's immediate availability in the community due to the limited participation of community oncologists in CAR-T trials, thus impacting its clinical availability at community practices and resulting in further outcome disparities. Thus, strategies to overcome and minimize these barriers are desperately needed to improve outcomes for high-risk hematologic malignancies. These strategies include enhancing community oncology access to clinical trials and the participation of diverse populations, including our patient.

We recommend the adoption of our 5-step strategy at research sites and sponsors to overcome these disparities and to promote clinical research in community oncology practices. DRIVE[33] is a practical, immediately actionable 5-step strategy for promoting and improving diversity, equity, inclusion, and access (DEIA) in clinical trials for minority patients. DRIVE aims to use these strategies to correct inequalities and promote the overall health of humankind, as established in the Greenberg Report,[34] equating diversity and safety due to the potentially generalizable clinical data generated. Additionally, DRIVE uses proven principles and techniques to create meaningful improvements in cancer care and outcomes.

DRIVE

D: Diversity officer for clinical research studies; to develop an actionable, flexible, and prospective diversity plan for clinical research. Diversity officers should be funded by study sponsors and institutions as part of the clinical trial enterprise, very similarly to funding of data safety and monitoring boards.

R: Ranking of clinical studies for diversity; generating an informational tool for determining clinical research diversity (Figure 4). Ranking should be based on independent audits of self-reported

Score calculation:

1. Identify disease burden (eg, prevalence) by demographic mix, in this case race and ethnicity.

2. Calculate the proportion of the disease burden that minority groups* account for individually and as a group (ie, the proportion of the disease burden for all minority groups combined).

3. Calculate the proportion of the trial enrollees that minority groups account for individually and as a group (ie, the proportion of enrollees for all minority groups combined).

4. Divide the proportions of enrollees from each group, and minority groups combined, by their proportionate disease burden (eg, if Hispanic disease burden is 18% and Hispanic enrollment is 12%, 0.12/0.18 = 67%). This proportion is referred to as the diversity proportion.

Scoring:

Score†	Overall diversity proportion	Individual diversity proportions by race-ethnicity
0	≤20%	N/A
1	21%-40%	None >50%
2	21%-40%	At least 1 proportion >50%
3	41%-60%	At least 2 proportions >60%
4	61%-80%	At least 3 proportions >80%
5	>80%	At least 3 proportions >80%

Figure 4. DRIVE rank score. *Minority groups in the US are self-defined by the participants and are listed as follows: Hispanic White and Hispanic and non-Hispanic African American or Black, Native American, Asian, Pacific Islander, and mixed race. In other countries, minorities should be defined as appropriate, based on societal norms and internationally medically acceptable groups/nationalities. †Studies will be ranked at the next lower rank if all criteria for next higher rank are not reached. Reproduced with permission from Birhiray and Birhiray.[33]

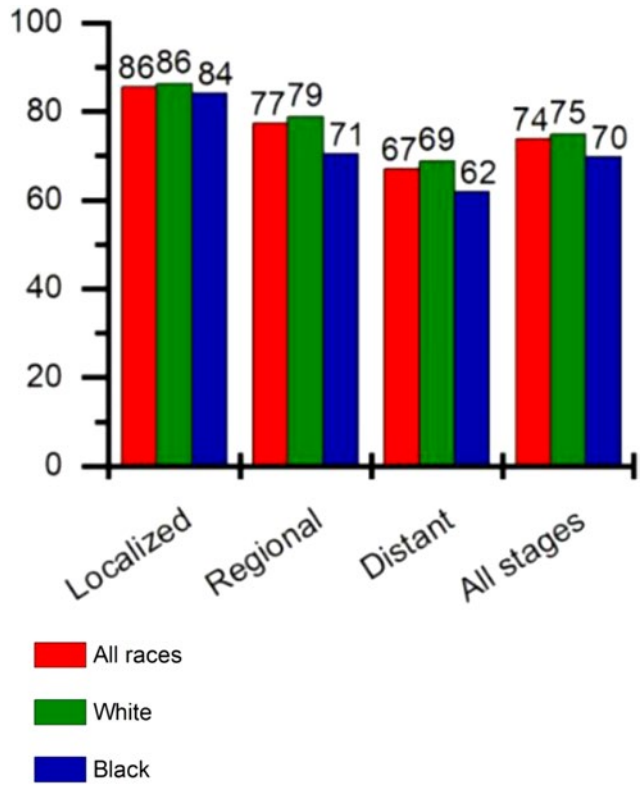

Figure 5. Lymphoma survival. Five-year relative survival for selected cancers by race and stage at diagnosis, United States, 2012 to 2018. White and Black race categories are exclusive of Hispanic ethnicity. Reproduced with permission from Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17–48.[23]

accrual data and generated by DEIA promoting heath care organizations and major medical societies.

I: Individual DEIA plan; created by each investigator to promote enrollment of diverse subjects.

V: Verification of study diversity; creating a process for confirming research diversity. Verification should be based on the same auditing used for evaluating clinical trial data for efficacy and safety.

E: Elevate and enhance training of minority investigators and research team members; to promote minority patient participation by all clinical trial stakeholders

Conclusion

Strategies to enhance the participation of patients with high-risk malignancies in community practices can address health disparities and promote the early adoption of potentially curative therapies in community oncology practices. Outcomes for lymphomas are significantly affected by race, as shown in the clinical case presented, as well as in the other high-risk hematologic malignancies (Figure 5). Thus, efforts to overcome barriers that potentially prevent clinical trial availability and enrollment in community oncology practices are important steps in this direction. Our recommendations include adoption of the DRIVE strategy, as well as a full-throated adoption of the elements promulgated in the US Food and Drug Administration Omnibus Act of 2022 by the pharmaceutical industry, including the creation of a prospective diversity plan for major clinical trials and expanding clinical trials to community oncology practices. Additionally, we suggest promoting the NCORP program's goals. These steps would further enhance clinical research availability in community oncology for high-risk hematologic malignancies.

Conflict-of-interest disclosure

Maya N. Birhiray is the daughter of Ruemu E. Birhiray.
Ruemu Ejedafeta Birhiray: no competing financial interests to declare.
Maya Nicole Birhiray: no competing financial interests to declare.

Off-label drug use

Ruemu Ejedafeta Birhiray: Nothing to disclose.
Maya Nicole Birhiray: Nothing to disclose.

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Current use of bispecific antibodies to treat multiple myeloma

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Targeted immunotherapy has significantly improved the outcome of patients with hematological malignancies by leveraging the power of the immune system to eliminate tumor cells. In multiple myeloma (MM), bispecific T-cell engagers (BsAb) targeting B-cell maturation antigen (BCMA), G protein–coupled receptor, class C, group 5, member D (GPRC5D), and Fc receptor-like 5 (FcRL5) have already demonstrated remarkable clinical activity in triple-class refractory patients. However, responses to BsAb are not universal, and resistance often emerges while on therapy. Mechanisms mediating resistance are tumor intrinsic or immune dependent. Reported tumor intrinsic factors include antigenic loss (biallelic or functional) through deletions or mutations of target genes, increased soluble BCMA (for BCMA targeting BsAb), high tumor burden, and extramedullary disease. Immune-mediated resistance are largely dependent on T-cell fitness and tolerant immune environment. Understanding these mechanisms will allow the design of optimized BsAb therapy and an informed approach to sequencing and combining these molecules with other anti-MM agents and immune therapies.

LEARNING OBJECTIVES

- Current use, efficacy, and sequencing of bispecific antibodies in multiple myeloma
- Understanding the mechanisms of action and escape to bispecific antibodies in multiple myeloma

CLINICAL CASE

A 76-year-old female with Revised International Staging System (R-ISS) stage III multiple myeloma (MM) has disease relapse following four lines of therapy, including cyclophosphamide, bortezomib, and dexamethasone (first line), daratumumab, lenalidomide, and dexamethasone (second line), pomalidomide and dexamethasone (third line), and carfilzomib and dexamethasone (fourth line). This patient with penta-refractory MM enrolls in a clinical trial and receives anti-BCMA bispecific antibody as her fifth line of therapy. She attains complete remission with a duration of response of 12 months, after which she has a recurrence of her disease.

Introduction

The advent of T-cell redirecting immunotherapies such as chimeric antigen receptor T cells (CAR T) and bispecific antibodies (BsAb) has revolutionized the treatment of multiple myeloma (MM). While these therapies have demonstrated promising results in inducing deep remissions in patients with relapsed refractory MM, universal disease responses remains a challenge and relapses invariably occur. Clinical trials of single-agent CAR T or BsAb targeting MM-specific antigen in heavily pretreated patients resulted in high responses with variable duration of remission lasting from a few months to over two years.[1-8] Given the increasing number of CAR T and BsAb agents at various stages of clinical development in MM, there is a pressing need to integrate biological correlates and clinical parameters to generate predictive markers that can guide the selection of therapy sequences and optimal combinatorial strategies for individual patients. This article focuses on the current use of BsAb in MM, reviewing the pre-clinical and clinical studies that support data-driven clinical decisions in the rapidly advancing era of immunotherapy.

Mechanism of action of BsAb

T-cell–engaging BsAb are synthetic molecules that facilitate the interaction between effector T cells and target cells, promoting the eradication of tumors through T-cell–mediated cytotoxicity. By inducing target and effector cells proximity, BsAb support the formation of cytolytic immunologic synapses. This leads to the release of

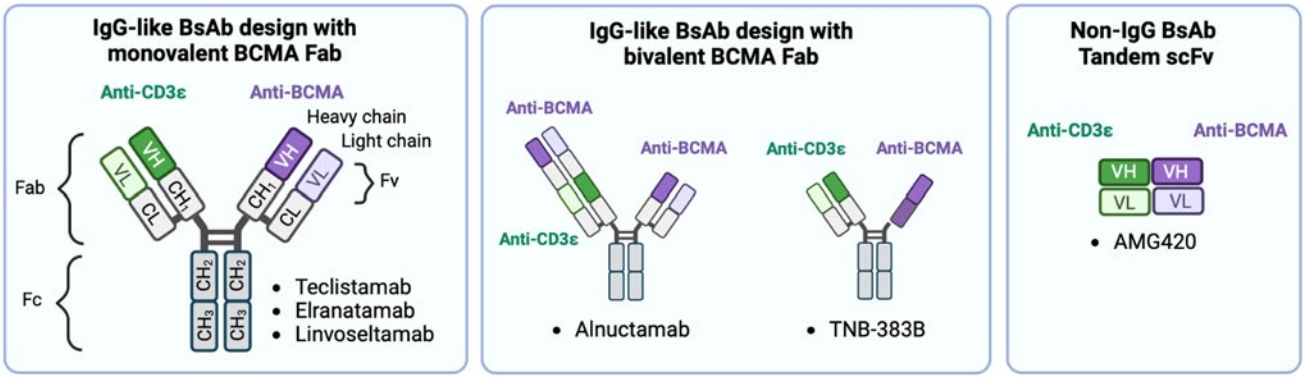

Figure 1. Structure of anti-BCMA bispecific antibodies. Anti-BCMA BsAb can be classified into immunoglobulin G (IgG)-like and non-IgG-like based on their structures. IgG-like BsAb consist of antibody binding fragments (Fab) which recognize target antigens, and a crystallizable fragment (Fc). In addition to having one Fab targeting CD3 (anti-CD3ε), BsAb with monovalent BCMA Fab consists of one Fab targeting BCMA (anti-BCMA). BsAb with bivalent BCMA Fabs are designed with either two anti-BCMA Fabs or two anti-BCMA variable domain of heavy chains (VH). Non-IgG BsAb, AMG420, is synthesized as tandem single chain Fvs and lacks Fc. CH, constant domain of heavy chain; CL, constant domain of light chain; Fv, variable fragment; VL, variable domain of light chain.

cytotoxic effectors such as perforin and granzyme B from the activated T cells, triggering tumor cell apoptosis and pyroptosis. In contrast to canonical T-cell activation, which requires T-cell receptor (TCR) recognition of a peptide bound to major histocompatibility complex (MHC) (signal 1) and co-stimulation (signal 2), BsAb mediate both TCR and co-stimulation independent polyclonal T-cell activation.

BsAb exert their therapeutic effect based on their unique structural design, which includes variations in epitope specificities and affinities, IgG subclasses, Fc modifications, and Fab valency, among others. While over 100 different BsAb forms are developed, they can be broadly categorized into IgG-like and non–IgG-like formats, based on the presence or absence of the fragment crystallizable domain (Fc). Currently, all the BsAb in active clinical development for MM have IgG-like structures, with one Fab targeting CD3 on T cells and the other targeting the MM cell surface antigen, such as BCMA (Figure 1). The most advanced anti-BCMA BsAb in clinical development for MM, teclistamab, elranatamab, and linvoseltamab, have monovalent anti-BCMA Fab, while alnuctamab and ABBV-383 contain bivalent BCMA binding fragments. In contrast, the anti-BCMA BsAb AMG420, initially developed as a non–IgG-like format in a tandem single-chain variable fragment (scFv) lacking Fc region, had reduced serum half-life, which necessitated frequent administration schedules. Consequently, the development of AMG420 and its variations have been discontinued.[9]

The therapeutic activity of BsAb relies on the expression of target antigens on the surface of MM cells. BCMA, a type III transmembrane protein, is highly expressed on malignant plasma cells and promotes prosurvival signals upon ligand binding. Other targets for T-cell immunotherapy development with BsAb include G protein–coupled receptor, class C, group 5 (GPRC5D), fragment crystallizable receptor-like 5 (FcRL5, also referred to as FcRH5), and CD38.[10] While CD3-expressing T cells are the primary targets for effector cell engagement, several preclinical studies are currently assessing the tumoricidal efficacy of BsAb that bind to CD16A, NKp30, NKG2D, or MICA for recruiting innate immune effectors such as NK cells. A summary of the BsAb currently in clinical development is provided in Table 1.

Current use of bispecific antibodies

Until recently, patients with triple-class- or penta-refractory MM had limited options for salvage therapy, leading to poor clinical outcomes with median overall survival rates of less than 12 months and 6 months, respectively.[11] The introduction of T-cell–based anti-MM immunotherapies into clinical practice was highly anticipated with the prospect that therapeutically reinstating the host anti-cancer immunity could induce deep remissions in patients with end-stage myeloma.

Teclistamab is the first anti-BCMA BsAb that has been approved by regulatory agencies for the treatment of patients with MM who have received 4 or more lines of therapies including proteasome inhibitor (PI), immunomodulatory agent (IMiD), and anti-CD38 monoclonal antibody. The multicenter phase 1/2 MajesTEC-1 trial, which included 165 patients, including 77% and 30% of triple-class and penta-refractory patients, respectively, demonstrated that single-agent weekly administration of teclistamab (1.5 mg/kg) generated an overall response rate (ORR) of 63.0%. At a median follow-up of 14.1 months, the median duration of response was 18.4 months, with a median progression-free survival (PFS) of 11.3 months.[2] Subgroup analysis of MajesTEC-1 revealed that patients with high-risk disease features, including R-ISS stage III, extramedullary plasmacytoma, and increased bone marrow plasma cell burden ≥60%, tended to have lower response rates compared with those with standard-risk MM.[2,12] Of interest, while advanced-stage ISS is traditionally associated with poor outcome with standard myeloma therapeutics, the response rates of patients with high-risk cytogenetics was comparable to those with standard-risk cytogenetic profiles.[2] Albeit small patient numbers, the data suggest that traditional tumor cytogenetic risk profiles may have a less important role in stratifying patient response to BsAb therapies.

In the ongoing phase 1 MagnetisMM-1 study of elranatamab,[13] among 55 patients (91% triple-class refractory) at a median follow-up of 12 months, ORR was 64%, with 38% achieving ≥ complete response. Importantly, this trial included 13 patients with prior BCMA therapies, including antibody drug conjugate, CAR T, or both. Within this subgroup, overall response rate was 54% (7/13), with 6 patients achieving ≥ very good partial

Table 1. T-cell–engaging bispecific antibodies in clinical development

Bispecific antibody	Target	Format	Valency	Route of administration	Properties
Teclistamab (JNJ-640079957)	BCMA × CD3	IgG4 duobody	Monovalent CD3 binding Monovalent BCMA binding	Subcutaneous	Fc mutated ↑ half-life, and ↓ immunologic effector function–mediating CRS
Elranatamab (PF-06863135)	BCMA × CD3	IgG2a BsAb	Monovalent CD3 binding Monovalent BCMA binding	Subcutaneous	Fc mutated ↑ half-life, heterodimerization, and ↓ immunologic effector function–mediating CRS
TNB-383B (ABBV-383)	BCMA × CD3	IgG4 BsAb	Low-affinity CD3 binding Bivalent BCMA binding	Intravenous	Decouples T-cell activation from CRS and activates Teff over Tregs
Linvoseltamab (REGN5458)	BCMA × CD3	IgG4 BsAb	Monovalent CD3 binding Monovalent BCMA binding	Intravenous	Fully human, VelociBi™ Fc silent region, mutation in protein A binding site
Alnuctamab (CC-93269)	BCMA × CD3	IgG1 BsAb, asymmetrical 2 + 1 format	Monovalent CD3 binding Bivalent BCMA binding	Intravenous/ subcutaneous	Heterodimeric mutated Fc with intact FcRn, and ↓ immunologic effector function–mediating CRS
Talquetamab (JNJ-64407564)	GPRC5D × CD3	IgG4 duobody	Monovalent CD3 binding Monovalent GPRC5D binding	Subcutaneous	Fc-mutated ↑ half-life, and ↓ immunologic effector function–mediating CRS
Forimtamig RG6234 (RO7425781)	GPRC5D × CD3	IgG1, asymmetrical 2 + 1 format	Monovlent CD3 binding Bivalent GPRC5D binding	Intravenous/ subcutaneous	Fc-mutated (silent Fc) ↑ half-life
Cevostamab (BFCR4350A)	FcRL5 × CD3	Humanized IgG1-based Ab	Monovalent CD3 binding Monovalent FcRL5 binding	Intravenous	FcRL5 expressed on B and plasma cells

BCMA, B-cell maturation antigen; CD3, cluster of differentiation 3; CRS, cytokine release syndrome; FcRL5, fragment crystallizable receptor-like 5 (FcRL5) also referred to as FcRH5; GPRC5D, G protein–coupled receptor, class C, group 5.

response. Similarly, in the MagnetisMM-3 phase 2 study cohort of BCMA-naïve patients (N = 123), ORR was 61%. Predictors of poor response included ISS stage III disease and the presence of extramedullary disease. At a median follow-up of 10.4 months, median PFS and overall survival were not reached.[5]

The MonumenTAL-1 trial examined the safety and efficacy of talquetamab, an anti-GPRC5D BsAb in patients with a median of 6 prior lines of therapy.[4] At the active subcutaneous and intravenous doses, 68% and 72% of patients responded, respectively. At 11.7- and 4.2-month follow-up for the 405 µg/kg and 800 µg/kg doses, the response was 10.2 months and 7.8 months, respectively. Responses were seen in 55.6% to 66.7% of patients with high-risk cytogenetics, 40% to 45% of patients with extramedullary disease, and 50% of patients previously treated with anti-BCMA CAR T or BsAb.[4]

In addition to the aforementioned BsAbs listed, other BCMA (linvoseltamab, alnuctamab, ABBV-383) and non-BCMA targeting BsAb (cevostamab, forimtamig) have reported similar efficacy in advanced MM.[7,8,14-16] As clinical trials continue to provide encouraging updates on the efficacy of single-agent BsAb in inducing deep remission in heavily pretreated MM patients, important questions remain regarding the optimal duration of therapy (fixed vs. continuous schedules), sequencing of BsAbs and CAR T, and combinations with other anti-MM backbone therapies (summarized in Table 2). Of note, while BsAbs have similar reported activity in early-phase 1/2 trials, they do differ based on their target, valency, affinity, and structural design (Table 1). These differences may have therapeutic relevance, as discussed further below. Emerging data regarding the sequencing of BsAb with adoptive cellular therapies support sustained efficacy of BsAb post CAR T therapy (CAR T → BsAb), while the reverse sequence (BsAb → CAR T) significantly reduces the PFS of CAR T recipients.[4,5,8,17-19]

Resistance to bispecific antibody therapies

Resistance to BsAb therapy in MM can occur through various mechanisms, which can be broadly classified into two categories: (1) immune dysfunction (T cell or immune microenvironment) and (2) tumor-intrinsic adaptation (antigenic drifting and disease burden). A summary of these mechanisms are illustrated in Figure 2. Understanding the interplay among these mechanisms and developing strategies to overcome them are critical to improving the efficacy of BsAb.

Immune-related factors

T-cell dysfunction

The efficacy of BsAb therapy depends on the fitness of the immune effector cells. MM is characterized by progressive T-cell dysfunction. Marrow-infiltrating lymphocytes (MIL) in MM consist of heterogeneous T-cell populations that undergo exhaustion characterized by terminal differentiation, loss of effector functions, and expression of inhibitory receptors from suboptimal priming and chronic antigen stimulation in the immunosuppressive tumor microenvironment.[20-24] Functional and numerical defects in T cells not only promote MM disease progression but also portend poor response to BsAb therapy. Correlative studies from the MajesTEC-1 trial presented at ASH 2022 suggested

Table 2. Selected clinical trials of bispecific antibodies in multiple myeloma

Trial	Patient	Intervention	Phase
MajesTEC-4 (NCT05243797)	NDMM	Post-autologous stem cell transplant: TEC + Len vs. Len vs. TEC	Phase 3
MajesTEC-5 (NCT05695508)	NDMM transplant eligible	Induction with: TEC + Dara + Len + dex vs. TEC + Bort + Len + Dara + dex vs. Standard of care Followed by: TEC + Dara + Len maintenance	Phase 2
MajesTEC-7 (NCT05552222)	NDMM transplant noneligible	TEC + Dara + Len vs. Dara + Len + dex	Phase 3
MagnetisMM-7 (NCT05317416)	NDMM	Post-autologous stem cell transplant: ELRA vs. Len	Phase 3
MajesTEC-2 (NCT04722146)	NDMM and RRMM	TEC + Dara + Pom vs. TEC + Dara + Pom + Bort (21- or 28-day cycle) vs. TEC + nirogacestat vs. TEC + Len vs. TEC + Dara + Len	Phase 1b
MajesTEC-3 (NCT05083169)	RRMM, 1–3 prior lines including PI and Len	TEC + Dara vs. Dara + Pom + dex or Dara + Bort + dex	Phase 3
MajesTEC-9 (NCT05572515)	RRMM, 1–3 prior lines including anti-CD38 and IMiD	TEC vs. Pom + Bort + dex or Carf + dex	Phase 3
MagnetisMM-6 (NCT05623020)	Part 1: RRMM 1–2 prior lines of therapy including IMiD and PI or NDMM transplant noneligible Part 2: NDMM transplant noneligible	ELRA + Dara + Len vs. Dara + Len + dex	Phase 3
MagnetisMM-1 (NCT03269136)	RRMM, triple-class refractory	ELRA vs. ELRA + dex vs. ELRA + Len vs. ELRA + Pom	Phase 1
MagnetisMM-3 (NCT04649359)	RRMM, triple-class refractory Cohort A: no prior anti-BCMA Cohort B: prior anti-BCMA ADC or CAR T	ELRA	Phase 2
MagnetisMM-4 (NCT05090566)	RRMM, triple-class refractory	ELRA + nirogacestat vs. ELRA + Len + dex	Phase 1b/2
MagnetisMM-5 (NCT05020236)	RRMM, prior therapy including IMiD and PI	ELRA vs. ELRA + Dara vs. Dara + Pom + dex	Phase 3

Table 2. Selected clinical trials of bispecific antibodies in multiple myeloma (*Continued*)

Trial	Patient	Intervention	Phase
TRIMM-2 (NCT04108195)	RRMM, 3 prior lines including PI and IMiD	TEC + Dara vs. TALQ_+ Dara vs. TEC + Dara + Pom vs. TALQ_+ Dara + Pom	Phase 1b
RedirecTT-1 (NCT04586426)	RRMM	TALQ_+ TEC + Dara	Phase 1b
TRIMM-3 (NCT05338775)	RRMM	TALQ_+ TEC + PD-1 inhibitor	Phase 1b
MonumenTAL-2 (NCT05050097)	NDMM	TALQ_+ Carf vs. TALQ_+ Dara + Carf vs. TALQ_+ Len vs. TALQ_+ Dara + Len vs. TALQ_+ Pom	Phase 1b
MonumenTAL-3 (NCT05455320)	RRMM	TALQ_+ Dara + Pom vs. Dara + Pom + dex vs. TALQ_+ Dara + dex	Phase 3
IFM 2021–01 (NCT05572229)	Older adults, age ≥65	TEC + Dara vs. TEC + Len	Phase 2
Immuno-PRISM (NCT05469893)	High-risk SMM	Len/dex vs. TEC	Phase 2

ADC, antibody drug conjugate; Bort, bortezomib; Carf, carfilzomib; Dara, daratumumab; dex, dexamethasone; ELRA, elranatamab; IMiD, immunomodulatory drug; Len, lenalidomide; NDMM, newly diagnosed multiple myeloma; PI, proteasome inhibitor; Pom, pomalidomide; RRMM, relapsed refractory multiple myeloma; SMM, smouldering multiple myeloma; TALQ_, talquetamab; TEC, teclistamab.

that a higher number of baseline peripheral T cells, increased frequency of naïve CD8 T cells (CD45RA+ CD27+), and lower regulatory T cells (Treg) were seen in the peripheral blood of responding patients than in nonresponders. Clinical response to teclistamab was also associated with lower-expression inhibitory receptors (PD-1, TIM3, and CD38) on T cells.[12] A comprehensive single-cell interrogation of marrow and peripheral blood T cells demonstrated that increased frequency of exhausted CD8+ TOX+T cells is associated with response failure while clonal expansion of fit preexisting T cells is found in patients with clinical response.[25] In this study, CXCR3-positive CD8 cells are demonstrated to be the main mediators of BsAb cytotoxicity. Furthermore, serial interrogation of the TCR repertoire from patients who respond to anti-BCMA BsAb suggest that the expansion and persistence of putatively tumor-reactive TCR clonotypes between the pre- versus post-therapy timepoints is associated with therapy response while such clonotypic persistence is lacking in nonresponders. BsAb, in effect, induce the pooling of peripheral blood T cells to the bone marrow–tumor microenvironment and enable the selective expansion of tumor-reactive clonotypic CD8+ T cells.[26] A preexisting global exhausted T-cell profile, in both the peripheral blood and bone marrow compartments, is therefore an impediment to effective BsAb response. In addition to preexisting T-cell exhaustion observed in MM patients prior to the initiation of BsAb therapy, repetitive T-cell engagement with BsAb administration may further exacerbate T-cell dysfunction[27]; integrating treatment-free interval into the therapy regimen may mitigate this effect, allowing for a more durable therapeutic benefit while attenuating treatment-emergent adverse events.[27]

Immunosuppressive bone marrow microenvironment

Malignant plasma cells in MM are in complex interplay with the bone marrow accessory cells within the tumor microenvironment, which together promote MM cell survival and growth while suppressing effective anti-tumor immune activity. The bone marrow microenvironment is characterized by the presence of stromal cells, osteoclasts, myeloid-derived suppressor cells, tumor-associated macrophages, plasmacytoid dendritic cells, and regulatory T or B cells.[28] These cells, in combination with the secretion of immunosuppressive cytokines, including IL-6, IL-8, IL-10, IL-15, IL-18, and VEGF, and the expression of inhibitors such as soluble MICA, TGF-β, and indoleamine 2,3-dioxygenase, impair effective T-cell responses.[21,29] The success of BsAb therapy relies on recruiting T cells into this immunosuppressive milieu, where they must overcome multiple mechanisms that hinder T-cell metabolism and counter their activation, expansion, and persistence.

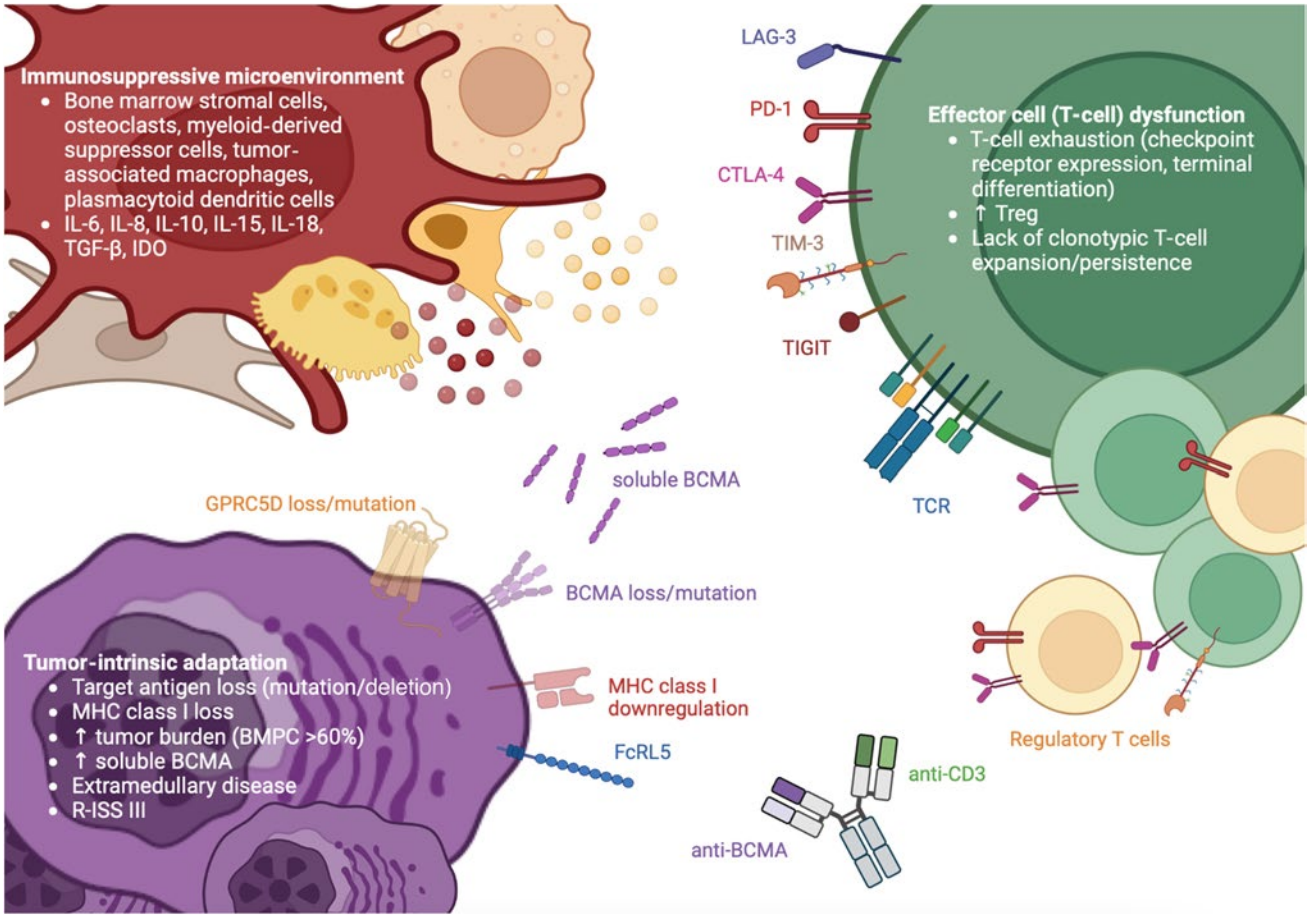

Figure 2. Mechanisms of resistance to bispecific antibodies in multiple myeloma. Immunosuppressive tumor microenvironment, reduced T cell fitness, and tumor-intrinsic mechanisms can contribute to multiple myeloma resistance to BsAb. BMPC, bone marrow plasma cells; CTLA-4, cytotoxic T-lymphocyte associated protein 4; LAG-3, lymphocyte activation gene-3; MHC, major histocompatibility complex; PD-1, programmed cell death protein 1; TCR, T cell receptor; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIM-3, T cell immunoglobulin domain and mucin domain-3; Treg, regulatory T cells.

The immunomodulatory effect of anti-MM agents, including immunomodulatory drugs (IMiD)[30] and daratumumab,[31] may further potentiate BsAb activity, and these combinations are actively being investigated in clinical trials (refer to Table 2).

Tumor-related factors

Antigenic loss

While bispecific antibodies effectively eradicate the bulk of a tumor by targeting ubiquitously expressed surface antigens, such therapeutically enhanced anti-cancer immunity exerts clonal evolutionary selective pressures. This can facilitate the emergence of clones whose growth is fueled by compensatory mechanisms that allow immune evasion and convergent evolution, ultimately leading to clinical relapse. One of the key mechanisms of tumor-intrinsic adaptive resistance to targeted immunotherapies is antigen escape. Loss of BCMA expression post anti-BCMA CAR T is detected in around 4% of cases, while preexisting heterozygous deletions of *TNFRSF17* (gene encoding BCMA) is found in up to 4% of the immunotherapy-naïve MM patient population.[1,32] To date, there are 3 published reports of BCMA-negative MM relapse post anti-BCMA CAR T and two cases post anti-BCMA BsAb.[32-36] In these reports, BCMA antigen loss occurred through biallelic deletion at the *TNFRSF17* gene locus or monoallelic deletion coupled with a truncating nonsense mutation in the remaining *TNFRSF17* allele. In addition to biallelic deletions at the *TNFRSF17* gene locus, single amino acid mutations or deletions in the extracellular domain of BCMA were recently shown to confer resistance to some anti-BCMA BsAb.[36] Deletions or mutations on *TNFRSF17* were identified in 6 of 14 patients who progressed post anti-BCMA BsAb. Importantly, in this preclinical study, alternative BsAb design (asymmetrical bivalent anti-BCMA binding domains) or anti-BCMA CAR T overcame monovalent anti-BCMA BsAb resistance resulting from BCMA extracellular domain mutations.[36] Hence, resistance to one anti-BCMA BsAb may not preclude re-treatment with other BCMA-targeting agents.

GPRC5D loss or reduction of surface antigen expression was detected in all 6 of 6 patients who progressed post anti-GPRC5D CAR T.[3] Four additional cases of MM relapse with biallelic genomic events on *GPRC5D* (biallelic deletions or monoallelic deletion and mutations) post anti-GPRC5D BsAb have also been reported.[36] Of note, up to 15% of T-cell immunotherapy-naïve patients have preexisting heterozygous loss of *GPRC5D*.[32] Altogether, BCMA and GPRC5D antigen biallelic or functional loss are

important mechanisms of resistance to BsAb in MM. Such findings support the ongoing development of alternative or multiple antigen-targeting therapeutic approaches as well as optimizing the design of BsAb agents to better target antigen escape clones. Clinical trials are also underway to combine two BsAb that target BCMA and GPRC5D (NCT04586426, NCT05338775).

Soluble BCMA and myeloma disease burden

Clinical trials have shown that high disease burden, defined as ≥60% bone marrow plasma cells, is associated with primary refractoriness to single-agent anti-BCMA or anti-GPRC5D BsAb in patients with RRMM.[2,4] Along with bone marrow plasma cell assessment, measuring soluble BCMA (sBCMA) levels is an important correlate for evaluating disease burden and predicting BsAb therapy response. High sBCMA levels have been associated with lower response rates to single-agent teclistamab and were correlated with high R-ISS stage, increased bone marrow plasma cells (>60%), and the presence of extramedullary disease.[12] This observation raises several questions about the underlying mechanisms that drive the reduced efficacy of BsAb in the setting of elevated sBCMA levels. Does the lack of response reflect poor penetration and limited T-cell trafficking and engagement in the tumor bulk, as seen in the relatively ineffective response to BsAb in solid tumors?[37] Alternatively, could sBCMA serve as a ligand sink that traps anti-BCMA BsAb in serum, preventing their binding to MM cells' surface BCMA molecules?[12,38] Moreover, elevated sBCMA may result from a tumor-intrinsic evasion mechanism by which the MM cells enhance gamma secretase activity to downregulate surface BCMA expression. These mechanisms can be amenable to targeting by gamma secretase inhibitors,[39] and this is being tested in ongoing clinical trials (NCT04722146).

Debulking MM with cytotoxic agents prior to or in combination with BsAb therapy may help restore BsAb-mediated cytotoxicity in high disease burden settings.[40] Furthermore, the combination of novel cereblon E3 ligase modulator (CELMoD) such as mezigdomide[41] has been shown to sensitize MM cells to alnuctamab-mediated cytotoxicity, in part through the induction of DNAM-1 ligands on MM cell surface.[42] Ongoing studies of BsAb in combination with other anti-MM agents will provide valuable insight into whether combination therapy can overcome the current limitations of BsAb therapy in the setting of high disease burden.

CLINICAL CASE (continued)

Whole genome sequencing of the CD138+ sorted MM cells at relapse demonstrated that the patient had a newly detectable clonal mutation in *TNFRSF17* [p. Arg27Pro] coupled with a monoallelic loss of *TNFRSF17* gene locus. This immune-selected mutant clone completely abrogated BCMA affinity of the BsAb Fab moiety with which it was treated.[36] Subsequent immediate treatment with another BsAb targeting GPRC5D resulted in an ongoing molecular remission at 1 year of follow-up.

Conclusion

The rapid progress in the development of BsAb and other T-cell immunotherapies for MM is revolutionary. However, the adaptive nature of MM tumor cells and their ability to evade the immune system necessitates an ongoing parallel effort in both clinical and preclinical research. It is crucial to investigate the mechanisms of resistance to these therapies and develop innovative therapeutic strategies to overcome them. Moreover, identifying predictive biomarkers of response to BsAb will enable the deployment of personalized immune-therapy.

Conflict-of-interest disclosure

Holly Lee: no competing financial interests to declare.
Paola Neri: received speaker's bureau honoraria from BMS, Janssen, and Sanofi and is a consultant/advisory board member for BMS and Janssen.
Nizar J. Bahlis: has received research funding from Pfizer, and received speaker's bureau honoraria from Amgen, BMS, Sanofi, Pfizer, and Janssen and is a consultant/advisory board member for BMS, Janssen, and Pfizer.

Off-label drug use

Holly Lee: There are no off-label drug uses to disclose.
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Current use of CAR T cells to treat multiple myeloma

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Anti–B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) T-cell therapies currently approved by the US Food and Drug Administration (FDA) have dramatically improved clinical outcomes for patients with heavily pretreated multiple myeloma who have disease refractory to conventional proteasome inhibitors, immunomodulatory drugs, and anti-CD38 monoclonal antibodies. However, despite this progress, multiple myeloma remains an incurable hematologic malignancy. In this review, we discuss practical considerations for currently FDA approved CAR T-cell therapies, including newer data evaluating those agents in earlier lines of therapy. We also discuss considerations for patients following relapse from anti-BCMA CAR T-cell therapy, which currently represents an unmet clinical need.

LEARNING OBJECTIVES

- Discuss practical considerations for CAR T-cell products currently approved by the US Food and Drug Administration and potential expanded indications for those agents
- Explore currently available research regarding challenges with therapy
- Evaluate treatment options following relapse from CAR T-cell therapy

CLINICAL CASE

A 62-year-old man was diagnosed with IgG κ multiple myeloma with cytogenetic studies notable for deletion of 17p and a t(4;14) translocation. In the first 3 years since his diagnosis, he has had progressive disease following 5 different lines of therapy, which have collectively included 2 proteasome inhibitors, 2 immunomodulatory drugs, anti-CD38 and anti-SLAMF7 monoclonal antibodies, and an autologous stem cell transplant. He was referred by his local oncologist to a major academic medical center for consideration for chimeric antigen receptor (CAR) T-cell therapy. After discussions with his medical team, he undergoes apheresis for planned treatment with commercial ciltacabtagene autoleucel (cilta-cel) after observed biochemical disease relapse.

Introduction

Although multiple myeloma (MM) remains an incurable malignancy,[1] novel T-cell redirecting therapies, including chimeric antigen receptor (CAR) T-cell and bispecific antibody (BsAb) therapies, have shown tremendous promise in heavily pretreated relapsed/refractory (RR) disease.[2-5] "While patients with high-risk disease, often defined by revised international staging system (R-ISS) III disease or by the presence of either high-risk cytogenetic abnormalities or extramedullary disease, typically have vastly inferior outcomes with systemic therapies, cur- rent prospective data sets have shown less disparate results among patients treated with CAR T-cell therapy" (Figure 1).[6,7] Despite success in the aggregate, outcomes for patients with myeloma receiving cellular therapies remain highly variable, with some patients having brief or no responses and some with years of progression-free survival (PFS) following treatment. However, given MM's current incurability, newer therapies inevitably lead to new challenges, with there now being a need to identify appropriate treatment options for patients following relapse from CAR T-cell therapy.

CAR T-cell therapy in multiple myeloma: patient and product selection

CAR T-cell products for MM currently approved by the US Food and Drug Administration (FDA) include the B-cell maturation antigen (BCMA) targeting products idecabta-

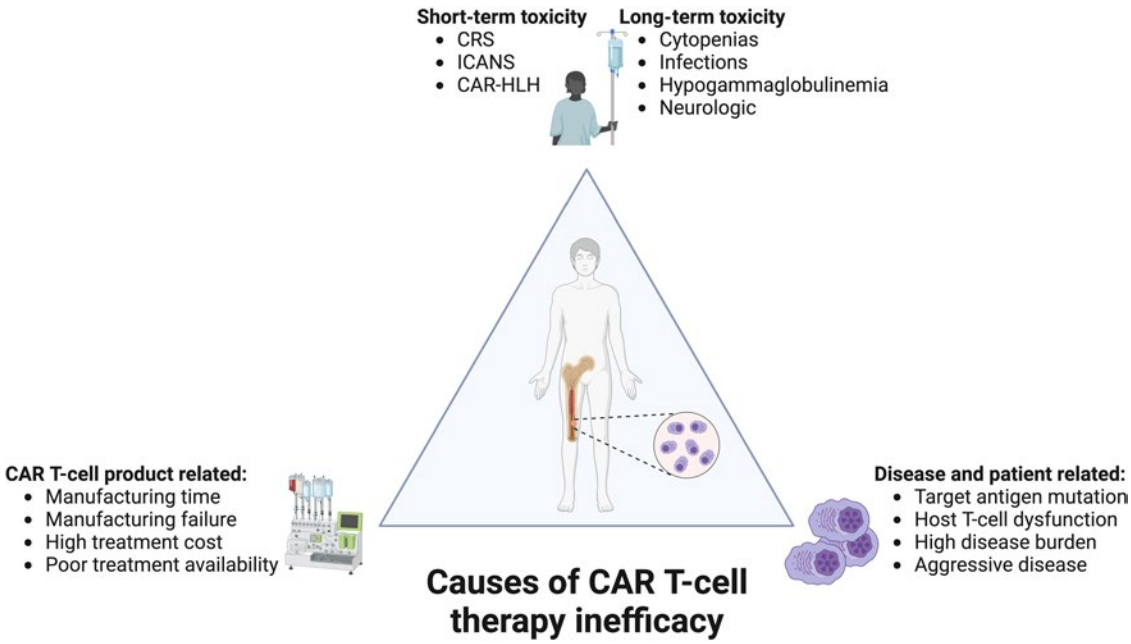

Figure 1. Causes of CAR T-cell therapy inefficacy: therapy inefficacy for patients with MM receiving CAR T-cell therapy can be due to disease- or patient-related factors, although logistical concerns relating to product manufacturing and cost/availability are also significant. Therapy-related toxicity can also represent a challenge even in patients with strong responses. CAR-HLH, Chimeric antigen receptor T cell-related hemophagocytic lymphohistiocytosis.

gene-vicleucel (ide-cel) and cilta-cel.[2,3] Both agents were approved based on results of single-arm phase 2 trials with commercial use permitted for patients with MM with at least 4 prior lines of therapy, including a proteasome inhibitor, an immunomodulatory drug, and an anti-CD38 monoclonal antibody. The initial KarMMA-1 (ide-cel) and CARTITUDE-1 (cilta-cel) trials demonstrated high response rates to their respective CAR T-cell products in their heavily pretreated patient populations, with both trials having a significant percentage of patients with triple-class refractory (84% and 88%, respectively) and penta-drug refractory (26% and 42%, respectively) disease. These data indicate that CAR T-cell therapies are an appropriate treatment option for heavily pretreated patients, including "penta-refractory" patients. This population has been estimated to have a median overall survival (OS) of less than 6 months,[8] with available treatment options in this setting having poor efficacy and high toxicity.[9]

There is currently no consensus with regards to patient selection for CAR T-cell therapies from available data sets. With regards to disease status, patients with several disease features typically associated with aggressive myeloma, including extramedullary disease, high-risk cytogenetic abnormalities, and high tumor burden, were included in the KarMMa-1 and CARTITUDE-1 trials. For patient-specific factors, patients included in these trials typically had good performance status with less than 5% of patients in all trials having an Eastern Cooperative Oncology Group performance score of 2 or higher. Performance status considerations with regards to CAR T-cell therapy may be due to both tolerating lymphodepleting therapy prior to CAR T-cell infusion, which included combination therapy with fludarabine (30 mg/m^2 body surface area) and cyclophosphamide (300 mg/m^2 body surface area) in all trials and expected therapy associated toxicities. Additionally, the contribution of rapidly progressing disease to poor performance status during the time required for CAR T-cell manufacturing may make this treatment option less feasible for this population. As patient age was highly variable in all trial populations, assessments of fitness is often done clinically.

There remain no reported or pending prospective data sets comparing outcomes between available FDA-approved BCMA targeting CAR T-cell products, leaving product selection up to the discretion of individual clinicians. The patient populations for both the KarMMa-1 and CARTITUDE-1 studies were similar (Table 1) with regards to prior therapy exposure and patient fitness, although the patients included in CARTITUDE-1 were less likely to have extramedullary disease or high-risk cytogenetic abnormalities. Nevertheless, the outcomes between these 2 trials are distinct, with cilta-cel patients included in CARTITUDE-1 achieving a 94% overall response rate (ORR) with 67% reaching a complete response (CR) while ide-cel patients achieved a 73% ORR with 25% reaching CR in KarMMA-1. Safety profiles were notable for similar rates of any grade and grade 3 or 4 cytokine release syndrome (CRS) with both cilta-cel and ide-cel (95%, 4% vs 84%, 5%) and similar rates of any grade neurotoxicity (21% vs 18%) but perhaps higher rates of grade 3 or 4 neurotoxicity with cilta-cel (9% vs 3%).

With regards to real-world data sets, 1 real-world analysis of 159 commercial ide-cel–infused patients, 75% of whom would have been ineligible for KarMMa-1 based on comorbidities, showed an ORR of 84% (42% ≥ CR) compared with 76.4% (30% ≥ CR) in KarMMa-1.[10] However, a similar real-world study of commercial ide-cel outcomes in 190 KarMMa-1–eligible patients identified significantly inferior outcomes when compared to KarMMA-1 data, with an ORR of 32.2%, with these results remaining when

Table 1. Review of clinical data sets for currently FDA-approved CAR T-cell therapies

	Ide-cel (KarMMa-1)	Ide-cel (KarMMa-3)	Cilta-cel (CARTITUDE-1)	Cilta-cel (CARTITUDE-4)
Trial phase	2	3	1b/2	3
No. of patients infused (enrolled)	128 (140)	225 (254)	97 (113)	208 (176)
Median age (range),† y	61 (33–78)	63 (30–81)	Not reported	61.5 (27–78)
Median time since diagnosis (range),† y	6 (1–18)	4.1 (0.6–21.8)	5.9 (4.4–8.4)	3.0 (0.3–18.1)
Median No. of prior lines (range)†	6 (3–16)	3 (2–4)	6 (4–8)	2 (1–3)
EMD,† No. (%)	50 (39)	61 (24)	13 (13)	44 (21.2)
ECOG,† No. (%)				
0	57 (45)	120 (47)	39 (40)	114 (54.8)
1	68 (53)	133 (52)	54 (56)	93 (44.7)
≥2	3 (2)	1 (<1)	4 (4)	1 (0.5)
R-ISS†				
I	14 (11)	50 (20)	61 (63)	136 (65.4)
II	90 (70)	150 (59)	22 (23)	60 (28.8)
III	21 (16)	31 (12)	14 (14)	12 (5.8)
Unknown	3 (2)	23 (9)	0	
Cytogenetic abnormalities†				
High risk	45 (35)	107 (42)	23 (24)	123 (59.4)‡
del(17p)	23 (18)	66 (26)	19 (20)	49 (23.7)
t(4;14)	23 (18)	43 (17)	2 (2)	30 (14.5)
t(14;16)	6 (5)	8 (3)	3 (3)	3 (1.4)
Prior ASCT†	120 (94)	214 (84)	87 (90)	Not reported
Prior treatment refractory status†				
IMiD	126 (98)	224 (88)	Not grouped (highest is Len with 96, 99%)	208 (100)
PI	116 (91)	189 (74)	Not grouped (highest is V with 92, 95%)	Not grouped (highest is V with 55, 26.4%)
Anti-CD38	120 (94)	242 (95)	94 (97)	50 (24)
Triple-class refractory	108 (84)	164 (65)	85 (88)	30 (14.4)
Penta drug refractory	33 (26)	15 (6)	41 (42)	2 (1)
No. (%) requiring bridging therapy†	112 (88)	213 (84)	Not reported	All
Response rate				
MRD negative, No. (%)	33 (24)	51 (20)*	43 (38)	126 (60.6)
sCR or CR	42 (30)	98 (39)	80 (71)	152 (73.1)
≥ VGPR	68 (48)	153 (60)	92 (81)	169 (81.3)
≥ PR/ORR	85 (67)	181 (71)	95 (84)	176 (84.6)
Median PFS (95% CI),† mo	8.8 (5.6–11.6)	13.3 (11.8–16.1)	34.9 (25.2-NR)[39]	NR (Not reported)
Median OS (95% CI),† mo	19.4 (18.2-NR)	Not reported	NR (NE)	NR (NE)
Grade 3+ CRS,† No. (%)	8 (6)	11 (4)	4 (4)	2 (1.1)
Grade 3–4 heme tox,† No. (%)	114 (89)	218 (87)	96 (99)	196 (94.2)

ASCT, Autologous stem cell transplantation; ECOG, Eastern Cooperative Oncology Group; IMiD, immunomodulatory drug; NE, Not estimable; NR, Not reached; PI, proteasome inhibitor; PR, partial response; R-ISS, Revised International Staging System; sCR, stringent complete response; VGPR, very good partial response.

*ORR are for all patients enrolled (and not enriched for those receiving cell infusion as reported in the manuscript).

†Values reported for only the patients who received ide-cel infusion (full data not reported) in the KarMMa-1 and CARTITUDE-1 population. Values include all enrolled patients in the KarMMa-3 and CARTITUDE-4 populations.

‡High risk in this trial included +1q in addition to del(17p), t(4;14), and t(14;16) as included in the other studies.

matching for KarMMa-1 patient characteristics.[11] With regards to cilta-cel, there is a paucity of published data; however, 1 report of a 139-patient commercial cohort observed an ORR of 80% (40% CR) with a similar toxicity profile to that observed in CARTITUDE-1.[12]

Considerations for bridging therapy and manufacturing time

One of the major logistical challenges with CAR T-cell therapy administration is managing disease relapse during the product manufacturing time to avoid complications associated with myeloma-related end-organ damage, as is present in our patient case described above. Current manufacturing times for ide-cel and cilta-cel are estimated at ~28 days. However, factoring in the time required to confirm disease relapse and coordinate the logistics for cell collection in the standard care setting likely involves a longer functional time between disease reemergence and CAR T-cell infusion. This time delay, unique to CAR T-cell therapy when compared to off-the-shelf therapies, is associated with a frequent need for bridging therapy. Over 80% of ide-cel patients (included in both KarMMA-1 and KarMMA-3) required bridging therapy during product manufacturing time, which is likely shorter in the context of a clinical trial than it would be in a standard-of-care setting.[2,13] Additionally, all currently published MM CAR T-cell trials have a notable percentage of their intention-to-treat population who did not ultimately receive their cell infusion. Specifically, 8% to 14% of enrolled patients in currently published ide-cel and cilta-cel trials dropped out prior to infusion.[14] The reasons for these dropouts are not explicitly reported in all relevant trials but are often attributed disease progression, adverse events, or cell manufacturing failure.

Off-the-shelf allogeneic CAR T-cell products have been investigated as a potential solution to issues surrounding CAR T-cell manufacturing time, as patient-specific autologous product preparation is not required. The only published clinical trial of allogeneic CAR T-cell therapy for MM evaluated the safety and feasibility of Allo-715, an allogeneic anti-BCMA CAR T-cell therapy, demonstrating a 70.8% ORR with a median duration of response of 8.3 months.[15] Of note, none of the 43 infused patients in this study required bridging therapy (Table 3). In this study, grade ≥3 adverse events were rare, including CRS (2.3%) and neurotoxicity (0%), meaning that this approach could potentially be an option for patients with rapidly progressing disease.

CLINICAL CASE (continued)

Following apheresis, the patient reports progressive fatigue and bone pain while awaiting cilta-cel delivery. On evaluation, the patient is noted to have worsening anemia, acute kidney injury, and radiographic studies demonstrating several new lytic bone lesions. He is admitted for bridging therapy with dexamethasone, cyclophosphamide, etoposide, and cisplatin, and while he has a transient partial response to therapy, he is found to have actively progressing disease just prior to cilta-cel infusion. Following infusion with cilta-cel, the patient experienced grade 4 immune effector cell-associated neurotoxicity syndrome (ICANS) among other significant treatment-related toxicities. He ultimately recovered from these side effects after a prolonged stay in the hospital intensive care unit and achieved CR to therapy with no evidence of minimal residual disease seen following bone marrow biopsy.

Notable short- and long-term therapy toxicities

Short-term toxicities for CAR T-cell therapy are well described, and most notably include CRS and ICANS.[16] These are common short-term side effects of CAR T-cell therapy and in most of cases are associated with no long-term sequelae, although cases of CRS- and ICANS-related mortality have been reported. Both are thought to have increased incidence in patients with high pretreatment disease burden, further indicating the importance of bridging therapy in patients with evidence of rapid disease progression.[17]

While these short-term toxicities are well documented in relevant clinical trials and have a consensus with regards to management guidelines, long-term toxicities are less thoroughly described. One single-center report observed cytopenias can persist long after cell infusion, with 28% of patients with grade ≥3 cytopenias 120 days following cell infusion.[18] Long-term neurotoxicity, including parkinsonian-like movement disorders as well as neurocognitive events, has been observed in 5% of patients in 1 cohort of cilta-cel–treated patients, which, given the small number of affected patients, does not have a clear clinical management strategy.[17]

CAR T-cells in earlier lines of therapy

The recently published KarMMa-3 and CARTITUDE-4 studies evaluated the efficacy of ide-cel and cilta-cel in earlier treatment settings. KarMMa-3 recruited patients with RR MM with 2 to 4 prior lines of therapy to evaluate the potential role for ide-cel in earlier lines of therapy.[19] The study was designed as a phase 3 randomized trial (2:1 randomization favoring ide-cel) comparing ide-cel to a selection of standard therapy regimens chosen at the clinician's discretion. Non–ide-cel treatment options included daratumumab in combination with pomalidomide and dexamethasone, daratumumab in combination with bortezomib and dexamethasone, ixazomib in combination with lenalidomide and dexamethasone, carfilzomib in combination with dexamethasone, and elotuzumab in combination with pomalidomide and dexamethasone. The trial met its primary end point, demonstrating superior PFS in the ide-cel group when compared to the conventional therapy group (13.3 vs 4.4 months). However, there are some challenges with interpreting this trial as strictly favoring ide-cel over standard therapy. OS comparisons were not reported (noted as an immature data set in the published study) and a notable number of patients in the ide-cel–treated group died during the study. Deaths due to any cause were reported as marginally higher in the ide-cel group compared to the standard therapy group (30% vs 26%), with deaths related to treatment representing less than half of deaths in the ide-cel arm.

The available regimens in the standard therapy group lacked some therapy options for patients with 1 to 3 prior lines of therapy exposure. Carfilzomib-containing triplet regimens including carfilzomib in combination with dexamethasone and either lenalidomide, daratumumab, or isatuximab were not included as control. However, while these regimens showed superiority to carfilzomib in combination with dexamethasone in the RR MM setting in the phase 3 ASPIRE, CANDOR, and

Table 2. Ongoing phase 3 clinical trials of ide-cel and cilta-cel in earlier lines

	CARTITUDE-5	CARTITUDE-6
Setting	NDMM following VRd without planned ASCT	NDMM, transplant eligible, following DVRd
Product	Cilta-cel	Cilta-cel
Control arm	Rd maintenance	ASCT
Primary end point	PFS	PFS, sustained MRD-CR
Estimated enrollment	650	750
Study start date	June 2021	February 2022
Estimated primary completion date	June 2026	June 2026
NCT ID	NCT04923893	NCT05257083

DVRd, daratumumab, bortezomib, lenalidomide and dexamethasone; NDMM, newly diagnosed multiple myeloma; VRd, bortezomib, lenalidomide and dexamethasone.

IKEMA trials,[20-22] the KarMMA-3 population was nearly entirely refractory to anti-CD38-based therapy, which was not the case for patients recruited to those phase 3 studies. It should be noted that, in the standard care group, Kd was highly represented as a clinician-chosen treatment option, with clinicians for 23% of patients selecting it, indicating favor for carfilzomib's use in this setting. Furthermore, 38% of patients in the standard therapy group received daratumumab-containing regimens (daratumumab in combination with pomalidomide and dexamethasone or daratumumab in combination with bortezomib and dexamethasone) despite 94% of patients having daratumumab refractory disease. While not specific to daratumumab, prospective trials evaluating anti-CD38 monoclonal antibodies in the daratumumab refractory setting have shown 0% ORRs.[23]

CARTITUDE-4 specifically recruited patients with lenalidomide refractory disease with 1 to 3 prior lines of therapy, and patients were randomized (1:1) to either cilta-cel or physicians' selection of standard-of-care therapies, including bortezomib in combination with pomalidomide and dexamethasone, as well as daratumumab in combination with pomalidomide and dexamethasone (DPd).[13] This trial also met its primary end point, showing superior PFS in the cilta-cel group when compared to the standard therapy group (PFS not reached vs 15.9 months). Like in KarMMa-3, carfilzomib-containing triplets were notably not included in the control arm of this study, despite carfilzomib in combination with dexamethasone and daratumumab being FDA approved within 2 months of trial enrollment. This is of particular relevance when comparing the outcomes of these 2 trials to each other as patients in the cilta-cel arm included in this study had far lower carfilzomib exposure compared to bortezomib exposure (37.0% vs 97.6%) and far lower daratumumab exposure than the patients included in KarMMa-3 (24.5% vs 95%). The control arm may have had more success if the protocol had been amended to include these regimens, particularly carfilzomib in combination with dexamethasone and lenalidomide as per the ASPIRE trial. The significant prior bortezomib exposure is reflected in the observation that, for patients included in the control arm, 86.7% were given DPd by their treating clinician as opposed to bortezomib in combination with pomalidomide and dexamethasone, likely to avoid retreatment with a previously received agent. Unlike the KarMMa-3 study, deaths on study due to any cause in the CARTITUDE-4 study were not higher in the cilta-cel arm than in the control arm (18.8% vs 21.8%). However, this did not include patients who had evidence of disease progression prior to receiving cilta-cel on trial and were subsequently given cilta-cel as postprotocol therapy per trial design (with 10/20 such patients dying at the time of trial publication). Side effects related to neurotoxicity, as represented by ICANS and movement disorders, showed lower frequency than in clinical trials evaluating cilta-cel in more heavily pretreated disease. This may be due to the fact that toxicity associated with CAR T-cell therapy has been observed more frequently in patients with higher disease burden at the time of cell infusion, as is the case with our patient described above.

Overall, it is difficult to compare the relative efficacy of ide-cel and cilta-cel in these trials, as they recruited patients in different settings, which is reflected in the disparate outcomes in their respective control arms. Further, comparisons of treatment arms across these trials should be done with significant caution as the ide-cel population in KarMMA-3 had a significantly higher percentage of triple-class refractory disease when compared to the cilta-cel population in CARTITUDE-4 (65% vs 25.5%). This discrepancy is primarily due to a significantly higher daratumumab refractory population in KarMMa-3 compared to CARTITUDE-4 patients (95% vs 24.5%), a population with notable poorer treatment outcomes.[8] It should be noted, however, that a recently reported real-world data set of 143 cilta-cel patients, 71% of whom were triple-class refractory, had an ORR of 89% with median PFS not reached, albeit with a short median follow-up time of 5.8 months.[12] Both the KarMMa-3 and CARTITUDE-4 studies appropriately analyzed data via intention-to-treat analysis, but neither phase 3 study has reported OS in either group, which, when available, will further inform clinical decision-making. Additionally, there are current ongoing clinical trials evaluating cilta-cel in the frontline setting (Table 2), which has to potential to further expand the indications for CAR T-cell therapy in MM.

Therapy considerations for patients with prior exposure to anti-BCMA targeting agents

There are no fully reported prospective data sets evaluating the efficacy of ide-cel or cilta-cel in patients with prior exposure to other BCMA targeting agents, including the antibody

Table 3. Non-FDA-approved CAR T-cell therapies with complete clinical trial data available

	Allo-715	MCARH109	Xuzhou GPRC5D CAR T cell therapy	OriCAR-017
Trial phase	1	1	2	1
Target	BCMA	GPRC5D	GPRC5D	GPRC5D
Specificity	Allogeneic	Autologous	Autologous	Autologous
Patients enrolled (infused)	48 (43)	19 (17)	33 (33)	13 (10)*
Median age (range), y	64 (46–77)	60 (38–76)†	58 (39–70)	64 (58–68)†
Median prior lines (range)	5 (3–11)	6 (4–14)†	4 (2–12)	5.5 (4–10)†
Triple-class refractory, No. (%)	39 (91)	16 (94)†	Not reported	Not reported
Penta refractory, No. (%)	18 (42)	Not reported	Not reported	Not reported
Prior anti-BCMA CAR T-cell therapy, No. (%)	Excluded	8 (47)†	9 (27)	5 (50)†
Received bridging therapy, No. (%)	0	16 (94)†	Not reported	2 (80)†
ORR, No. (%)	24 (56)†	12 (71)†	30 (91)	10 (100)†
CR, No. (%)	Not reported	6 (35)†	21 (64)	6 (60)†
ORR in patients with prior anti-BCMA CAR T-cell therapy, No. (%)		7/10 (70)†	9/9 (100)	5 (100)†
Median PFS	Not reported	Not reached	Not reached	Not reached
CRS grade 3+, No. (%)	1 (2)	1 (6)	0 (0)	0 (0)
ICANS grade 3+, No. (%)	0 (0)	1 (6)	1 (3)	0 (0)
Trial ID	NCT04093596	NCT04555551	ChiCTR2100048888	NCT05016778

*Thirteen patients were screened for the trial, but 1 was excluded due to low plasma cell GPRC5D expression.

†Values listed are for only the enrolled patients receiving product infusion.

drug conjugate belantamab mafodotin, other BCMA targeting CAR T-cell products, or BCMA targeting bispecific antibody therapies. In a small subset of patients receiving anti-BCMA CAR T-cell and anti-BCMA BsAbs, single-cell genomic sequencing of myeloma cells at relapse identified BCMA biallelic loss and BCMA missense mutations at relapse, indicating that prior BCMA-directed therapy exposure may limit the efficacy of further BCMA-directed therapies, although there are no clinical data available linking these.[24-26] In 1 retrospective study of real-world ide-cel outcomes, PFS following ide-cel infusion was found to be inferior in 33 patients with prior exposure to either belantamab mafodotin or anti-BCMA BsAbs with a median PFS of 9.0 months (7.6-not reached) in the anti-BCMA therapy-naive group and 3.2 months (2.8-not reached) in the anti-BCMA therapy-exposed population ($P \leq .001$).[10] While this may be due to resistance mechanisms to anti-BCMA therapies that follow anti-BCMA therapy, it is unclear if the anti-BCMA refractory population represents a more heavily pretreated population with more aggressive disease regardless. Further data sets will be required to determine if prior BsAb therapy is disruptive to lymphocyte apheresis prior to CAR T-cell manufacturing and if this represents a cause of CAR T-cell therapy failure with clinical significance.

An early report of CARTITUDE-2 cohort C, a phase 2 study evaluating the efficacy of cilta-cel in patients with prior noncellular anti-BCMA therapy exposure, has been reported. Among 20 cilta-cel–infused patients, 7 with prior anti-BCMA BsAb and 13 with prior anti-BCMA antibody drug conjugate exposure, the ORR was 67%, compared with the 98% ORR among infused cilta-cel patients in CARTITUDE-1.[27] Notably, this patient population was more heavily pretreated, with a median of 8 prior lines of therapy compared to CARTITUDE-1's 6 median prior lines. While this data set reported worse outcomes for cilta-cel in the post-BsAb setting (ORR, 57.1%; median PFS, 5.3 months) than in the CARTITUDE-1 study, it is difficult to draw generalizable conclusions from a 7-patient data set.

Overall, these data indicate that anti-BCMA CAR T-cell therapy should remain a consideration for patients with relapse following other anti-BCMA therapies, although responses rates may be diminished. There may also be utility in assessing the genetic integrity of *TNRSF17*, the gene coding for BCMA, prior to consideration for therapy.

Response assessment and PFS prediction

Response assessments following MM treatment, classified according to International Myeloma Working Group criteria,[28] were strongly predictive of duration of response in both KarMMA-1 and CARTITUDE-1, with patients achieving a CR having drastically improved outcomes compared to those with very good partial response or partial response following infusion. Additionally, the prognostic role of minimal residual disease (MRD) negative status remains critical in this population. A recent single-center retrospective study of CAR T-cell therapy outcomes in MM identified that median PFS for MRD-positive vs MRD-negative patients was drastically different, with a median PFS of 2.9 vs 17.5 months, favoring the MRD-negative group.[29]

This indicates that, in clinical practice, discussions regarding subsequent therapy should be a priority in those not achieving MRD negativity following infusion. Additionally, this finding supports the need for further research into identifying patients most likely to have a strong response to therapy, given the high costs associated with CAR T-cell therapy.

Considerations for access and cost-effectiveness

CAR T-cell therapies are associated with considerable expense. Given the requirement for inpatient administration at specialized centers, only patients with means to travel to these institutions and those living in countries with an infrastructure for CAR T-cell therapy administration will have access to these cellular therapies. Wholesale acquisition costs for cilta-cel are currently estimated at $465,000 USD per patient with additional nonproduct costs, including inpatient and outpatient management as well as adverse event management, being estimated in 1 study to be an additional $160,933 per patient.[30] While cost-effectiveness analyses cannot be accurately performed until complete PFS and OS data are available for CARTITUDE-1 patients, this indicates that CAR T-cell therapy for RR MM will potentially be a cost-effective treatment option only for those patients who achieve multiyear remissions following therapy.[31] Treatments costs in CAR T-cell therapy nonresponders are not substantially mitigated when compared to strong responders, as opposed to other MM therapies that are administered continuously and are typically discontinued at relapse.[32]

When compared to CAR T-cell therapies for lymphoma, ide-cel and cilta-cel are not regarded as curative therapies, with patients relapsing following successful CAR T-cell therapies often proceeding to similarly expensive alternatives. Overall, this further indicates an unmet need for a robust system of identifying patients most likely to response to CAR T-cell therapy prior to product manufacturing.

Considerations for relapse

There are several data sets evaluating the efficacy of various treatment options in patients with disease relapse following previous response to anti-BCMA CAR T-cell therapies. Three currently published clinical trials evaluating the efficacy of anti-GPRC5D targeted CAR T-cell therapies included several patients with prior anti-BCMA CAR T-cell therapy exposure, with such patients in both trials having high response rates to therapy.[33-35] Overall response rates were >70% in all 3 studies, with median PFS not being reached in any and with grade ≥3 CRS being seen in <10% of patients in all studies.

A recently published retrospective multicenter study assessed 140 anti-BCMA CAR T-cell–treated patients, 79 of whom were evaluable and went on to receive subsequent antimyeloma therapy. Among these patients, there were 35 instances of salvage therapy with another T-cell redirection therapy, including either CAR T-cell or BsAb therapy, with an ORR of 91.4% for these instances.[36] Most of these agents included non-BCMA targeted T-cell redirection therapies, indicating that treatment options for anti-BCMA CAR T-cell refractory patients will be strong pending the approval of recently evaluated non-BCMA targeting T-cell redirection therapies.[5,33-35] Although not fully reported, safety and feasibility studies of CAR T-cell combination therapy and dual targeting approaches may also represent an effective treatment option, with 1 study of a BCMA/CD19 dual targeting CAR T-cell therapy showing a 93.1% ORR with a median duration of response of 37 months.[37] Other studies of dual targeting approaches are ongoing (NCT05509530, NCT05325801, NCT05431608).

BsAb therapy should be strongly considered in the post-CAR T-cell relapse setting. Preliminary retrospective data sets have evaluated teclistamab specifically in this context and demonstrated the potential for durable responses despite anti-BCMA CAR T-cell therapy exposure.[38]

Conclusions

Anti-BCMA CAR T-cell therapies represent highly effective treatment options for patients with heavily pretreated RR MM. While there remain questions with regards to patient selection, outcome heterogeneity, overall cost, patient access, and appropriate treatment timing, there is little doubt that these therapies have revolutionized clinical management of RR MM. Going forward, it will be critical to continue to evaluate new prospective studies evaluating these agents, particularly in earlier lines of therapy and in patients with prior exposure to anti-BCMA agents.

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Off-label drug use

Ross S. Firestone: There are no discussions of off label drug use in this article.
Sham Mailankody: There are no discussions of off label drug use in this article.

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Managing side effects: guidance for use of immunotherapies in multiple myeloma

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Chimeric antigen receptor T-cell therapy and bispecific T-cell recruiting antibodies have transformed the treatment landscape for relapsed/refractory multiple myeloma, with B-cell maturation antigen being the most common target and other targets in clinical development. However, these therapies are associated with unique and severe toxicities, including cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), delayed neurotoxicity, cytopenias, and infection. In addition, immune effector cell-associated hemophagocytic lymphohistiocytosis (HLH)–like syndrome (IEC-HS), which exhibits overlap between CRS and HLH, can be challenging to diagnose and treat. In this review, we provide an overview of toxicities associated with novel immunotherapies for treatment of multiple myeloma and describe management recommendations. The pathophysiology and risk factors behind these toxicities are not yet comprehensively understood. Based on consensus recommendations, treatment for CRS consists of tocilizumab and steroids, while treatment for ICANS includes steroids and anakinra in severe cases. Management of cytopenias and infection is similar to post–hematopoietic cell transplantation principles with antimicrobial prophylaxis, growth factor support, immunoglobulin replacement, and vaccinations. In contrast, effective treatments for delayed neurotoxicity and IEC-HS are lacking, although steroids and anakinra are commonly used. Management of all these toxicities should include a broad differential and multidisciplinary collaboration with infectious diseases, neurology, and/or critical care providers.

LEARNING OBJECTIVES

- Recognize immunologically mediated toxicities with novel immunotherapies for multiple myeloma (chimeric antigen receptor T-cell and bispecific antibodies)
- Identify standard and emerging treatment options for cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, and immune effector cell-associated hemophagocytic lymphohistiocytosis–like syndrome
- Evaluate and treat cytopenias following novel immunotherapies
- Understand key principles in preventing infection in patients with multiple myeloma treated with novel immunotherapies

CLINICAL CASE 1

A 65-year-old woman with a history of IgG κ multiple myeloma (MM) with high-risk features, including t(4;14), was initially treated with daratumumab, bortezomib, lenalidomide, and dexamethasone, resulting in a very good partial response. She received autologous hematopoietic cell transplantation (HCT), followed by daratumumab and lenalidomide maintenance. She relapsed within 1 year of HCT. After 3 subsequent lines of therapy, she underwent chimeric antigen receptor (CAR) T-cell therapy with ciltacabtagene autoleucel (cilta-cel). She received bridging therapy with a carfilzomib-based regimen, with progressive disease as best response. At time of lymphodepletion therapy, her M-spike was 2 g/dL, bone marrow showed 30% involvement with plasma cells, and positron emission tomography–computed tomography (PET-CT) had a few areas of fluorodeoxyglucose (FDG) avid disease.

On day 7 following CAR T-cell infusion, she developed fever and hypotension that responded well to intravenous (IV) fluids. Her absolute neutrophil count (ANC) count was 0.8×10^9/L. Infectious workup was sent, and she was started on broad-spectrum antibiotics for neutropenic fever. She was also deemed to have grade 2 cytokine release syndrome (CRS) and received tocilizumab 8 mg/kg IV and dexamethasone 10 mg IV once. C-reactive protein was

12 mg/dL and ferritin was 6000 ng/mL, both of which were significantly increased from baseline. Her fevers resolved with tocilizumab and dexamethasone, infectious workup was negative, and antibiotics were stopped after 48 hours. On day 9 following CAR T-cell infusion, she was noted to have word-finding difficulty, and immune effector cell-associated encephalopathy score was 8/10. She was deemed to have grade 1 immune effector cell-associated neurotoxicity syndrome (ICANS) and received dexamethasone 10 mg IV, with resolution of symptoms. She was discharged from the hospital on day 12.

Introduction

CAR T-cell and bispecific T-cell engaging antibodies have emerged as promising treatments for relapsed/refractory MM. The current treatments approved by the US Food and Drug Administration (FDA), including idecabtagene vicluecel (ide-cel), cilta-cel, teclistamab and elranatamab, target B-cell maturation antigen (BCMA), although other targets like Fc receptor homolog 5 (FcRH5) and G-protein-coupled receptor family C group 5 member D (GPRC5D) are being explored in clinical trials and may become available as standard of care in the near future. Although these immunotherapies can lead to high overall response rates and durable responses, their use is limited by potentially severe and life-threatening complications, such as CRS, ICANS, delayed neurotoxicity, cytopenias, and infection (Figure 1). Prevention, monitoring, and management of these complications are crucial to improving patient outcomes.

Cytokine release syndrome

CRS occurs due to T-cell activation, proliferation, and systemic inflammation. It is seen with both CAR T-cell therapy and bispecific antibodies in MM. Clinical manifestations include fever and hypotension, while severe cases result in shock and multiorgan failure.[1] CRS is usually accompanied by changes in laboratory parameters, including elevated C-reactive protein, ferritin, lactate dehydrogenase, and coagulation labs. It is graded according to American Society of Transplantation and Cellular Therapy (ASTCT) criteria.[1] Most patients undergoing CAR-T therapy and two-thirds of patients receiving bispecific antibodies experience CRS, although grade 3 or higher CRS is less common.[2-8]

Table 1 shows CRS after BCMA-targeted immunotherapies, although the incidence of CRS appears to be similar regardless of the target antigen. For example, CRS was seen in 88% of patients undergoing GPRC5D CAR T-cell therapy with MCARH109[9] and in 70% to 80% receiving the non-BCMA-bispecific antibodies talquetamab (CD3×GPRC5D) and cevostamab (CD3×FcRH5), respectively.[10,11] CRS typically happens in the first few days of initiating therapy, with median time to onset for ide-cel and cilta-cel being 1 and 7 days, respectively.[2,3] It is possible that this difference in onset of CRS relates to the properties of the CAR T construct, cell dose, and subsequent time to CAR T-cell expansion. The target dose of cilta-cel is 0.5 to 1×10^6 CAR T cells/kg, and that of ide-cel is 300 to 460×10^6 CAR T cells; for a patient who weighs 100 kg and receives the higher end of the dose of cilta-cel, the overall dose of ide-cel is still 3 to 4 times higher. For bispecific antibodies,

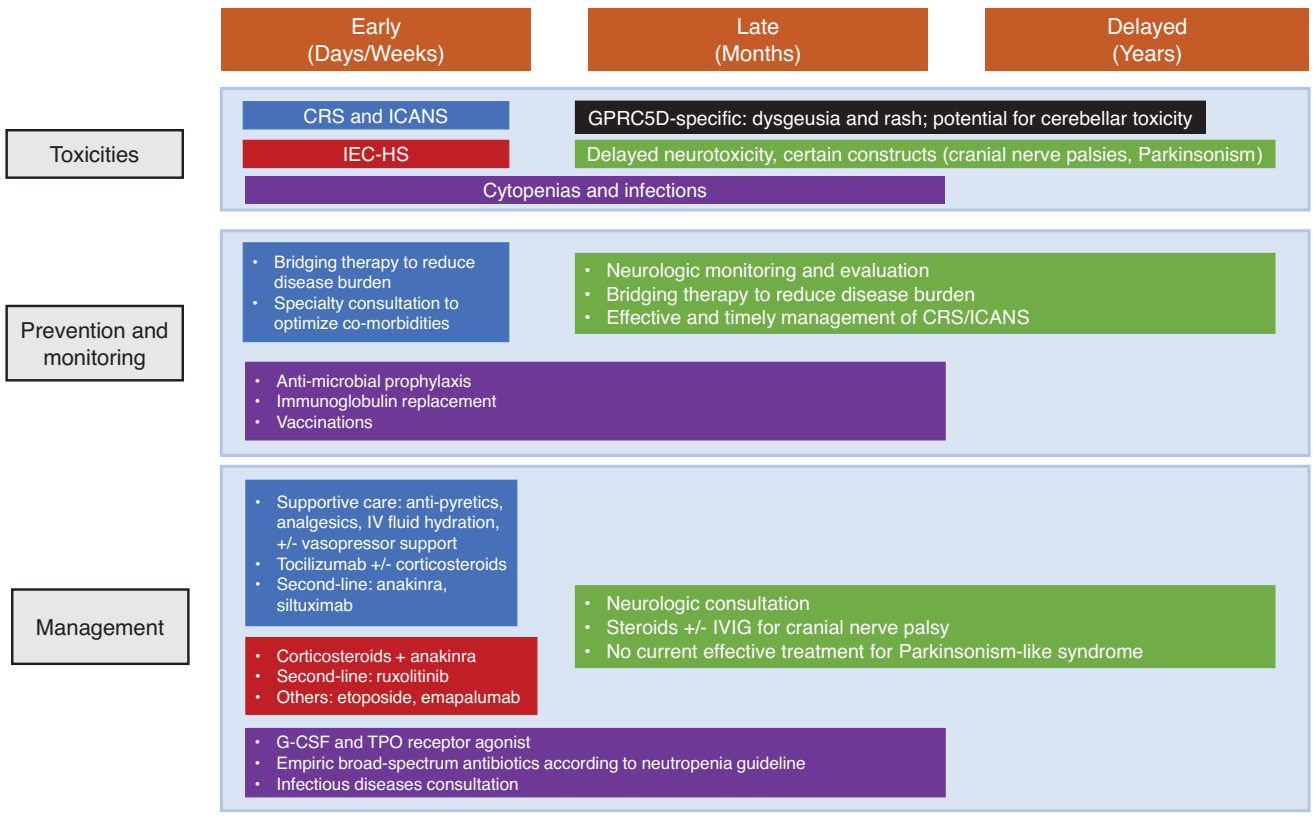

Figure 1. Early, late and delayed toxicities with CAR-T cell therapy and bispecific antibodies in multiple myeloma.

Table 1. CRS, ICANS, delayed neurotoxicity, and IEC-HS with BCMA CAR T-cell and bispecific T-cell recruiting antibodies

	Idecabtagene vicleucel (KarMMa)[1]	Ciltacabtagene autoleucel (CARTITUDE-1)[2]	Teclistamab (MajesTEC-1)[3]	Elranatamab (MagnetisMM-3)[4]*	Linvoseltamab (Linker-MM1)[5]*	Alnuctamab[6]*	ABBV-383[7]
CRS							
Any grade	84%	95%	72%	56%	44%	53%	57%
Grade ≥3	5%	4%	<1%	0%	1%	0%	2%
ICANS							
Any grade	18%†	17%	3%	3%	6%	3%	2%
Grade ≥3	3%	2%	0%	0%	1%	0%	N/A
Delayed neurotoxicity							
Any grade	—	12%	—	—	—	—	—
Grade ≥3	—	8%	—	—	—	—	—

1. Munshi et al,[2] *NEJM* 2021. 2. Berdeja et al,[3] *Lancet* 2021. 3. Moreau et al,[4] *NEJM* 2022. 4. Bahlis et al,[5] ASH 2022 presentation. 5. Bumma et al,[6] ASH 2022 presentation. 6. Wong et al,[7] ASH 2022 presentation. 7. D'Souza et al,[8] *JCO* 2022.

*Data from updated data presented at the 2022 American Society of Hematology Annual Meeting.

†Referred to as investigator-identified neurotoxicity.

CRS is usually limited to step-up doses and first full dose and occurs at median of 1 to 2 days after the dose.[4] Recurrence of CRS can be seen with subsequent bispecific antibody doses, although this is also typically limited to the first few doses.[5,12] Risk factors for development of CRS after CAR T-cell therapy, particularly severe CRS, are not well defined for myeloma, although disease burden is associated with higher-grade CRS after ide-cel,[13] similar to that seen for other hematologic malignancies treated with CD19-directed CAR-T cell therapy. The risk factors for CRS following bispecific antibody therapy are unknown.

Management of CRS is dependent on severity, and similar principles apply to CRS management with both CAR-T cell therapy and bispecific antibodies, with the notable addition for bispecific antibodies being to hold further doses until CRS has resolved. The FDA label for the only bispecific antibody approved to date, teclistamab, recommends step-up dosing with inpatient monitoring for CRS for 48 hours after administration of both step-up doses and first full dose and the lable for elranatamab recommends inpatient monitoring for 48 hours after first step-up dose and 24 hours after the second step-up dose. Both labels do not have specific guidelines on the use of tocilizumab and steroids for management, but these should be used similarly to CAR T-cell therapy. Patients with grade 1 CRS can be managed with close observation and supportive care, although many institutions use tocilizumab, an interleukin (IL) 6 receptor antagonist for grade 1 CRS, especially if persistent. Grade 2 CRS is managed with tocilizumab and steroids. Tocilizumab can be repeated every 8 hours usually for a maximum of 3 doses. For grade 2 CRS, steroid treatment is usually of limited duration and can be stopped once CRS resolves to grade 1. Grade 3 or 4 CRS is life-threatening and requires vasopressor support, along with tocilizumab, and high-dose corticosteroids in an intensive care unit, with potential use of other treatments if symptoms are not rapidly improving (Table 2).

While the role of tocilizumab and corticosteroids for CRS is well established,[2-4,7,8,14-18] the optimal treatment for CRS that is refractory to tocilizumab and corticosteroids remains unclear, and experiences are limited to retrospective or nonrandomized studies. Anakinra, an IL-1 receptor antagonist, is often used as second-line therapy for CRS.[19-22] For example, in 18 patients who developed CRS in the phase 1b/2 CARTITUDE-1 trial of cilta-cel, administration of anakinra at 100 to 200 mg every 8 to 12 hours over a median of 2.5 days led to CRS resolution in all but 1 patient.[21] Additional therapies for refractory CRS include siltuximab (anti–IL-6 monoclonal antibody),[23] etanercept (tumor necrosis factor α [TNF-α] inhibitor),[24] infliximab (anti–TNF-α monoclonal antibody),[16,25] and lenzilumab (anti–granulocyte-macrophage colony-stimulating factor monoclonal antibody).[26]

Similarly, prevention of CRS is an unmet need. All patients should have comorbidities optimized prior to CAR T-cell therapy. As disease burden is the strongest predictor for CRS,[14,27] prelymphodepletion bridging chemotherapy for patients with high tumor burden can be used as a mitigation strategy.[3] Other methods, such as prophylactic tocilizumab and anakinra, are currently being studied in patients with MM receiving bispecific antibodies[28] and in patients with B-cell non-Hodgkin lymphoma receiving CD19 CAR T-cell therapy[29-31] and, if effective, may be considered in the future.

CLINICAL CASE 1 (continued)

On day 16 of CAR T-cell therapy, the patient presented to the emergency department with right-sided facial droop consistent with a cranial nerve VII palsy. Workup for stroke, including magnetic resonance imaging (MRI) of the brain, was normal. Differential diagnosis included delayed neurotoxicity from cilta-cel vs cranial nerve VII palsy related to herpes zoster reactivation. There were no cutaneous lesions suggestive of herpes zoster, and she had also been on acyclovir prophylaxis. She was started on steroids with dexamethasone 4 mg twice daily and received intravenous immune globulin (IVIG). Her facial droop gradually improved over several days, although she developed side effects to steroids, which were decreased and then stopped after 2 weeks. Her facial droop continued to improve

Table 2. Prevention, monitoring, and management of CRS, ICANS, and delayed neurotoxicity

	Prevention	Monitoring	Management
CRS	Potential risk factors: high disease burden, aggressive disease • When clinically feasible, consider bridging therapy to reduce disease burden or use CAR-T cell therapy therapy in lower disease burden state	• Temperature and vital signs • Laboratory tests: CBC, chemistry, CRP, ferritin, coagulation studies	Any grade: • Supportive care: antipyretics • IV fluid hydration • Supplemental oxygen • Assessment of infection and, if neutropenic, empiric antibiotics per neutropenic guidelines Grades 1 and 2: consider tocilizumab for grade 1 CRS based on clinical features, especially if persistent. Recommend tocilizumab + corticosteroids for grade ≥2 CRS, with dosing and frequency based on severity. Grade ≥3: • Vasopressor support • Tocilizumab + corticosteroids • Second line: anakinra, siltuximab, etanercept, infliximab, lenzilumab
ICANS	Potential risk factors: disease burden, baseline elevated inflammatory markers, higher CAR T-cell dose • When clinically feasible, consider bridging therapy to reduce disease burden or use CAR T-cell therapy in lower disease burden state • Antiseizure prophylaxis	• Neurologic consultation in patients with preexisting neurologic disease • Baseline neurologic and mental status exams • ICE score every 8 hours • Neurologic checks at least every 8 hours • Airway monitoring	Any grade: • Supportive care: aspiration precautions, seizure prophylaxis • Corticosteroids: dosing and frequency dependent on severity • Consider CT head, MRI brain, EEG, and neurologic consultation Grade ≥3: • High-dose corticosteroids • Second line: anakinra, siltuximab
Delayed neurotoxicity	Risk factors: high disease burden, CRS, ICANS • Timely treatment of CRS/ICANS • When clinically feasible, consider bridging therapy to reduce disease burden or use CAR T-cell therapy in lower disease burden state	• Neurologic consultation in patients with preexisting neurologic disease • Neurologic evaluation up to 1 year after infusion to evaluate for cranial nerve palsies, neuropathy, and Parkinsonism	• Supportive care • Neurologic consultation • Cranial nerve palsies: corticosteroids, consider IVIG • No known effective therapy for Parkinsonian features
IEC-HS	Unknown	• As in CRS with close monitoring of blood counts, liver and renal function, and coagulation parameters	• Supportive care: antipyretics, analgesics, IV fluid hydration, vasopressor support, correction of coagulopathy • Corticosteroids + anakinra • Second line: ruxolitinib, etoposide, emapalumab, activation of CAR T-cell "kill switches" • Evaluation and treatment of alternative etiologies, including infection and progressive disease

CBC, complete blood count; CRP, C-reactive protein; EEG, electroencephalogram; ICE, immune effector cell-associated encephalopathy.

but had not completely resolved at last follow-up (8 weeks from CAR T-cell infusion).

Neurotoxicity

Neurotoxicity is another class effect seen with both CAR T-cell therapy and bispecific antibodies, with around 20% of patients experiencing it after CAR T-cell therapy, while the incidence is lower with bispecific antibodies (Table 1).[2-8] As shown in Table 1, up to 5% of patients develop ICANS after BCMA-targeted bispecific antibodies, though it has been seen in around 10% of patients after GPRC5D-targeted bispecific antibodies.[2-8] In a phase 1 dose escalation trial of GPRC5D CAR T-cell therapy with MCARH109, 1 patient experienced grade 4 ICANS at the highest dose level (450×10^6 CAR T cells).[9]

Neurotoxicity after immunotherapies typically manifests as ICANS in the first few days after infusion or initial doses, although other unusual delayed neurotoxicities have also been seen. Symptoms of ICANS include tremor, dysgraphia, expressive aphasia, and apraxia, and can progress to seizures and coma.[1] Neuroimaging is generally normal, but MRI can demonstrate cerebral edema or hyperintensities in the limbic system and brainstem.[1,32-34] Median time to onset of ICANS is 2 days after ide-cel[2] and 8 days after cilta-cel[3]; for bispecific antibodies, ICANS is usually restricted to the step-up doses and first full dose, with median time to onset of 2 to 3 days.[4,5,35]

While occasional cases of delayed neurotoxicities have been described with several BCMA-targeted CAR T-cell therapies, an unusually high incidence has been seen after cilta-cel. These delayed neurotoxicities include cranial nerve palsies, most commonly seventh nerve palsy, neuropathy, and Parkinsonism-like syndrome, which is characterized by movement, cognitive, and personality changes (also called movement and neurocognitive treatment-emergent adverse events, or MNTs). In the CARTITUDE-1 trial, cranial nerve palsies and MNTs occurred in 1 (1%) and 5 (5%) patients, respectively[36]; in CARTITUDE-4, the incidences of cranial nerve palsies and MNTs were 9% and 0.5% (n=1), respectively.[37] In addition, a real-world study

of cilta-cel observed a 12% incidence of delayed neurotoxicities, most of which were cranial nerve palsies (MNTs, 1%).[38] The median time to onset of these delayed neurotoxicities was 3 to 4 weeks, although they have been reported to occur more than 3 to 6 months after CAR T-cell infusion and can last through 1 year after infusion.[36,37,39-41] The mechanism behind these delayed neurotoxicities is unclear, but expression of BCMA at a low level in the parts of the central nervous system and trafficking of CAR T cells mediating on-target, off-tumor effects may play a role. Risk factors include preexisting CRS and ICANS and, similarly to CRS and ICANS, high disease burden and high CAR T-cell expansion.[36] Of note, all 6 patients who developed MNTs on CARTITUDE-1 and CARTITUDE-4 were male.[36,37] In the phase 1 studies of the GPRC5D-targeted CAR T-cell products MCARH109 and CC-95266, cerebellar neurotoxicity, such as dizziness and ataxia, were observed at incidences of 12% (n=2) and 13% (n=9), respectively, with a potential mechanism being GPRC5D expression in the cerebellum.[9,42] Patients should be educated and closely monitored for symptoms of delayed neurotoxicity, including by neurological exam that includes evaluation for gait, tremor, and handwriting changes and by neuroimaging, with the caveat that neuroimaging is often normal.[36,40]

Currently, treatment of ICANS consists of supportive care, corticosteroids, and anti-inflammatory agents such as anakinra and siltuximab in severe cases (Table 2).[15,17,34,36] Antiseizure prophylaxis is often used. Many centers use antiseizure prophylaxis in all patients undergoing CAR T-cell therapy, with dose increase at the time of ICANS development; in the case of bispecific antibodies, it is usually reserved for patients who develop symptoms of neurotoxicity given the low incidence of ICANS.

The treatment for delayed neurotoxicities is even more limited. Steroids are commonly used for treatment of cranial nerve palsies, often in conjunction with IVIG, and, in some cases, treatment for varicella zoster virus infection even in the absence of any lesions, although systematic data on efficacy are lacking. These cranial neuropathies often resolve, although time to resolution can be several weeks. MNTs are the most challenging delayed neurologic toxicities to manage, without any effective treatment option. Typical treatment for Parkinson disease has not been found to be effective. In patients who developed MNTs on CARTITUDE-1, steroids, systemic and intrathecal chemotherapy, anakinra, siltuximab, and neurologic agents such as carbidopa/levodopa did not improve symptoms.[36] Preemptive strategies include reducing tumor burden by use of effective bridging therapy and prompt treatment of CRS and ICANS. Neurologic consultation should also be performed prior to treatment in patients with preexisting neurologic disease to establish baseline symptoms and function, and patients should be monitored for up to 1 year following CAR T-cell infusion.[34,36]

Hemophagocytic lymphohistiocytosis (HLH)–like syndrome/immune effector cell-associated HLH-like syndrome

Hemophagocytic lymphohistiocytosis (HLH)–like syndrome/immune effector cell-associated HLH-like syndrome (IEC-HS) has been recently described as an entity distinct from severe CRS and is characterized as an hyperinflammatory syndrome with macrophage activation and HLH, worsening or new cytopenias, hyperferritinemia, coagulopathy, hypofibrinogenemia, and/or transaminitis.[43] Clinical trial reports of IEC-HS are limited to 1 patient on CARTITUDE-1.[3] In a single-center study of 55 patients undergoing BCMA CAR T-cell therapy, 12 (22%) developed IEC-HS.[44] Potential risk factors for IEC-HS include prior infection, longer CRS duration, grade ≥2 CRS, and neurotoxicity.[44]

Given the complexity and life-threatening nature of IEC-HS, a recent ASTCT working group developed consensus guidelines for diagnosing, grading, and treating IEC-HS.[43] Key components of management include rapid clinical identification; initial treatment with anakinra and corticosteroids; escalation to dual therapy with the addition of ruxolitinib, etoposide, and/or emapalumab; and evaluation of other etiologies of hyperinflammation, such as infection and progressive disease (Table 2).[43]

CLINICAL CASE 2

A 62-year-old man with standard-risk MM received teclistamab as 10th line for progressive disease and achieved a very good partial response after 6 months of therapy. IgG levels were low at 200 to 250 mg/dL, and he received IVIG once a month. During cycle 7 of teclistamab, he presented with fever and cough for 3 days. Chest imaging showed patchy ground-glass opacities bilaterally. He was thought to have coronavirus disease 2019 (COVID-19) pneumonia and was treated with a course of remdesivir with clinical improvement.

One month later, he presented again with fever. Infectious workup, including blood cultures and respiratory viral panel, was negative. He was started on broad-spectrum antibiotics. Given persistent fevers, he underwent CT chest, abdomen, and pelvis, which revealed pulmonary nodules. He was started on posaconazole. Four days after starting posaconazole, liver function tests were noted to be increased. He continued to have fevers, so workup of viral reactivation was pursued, and he was found to have adenoviremia with a viral load of 105 000 copies/mL. He was started on cidofovir, which was complicated by acute kidney injury. Gradually, his viremia decreased and his fevers resolved.

Cytopenias

Similar to CD19-targeted immunotherapies, BCMA-targeted immunotherapies frequently result in cytopenias. In addition to the clinical trial experiences described in Table 3, retrospective studies of patients receiving BCMA CAR T-cell therapy have demonstrated prolonged acute and delayed cytopenias and B-cell aplasia lasting >30 days following CAR T-cell infusion.[45,46] Predictors of delayed count recovery included increased bone marrow disease burden and longer CAR T-cell persistence.[46] Longer CAR T-cell persistence was also associated with slower recovery of IgA but not IgG or IgM.[45] There was no significant association between duration of cytopenias and CRS, number of lines of prior therapy, prior autologous hematopoietic cell transplantation, or peak CAR T-cell expansion.[46] In contrast, fewer lines of therapy predicted B-cell recovery at 3 months in both univariate and multivariable analyses.[45]

Table 3. Cytopenias and infection after BCMA CAR T-cell and bispecific T-cell recruiting antibodies

	Idecabtagene vicleucel (KarMMa)[1]	Ciltacabtagene autoleucel (CARTITUDE-1)[2]	Teclistamab (MajesTEC-1)[3]	Elranatamab (MagnetisMM-3)[4]*	Linvoseltamab (Linker-MM1)[5]*	Alnuctamab[6]*	ABBV-383[7]
Neutropenia							
Any grade	91%	96%	71%	48%	25%	37%	37%
Grade ≥3	89%	95%	64%	48%	23%	32%	34%
Thrombocytopenia							
Any grade	63%	79%	40%	30%	19%	24%	23%
Grade ≥3	52%	60%	21%	22%	16%	9%	12%
Anemia							
Any grade	70%	81%	52%	48%	36%	38%	29%
Grade ≥3	60%	68%	37%	37%	31%	25%	16%
Hypogammaglobulinemia							
Any grade	41%[8]	94%[9]	75%	75%	N/A	N/A	14%
Received IVIG	61%	38%	39%	41%	N/A	N/A	N/A
Infection							
Any grade	69%	58%	76%	67%	54%	34%	41%
Grade ≥3	22%	20%	45%	35%	29%	9%	N/A

1. Munshi et al,[2] *NEJM* 2021. 2. Berdeja et al,[3] *Lancet* 2021. 3. Moreau et al,[4] *NEJM* 2022. 4. Bahlis et al,[5] ASH 2022 presentation. 5. Bumma et al,[6] ASH 2022 presentation. 6. Wong et al,[7] ASH 2022 presentation. 7. D'Souza et al,[8] *JCO* 2022. 8. ABECMA FDA package insert. 9. CARVYKTI FDA package insert.

*Data from updated data presented at the 2022 American Society of Hematology Annual Meeting.

Management of cytopenias following BCMA-targeted immunotherapies is supportive. For early cytopenias (<30 days after CAR T-cell infusion), infectious prophylaxis and management as described below are critical. Granulocyte-colony stimulating factor (G-CSF) should be used during periods of prolonged neutropenia (ANC <500×10^9/L).[47] Protocols vary at each center, with some centers recommending G-CSF for ANC <1000 and others restricting it to ANC <500×10^9/L. Some centers also restrict use of G-CSF in patients with active or high-risk CRS due to initial reports of longer or more severe CRS after G-CSF administration in patients receiving CD19 CAR T-cell therapies,[48,49] although other studies report no association of G-CSF use with CRS or ICANS in BCMA CAR T-cell therapies.[50,51] In real-world studies of BCMA CAR T-cell therapy, approximately 90% of patients required G-CSF within 1 month of CAR T-cell infusion, with requirements decreasing over time.[52,53]

Treatment of prolonged or late cytopenias (>30 days after CAR T-cell infusion) consists of growth factor support with G-CSF, thrombopoietin-receptor agonists, and, for prolonged and late multilineage cytopenias, stem cell boost.[47] At this time, bone marrow evaluation for the presence of persistent or recurrent disease, opportunistic viral infections, marrow fibrosis, or secondary malignancy should be considered, particularly if there is no or minimal response to G-CSF.[47]

Infections

Infections occurred in over half of patients receiving BCMA-targeted immunotherapies on the pivotal clinical trials (Table 3).[2-5] Viral and bacterial infections are most common, although infections with fungal organisms such as *Aspergillus* and *Rhizopus* have also been observed.[2,52-56]

While prolonged hypogammaglobulinemia is a complication of both CD19- and BCMA-targeted immunotherapies, BCMA-targeted immunotherapies cause additional humoral immunodeficiency by destroying all plasma cells.[57,58] While rates of hypogammaglobulinemia and IVIG use have not been reported consistently across clinical trials (Table 3), retrospective studies of BCMA CAR T-cell therapy have demonstrated high rates of hypogammaglobulinemia.[45,52,54] Patients with severe infections had lower serum IgG concentrations than those with mild or moderate infections,[45] and infections tended to occur during periods of hypogammaglobulinemia.[52] In addition, patients receiving BCMA CAR T-cell therapy experienced a decline in pathogen-specific antibody titers to vaccinations[54] and, in 1 cross-sectional study, were half as likely to have seroprotective antibody titers and had fewer IgG-targeted pathogen-specific epitopes compared to patients receiving CD19 CAR T-cell therapy.[59]

Thus, prevention of infections after BCMA-targeted immunotherapies is critical. Following CAR T-cell therapy, patients should receive polymicrobial prophylaxis, including with trimethoprim-sulfamethoxazole for *Pneumocystis jirovecii* pneumonia prophylaxis and, during periods of prolonged severe neutropenia (ANC <0.5×10^9/L), levofloxacin and fluconazole (Table 4).[47,60] While similar principles of antimicrobial prophylaxis apply after bispecific antibody therapy, the duration of prophylaxis would depend on an individual patient's treatment duration and resulting cytopenias.

Table 4. Prevention of infectious complications and management of cytopenias resulting from CAR T-cell and bispecific T-cell recruiting antibodies

Intervention category	Intervention description	Indications	Notes
1. Antimicrobial Prophylaxis			
All patients			
Antiviral	Acyclovir or valacyclovir: prevention of HSV and VZV reactivation	All patients • CAR T-cell therapy: 12–18 months after infusion, at least until CD4 count >200/μL • Bispecific antibodies: during treatment and until 1 month after treatment discontinuation	
Pneumocystis jirovecii	Trimethoprim-sulfamethoxazole	All patients • CAR T-cell therapy: 12–18 months after infusion, at least until CD4 count >200/μL • Bispecific antibodies: during treatment and until 1 month after treatment discontinuation	Alternatives: dapsone, atovaquone, pentamidine (disadvantages include lack of activity against encapsulated organisms)
Selected patients			
Antiviral	Entecavir	Prevention of HBV reactivation in patients with history of HBV infection or known exposure	
Antibacterial	Levofloxacin	Consider during periods of prolonged severe neutropenia (ANC <0.5×10^9/L)	
Antifungal	Fluconazole or posaconazole	During periods of severe prolonged neutropenia (ANC <0.5×10^9/L) or prolonged steroid therapy	
2. Growth factors	G-CSF Thrombopoietin-receptor agonist	• Consider G-CSF if ANC <1.0×10^9/L; strongly recommend for ANC <0.5×10^9/L, especially if prolonged • Give G-CSF for active neutropenic infection • Consider TPO agonist if prolonged severe thrombocytopenia that persists beyond 30 days with high transfusion needs	• Caution in patients with active or high risk of CRS
3. Immunoglobulin replacement	IVIG 400–500 mg/kg	Serum IgG ≤400 mg/dL	Monitor serum IgG levels every 4 weeks
4. Vaccinations	Influenza COVID-19	Influenza vaccine repeated annually COVID-19 vaccine series repeated ≥3 months after CAR T-cell therapy If feasible, patients should be vaccinated prior to therapy.	Consider measuring serum pathogen-specific IgG titers after vaccination to evaluate for seroprotection. There are limited data to comment on repeating routine immunizations following CAR-T therapy and bispecific antibodies.

HBV, hepatitis B virus; HSV, herpes simplex virus; VZV, varicella zoster virus.

There are limited data to recommend standard post-cellular therapy vaccinations, although COVID-19 revaccination and influenza vaccination are highly recommended. If feasible, patients should be up to date on all appropriate vaccines prior to the start of therapy. Pathogen-specific IgG titers should be considered and more data are needed to recommend universal revaccination after CAR T-cell therapy, similar to post-HCT management (Table 4).

There is no clear consensus for monitoring or treating hypogammaglobulinemia after CAR T-cell or bispecific therapy. Rates of immunoglobin replacement therapy in clinical trial and real-world studies range from 13% to 62%.[2,52,53] According to consensus and expert recommendations, IgG replacement should be considered in patients with serum IgG ≤400 mg/dL, those with serious or recurrent bacterial infections, and those with low pathogen-specific antibody titers (Table 4).[17,60-62] Lastly, during periods of prolonged neutropenia or active neutropenic infection, G-CSF should be considered.[17,47]

It is important to note that viral reactivations with viruses like cytomegalovirus, human herpesvirus 6, Epstein-Barr virus, and adenovirus have been seen after both CAR T-cell therapy and bispecific antibodies.[10,55,56,63,64] Viral reactivation

should be strongly considered in the differential diagnosis when patients present with fever or other unexplained laboratory abnormalities. Viral levels should be evaluated in patients with fever without a clear explanation, especially in conjunction with findings such as liver function abnormalities and cytopenias.

The risk of infections with novel immunotherapies may be target dependent, as the risk of grade ≥3 infections, neutropenia, and hypogammaglobulinemia has been seen to be higher with BCMA-targeted bispecific antibodies compared with GPRC5D-targeted antibodies,[4,5,8,10,56] although additional follow-up and data are needed.

Unique toxicities of other MM targets

Other MM targets under investigation include FcRH5, which is expressed only on B cells, and GPRC5D, which is expressed on plasma cells and keratinized tissues. Similarly to the BCMA-targeted immunotherapies, common toxicities include CRS and cytopenias.[10,65,66]

Unique toxicities with GPRC5D-targeted immunotherapies relate to their effect on keratinized tissues. Such toxicities include skin-related events (dry skin, eczema, pruritus, hyperpigmentation), nail-related events (discoloration, dystrophy, hypertrophy, onycholysis), and dysgeusia, which occurred in up to 70% of patients on the phase 1 study of talquetamab.[10] These events tended to occur within 1 to 3 months of treatment and tended to resolve within 3 months, although nail changes can persist beyond 3 months; there were no treatment discontinuations related to these events.[10] Supportive care, including emollient creams and oral rinses, can be used for these symptoms.[66] Two patients (12%) experienced grade 1 dysgeusia after MCARH109, and it resolved in both patients without intervention.[9]

Conclusions

In conclusion, immunotherapies comprise a new treatment paradigm for patients with relapsed/refractory MM but are associated with unique, potentially prolonged immunologic, neurologic, hematologic, and infectious toxicities. As more of these promising therapies are developed, it is crucial for treating physicians to be able to recognize and treat these toxicities. Future work should focus on elucidating the pathophysiology and predictors of these toxicities and developing evidence-based management strategies to treat these toxicities.

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Emily C. Liang: no competing financial interests to declare.

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Off-label drug use

Emily C. Liang: There is no off label drug use.
Surbhi Sidana: There is no off label drug use.

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CAR T-cell therapy in aggressive lymphomas—identifying prognostic and predictive markers

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We discuss different pre-infusion, post-infusion and post-CAR T-cell relapse prognostic factors influencing the outcomes of anti-CD19 CAR T-cell therapy in patients with relapsed or refractory large B-cell lymphomas. Despite the overall positive results of anti-CD19 CAR T-cell therapy, a significant percentage of patients relapse. We summarize the efforts made to identify predictive factors for response and durable remissions and survival. In the pre-infusion setting, the patient-related factors discussed include Eastern Cooperative Oncology Group performance status, age, and comorbidities. Disease-related factors like tumor burden, histology, and biological features are also considered. In addition, inflammation-related factors and CAR T-cell product-related factors are considered. After CAR T-cell infusion, factors such as disease response assessed by 18FDG-PET/CT scan, liquid biopsy monitoring, and CAR T-cell expansion become crucial in predicting survival outcomes. Response to 18FDG-PET/CT scan is a widely used test for confirming response and predicting survival. Liquid biopsy, in combination with 18FDG-PET/CT scan, has shown potential in predicting outcomes. CAR T-cell expansion and persistence have shown mixed effects on survival, with some studies indicating their association with response. In the setting of post-CAR T-cell relapse, prognostic factors include refractory disease, time of relapse, and elevated lactate dehydrogenase levels at CAR T-cell infusion. Enrollment in clinical trials is crucial for improving outcomes in these patients. Overall, we discuss a comprehensive overview of prognostic factors that can influence the outcomes of anti-CD19 CAR T-cell therapy in patients with relapsed or refractory large B-cell lymphomas, highlighting the need for personalized approaches in treatment decision-making.

LEARNING OBJECTIVES

- Identify prognostic factors related to CAR T-cell for lymphoma therapy in the pre-infusion, post-infusion, and post-CART relapse setting
- Interpret how the presence of risk factors could have an impact on treatment or a follow-up approach

Introduction

Anti-CD19 CAR T-cell therapy dramatically changed the clinical scenario of patients affected by B-cell malignancies. In adult patients, the pivotal clinical studies ZUMA-1, JULIET, and TRANSCEND showed the curative potential of such cell therapies in the setting of large B-cell lymphomas beyond second line therapy.[1-3] Considering such promising results, anti-CD19 CAR T-cell therapy was also tested in second-line therapy. The ZUMA-7 and the TRANSFORM trials demonstrated the superiority of CAR T-cell vs autologous stem cell transplant.[4,5] Newer CAR T-cell products and their use in first-line therapy and in patients who are not candidates for autologous stem cell transplant are currently being tested.

CLINICAL CASE

In August 2018, a 48-year-old man presented to our department for multiple peripheral enlarged lymph nodes and the presence of B-symptoms. Later, a cervical lymph node biopsy was made with a diagnosis of diffuse large B-cell lymphoma (DLBCL) not otherwise specified (NOS), non-germinal center subtype. Molecular analysis detected a TP53 mutation, while fluorescence in situ hybridization studies showed no evidence of MYC, BCL2, or BCL6 rearrangements. The staging positron emission tomography-computed tomography scan (18FDG-PET/CT) revealed multiple hypermetabolic adenopathies with a bulky abdominal lesion infiltrating the pancreas, spleen,

left kidney, adrenal gland, and gastroesophageal junction effusion. The revised International Prognostic Index (IPI) was 4; the Eastern Cooperative Oncology Group (ECOG) status was 2. The patient received three lines of immunochemotherapy (R-CHOP, R-GDP, and R-ESHAP), with refractoriness to treatment. Therefore, he was a candidate to receive anti-CD19 CAR T-cell therapy with tisagenlecleucel (tisa-cel). After lymphoapheresis, bridging therapy was administrated with one cycle of Rituximab-Bendamustine-Polatuzumab with progression of disease at pre-lymphodepletion 18FDG-PET/CT scan evaluation. A patient-specific circulating tumor DNA (ctDNA) analysis was generated using a KMT2D p.Glu4385Gly tumor-specific mutation identified at diagnosis biopsy material. The patient received standard lymphodepletion therapy with fludarabine/cyclophosphamide. Elevated levels of lactate dehydrogenase (LDH) and C-reactive protein (CRP) were detected at the day of infusion.

Pre-infusion prognostic factors

Almost 60% of patients treated with anti-CD19 CAR T-cell will relapse.[1-3,6] A prognostic score to predict the outcomes of all patients eligible for anti-CD19 CAR T-cell therapy is currently missing. However, predictive factors for response and durable remissions have been identified (Table 1).

Patient-related

ECOG performance status ≥2 was independently associated with an inferior overall response rate (ORR), progression-free survival (PFS), and overall survival (OS).[7-9] These findings were further validated in the largest real-word prospective study by Jacobson et al and in a recent comparison study between tisa-cel and axicabtagene ciloleucel (axi-cel).[10,11]

Age is not considered a strict determinant for eligibility to receive anti-CD19 CAR T-cell therapy. In a large study conducted by the Center for International Blood and Marrow Transplant Research for axi-cel, patients age ≥65 were associated with higher ORR than younger patients.[12] The superior efficacy observed may be attributed to a selection bias, but further studies are needed to deepen the disease biology features in this setting. Age and performance status are part of the International Prognostic Index score. As described later, this score has a prognostic significance in this setting.

Comorbidities have a prognostic impact also in this setting. In a first evaluation of the impact of the Cumulative Illness Rating Scale (CIRS) on survival, the high comorbidity burden (defined as CIRS ≥7 or CIRS 3/4 in one system) was significantly associated with inferior OS and PFS.[8] In a recent multicenter retrospective real-world evidence analysis, a simplified CIRS-based index called "Severe4" was developed that included CIRS >2 at the respiratory, hepatic, renal, and upper gastrointestinal levels. This index was independently associated with inferior PFS, OS, and relapse-related mortality in DLBCL patients eligible for CAR T-cell therapy.[13]

Considering the crucial role of the gut microbiota in the antitumor immune-response to therapy, two recent retrospective and prospective studies showed that lower diversity and a specific microbiota composition are associated with survival outcomes after anti-CD19 CAR T-cell therapy in patients with B-cell malignancies.[14] Nevertheless, further studies are needed to deepen our understanding in this area.

Disease-related

In the context of R/R DLBCL with anti-CD19 CAR T-cell, conventional tumor-related predictive features that have prognostic significance in newly diagnosed DLBCL, such as activated B-cell-like phenotype, cell of origin, and double-hit rearrangements, have no prognostic value.[2,7,9] Nevertheless, several tumor intrinsic factors contribute to CAR T-cell failure, including tumor burden, histology, and biological features.

Intrinsic anti-CD19 CAR T-cell resistance was observed in a small cohort of 9 patients with T-cell/histiocyte-rich large B-cell lymphoma who were treated with axi-cel or tisa-cel, with all patients progressing by day +90.[15] Although the sample size was limited, an explanation was the unique immune environment that defines this disease. However, no clear reduction in the effectiveness of anti-CD19 CAR T-cell in pivotal and real-world studies involving high-grade B cell lymphoma patients was observed.[16]

IPI and age-adjusted-IPI (aaIPI) are two simple and widely used tools in the management of patients with DLBCL. A retrospective study evidenced the strong correlation between aaIPI ≥2 at the time of lymphodepletion with inferior PFS and OS in patients treated with commercial anti-CD19 CAR T-cell products.[17] LDH is part of IPI score and is independently associated with clinical outcomes. A real-world report of the Lymphoma CAR T-cell Consortium, by Nastoupil et al, found that elevated LDH before lymphodepleting chemotherapy, as a surrogate of metabolic tumor burden, was significantly associated with inferior PFS and OS.[9] Further analysis supported the negative prognostic influence of elevated LDH at CAR T-cell therapy election, at apheresis, and at pre-infusion.[9,16,17]

The total metabolic tumor volume (TMTV) measured on 18FDG-PET/CT is a cumulative volume measure of lesions. When the volume of the tumor was calculated before CAR T-cell infusion, patients in the low baseline TMTV group exhibited significantly improved OS and PFS compared to those in the high TMTV group.[18] The negative prognostic significance of a high TMTV was confirmed even after the bridging treatment in a French real-world analysis of 116 patients treated with axi-cel or tisa-cel.[19] Notably, the presence of two or more extranodal sites, when combined with high pre-infusion TMTV, showed the highest hazard ratio and was identified as an independent prognostic factor for relapse and early progression in multivariate analyses.

Furthermore, the need for a bridging therapy aligns closely with the escalating tumor burden and was associated with worse OS as well.[9] However, when effective bridging chemotherapy is able to reduce the burden of disease pre-infusion, its negative impact disappear.[20,21]

Analysis of TP53 alterations through next-generation sequencing has shown a predictive role in this setting. Shouval et al observed a notable independent association between TP53 alterations and inferior complete response (CR) and OS in a multivariable Cox regression model, especially with CAR T-cell product with 4-1 co-stimulation domain compared to CD28 (1-year PFS 10% vs 34% and 1-year OS 36% vs 51%).[22] Also, the pretreatment presence of complex structural variants, APOBEC mutational signatures, and genomic damage from reactive oxygen species predict anti-CD19 CAR T-cell resistance.[23] A retrospective analysis conducted by Cherng et al found that a high focal copy number alteration score detected with low-pass whole-genome sequencing of cell-free DNA at time of

Table 1. Baseline risk factors associated with outcomes for lymphoma patients treated with anti-CD19 CAR T-cell (on multivariate analysis)

Risk factor	Hazard ratio HR
• ECOG ≥2 (baseline)	**PFS**: HR 1.7 (95% CI: 1.1–2.7; $p = 0.010$) **OS**: HR 1.8 (95% CI: 1.10–3.00; $p = 0.020$)[9] **PFS**: HR 2.61 (1.90–3.60) **OS**: HR 3.27 (2.37–4.52)[10] **PFS**: HR 5.446 (95% CI: 2.354–12.597; $p < 0.001$) **OS**: HR 4.306 (95% CI: 1.841–10.071; $p = 0.001$)[11] **OS**: HR 1.63 (95% CI: 1.06–2.51; $p = 0.03$), ECOG considered as 1-unit increase[8]
• Age ≥65 years old • Age >60 years old	**ORR**: OR 1.39 (95% CI: 1.05–1.83)[10] **PFS**: HR 1.6 (95% CI: 1.1–2.3; $p = 0.01$)[9]
• Chemo-resistant disease prior to infusion	**PFS**: HR 1.48 (95% CI: 1.21–1.79) **OS**: HR 1.44 (95% CI: 1.15–1.81)[10]
• Disease status (PD vs other)	**PFS**: HR 1.804 (95% CI: 1.096–3.507; $p = 0.018$) **OS**: HR 2.561 (95% CI: 1.812–3.999; $p = 0.018$)[11]
• CIRS ≥7 or CIRS-3+ (baseline) • "Severe4" score (baseline)	**OS**: HR 2.39 (95% CI: 1.10–5.20; $p = 0.03$) **PFS**: HR 2.15 (95% CI: 1.54–2.99; $p<0.001$)[8] **OS**: HR 1.94 (95% CI: 1.35–2.78; $p<0.001$)[13]
• aaIPI ≥2 at time of lymphodepletion	**PFS**: HR 6.76 (95% CI: 2.21–20.69; $p = 0.001$) **OS**: HR 7.91 (95% CI: 1.74–35.85; $p = 0.007$)[17]
• High LDH at CAR T-cell election	**Relapse**: HR 2.04 (95% CI: 1.19–3.49; $p = 0.009$) **Early relapse**: 9.61 (95% CI: 1.23–75.41; $p = 0.031$)[19]
• High LDH at apheresis	**PFS**: HR 2.181 (95% CI: 1.303–3.651; $p = 0.003$) **OS**: HR 1.809 (95% CI: 1.084–3.021; $p = 0.023$)[11]
• High LDH before lymphodepletion	**PFS**: HR 1.9 (95% CI: 1.3–2.9; $p = 0.001$) **OS**: HR 3.0 (95% CI: 1.7–5.4; $p = 0.0001$)[9]
• Extranodal sites ≥2 at infusion	**Relapse**: HR 2.50 (95% CI: 1.44–4.35; $p = 0.00111$) **Early relapse**: HR 4.67 (95% CI: 1.55–14.11; $p = 0.0063$) **Death**: HR 3.61 (95% CI: 1.55–8.38; $p = 0.00283$[19]
• High MTV pre-lymphodepletion • Low MTV (baseline) • High MTV (baseline)	**Relapse**: HR 2.18 (95% CI: 1.23–3.89; $p = 0.00794$) **Early relapse**: HR 4.35 (95% CI: 1.32–14.37; $p = 0.016$) **Death**: HR 3.41 (95% CI: 1.41–8.26; $p = 0.0651$)[19] **PFS**: HR 0.40; (95% CI: 0.18–0.89). **OS**: HR 0.25; (95% CI: 0.10–0.66)[18] **PFS**: HR 3.44 (95% CI: 1.18–10.1; $p = 0.02$)[42]
• Use of bridging therapy • Refractory to bridging therapy	**OS**: HR 1.7 (95% CI: .04–2.70, 0.0300)[9] **PFS**: HR 2.273 (95% CI: 1.484–3.481; $p = 0.001$) **OS:** HR 2.273 (95% CI: 1.324–3.901; $p = 0.003$)[21]
• Increased CRP at infusion	**Relapse**: HR 1.12 (95% CI: 1.07–1.17; $p = 0.0001$) **Early relapse**: HR 1.15 (95% CI: 1.03–1.29; $p = 0.016$) **Death**: HR 1.12 (95% CI: 1.06–1.17; $p = .0001$)[19]
• Presence of TP53 gene alterations	**CR**: OR 3.61 (95% CI: 1.31–10.7; $p = 0.016$) **OS**: HR 2.03 (95% CI: 1.02–4.03; $p = 0.044$)[43]
• High focal copy number alterations before infusion	**PFS**: HR 2.11 (95% CI: 1.36–3.275; $p = 0.0007$) **OS**: HR 2.10 (95% CI: 1.28–3.43; $p = 0.0026$)[24]
• CAR T-cell type Tisa-cel vs axi-cel Axi-cel vs tisa-cel	 **PFS**: HR 1.475 (95% CI: 1.122–1.942; $p = 0.005$)[21] **PFS**: HR 0.61 (95% CI: 0.46–0.79; $p = 0.0003$) **OS**: HR 0.63 (95% CI: 0.45–0.88; $p = 0.0072$)[28]

aaIPI, age-adjusted International Prognostic Index; CIRS, Cumulative Illness Rating Scale; CRP, C-reactive protein; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; HR, hazard ration; LDH, lactate dehydrogenase; MTV, metabolic tumor volume; OS, overall survival; PD, progression of disease; PFS, progression-free survival.

leukapheresis, as a surrogate of genomic instability, was correlated with inferior +3 months CR ($p = 0.0029$), PFS, and OS.[24]

Inflammation-related

The inflammatory state, directly related to tumor burden, appears to be inversely correlated with in vivo CAR T-cell cell expansion and durable response. In a real-world analysis, day 0 CRP <30 mg/L correlated with improved duration of response (median not reached [NR] vs 3.6 months; $p = 0.0030$), PFS (median NR v 2,5 months; $p = 0.001$), and OS (median NR v 6,5 months; $p = 0.001$).[7] Moreover, a reduced peak ferritin was associated to an improved PFS (median 6.8 vs 2.2; $p = 0.020$) and

OS (median NR vs 2.2; $p = 0.001$). Locke et al analyzed samples from ZUMA-1 patients and found that systemic inflammation markers (LDH, IL6, ferritin) were the most significant risk factors for durable response along with CAR T-cell phenotype.[25] An elevated CRP at infusion level was confirmed as a predictive factor of relapse, early relapse, and death in multivariate analysis.[19]

CAR T-cell product-related

The heterogeneity in T-cell composition in the peripheral blood, including the proportion of T "naive" (T_N), T central memory (T_{CM}), and T effector memory (T_{EM}) cells along with their exhaustion phenotype related to age and chemotherapy regimens administered, can significantly influence the quality of lymphocyte apheresis product and subsequent CAR T-cell production. A strong correlation between the infusion of poorly differentiated memory anti-CD19 CAR T-cell and their enhanced expansion and prolonged persistence have been demonstrated. Notably, in the ZUMA-1 trial, a CAR T-cell product with a higher proportion of CD8 T_N/stem cell memory T cells (T_{SCM}) was associated with an objective ($p = 0.0327$) and durable response ($p = 0.0301$).[25] Finally, Monfrini et al. found that an enrichment of CD8 T_{CM} CAR T-cell products was associated with increased CAR T-cell expansion in vivo, which correlated with higher efficacy (odds ratio = 5.6, 95% CI (confidence interval), 1.681-18.65, $p < 0.005$) and PFS (median PFS NR vs 3.7 months, respectively; $p < 0.05$).[26] In patients treated with axi-cel, a higher number of CD8+ CAR T-cells expressing memory signatures was associated with better responses at +3 months, while the presence of CD8+ CAR T-cells with an exhaustion profile was associated with poorer clinical response.[27]

Finally, the type of CAR T-cell is associated with clinical outcomes. Axi-cel seems to provide superior outcomes in terms of PFS and OS compared to tisa-cel, while tisa-cel showed lower toxicity levels.[11,21,28]

Back to the CLINICAL CASE

The post-infusion course was complicated by cytokine release syndrome (CRS) grade 3 and immune effector cell-associated neurotoxicity syndrome grade 1 at day +4 treated with tocilizumab. At day +6, CRS worsened, requiring the administration of high doses of dexamethasone with progressive resolution. The patient was discharged on day +45. The 18FDG-PET/CT scan performed at +1 month showed a partial response (PR), and concomitant levels of ctDNA at +1 month and +2 months progressively decreased and became undetectable. Unfortunately, the 18FDG-PET/CT at +3 months and a concurrent liquid biopsy showed a radiological and serological disease relapse. In the absence of salvage therapies, palliative care was initiated, and the patient died 4 months after anti-CD19 CAR T-cell infusion.

Post-infusion prognostic factors

After CAR T-cell infusion, other prognostic factors become fundamental in predicting survival outcomes (Table 2). Response to the 18FDG-PET/CT scan is, at present, the most standardized and commonly used test to confirm a response and predict survival outcomes. In the long-term analysis of the ZUMA-1 trial, the estimated proportion of patients with PFS at +24 months was 72.0% (95% CI: 56.0-83.0) among those with CR at +3 months, 75.0% (95% CI: 31.5-93.1) among those with PR at +3 months, and 22.2% (95% CI: 3.4-51.3) among those with stable disease (SD) at +3 months from infusion.[6] In the JULIET trial, the estimated probability of survival at +12 months was 49% (95% CI: 39-59) among all patients and 90% (95% CI: 74-96) among patients with a CR.[2] In the TRANSCEND trial, patients who achieved CR at +1 year had OS of 86% (95% CI: 78.2-90.5) vs 58% (95% CI: 51.3-63.8) of the whole study cohort.[3] PFS at +1 year was 44% (95% CI: 37.3-50.7) for the total population and 65% (95% CI:56.1-72.7) among patients who had CR. The role of 18FDG-PET/CT response has been studied also in the real-life setting. Kuhnl et al. described the prognostic role of 18FDG-PET/CT at +1 month post-infusion, measured by the Deauville score (DS), in terms of response and survival outcomes.[29] Of 171 patients infused with commercial anti-CD19 CAR T-cell (axi-cel, tisa-cel), the risk of early progression was 15% for DS1 to 2, 32% for DS3, 37% for DS4, and 100% for DS5. Moreover, survival outcomes were associated with different scores. PFS at +1 year was 77.1% (DS1-2), 63.5% (DS3), 43.5% (DS4), and 0% (DS5). OS at +1 year was 87.1% (DS1-2), 86.2% (DS3), 62.7% (DS4), and 38.1% (DS5). Al Zaki et al found that the only factor associated with disease progression was having an SUV max ≥10 at day +30 post-infusion.[30] Finally, Guidetti et al combined DS at day +30 with SUV variation from pre-infusion to day +30.[31] Patients with DS4-5 and decreased SUV have +1 year PFS of 61%, which is similar to those with DS1-3. Patients with DS4-5 and increased SUV had a worse PFS at +1 year of 33% ($p = 0.04$).

Despite being considered experimental still, liquid biopsy proved to be an extremely powerful tool, when used in combination with 18FDG-PET/CT scan, in predicting survival outcomes. Frank et al reported the results of 69 patients treated with commercial axi-cel for whom liquid biopsy was available before CAR T-cell infusion and at different timepoints after infusion.[32] Patients with detectable day +28 ctDNA had a median PFS of 3 months vs

Table 2. Risk factors associated with survival outcomes for lymphoma patients after anti-CD19 CAR T-cell infusion

Risk factor	Prognostic role
• Higher CAR T-cell product expansion	**ORR**: OR 1.268 (95% CI: 1.062–1.676; $p < 0.05$)[26]
• +1 month 18FDG-PET/CT disease evaluation	• Prognostic role on relapse and PFS (Deauville score)[29] • SUV max useful in predicting progression for patients in PR/SD[30] • Deauville score combined with SUV variation allowed better stratification of patients at day +30 DS4–5[31]
• Tumor burden measured by liquid biopsy (VDJ ctDNA)	• Prognostic role after infusion at +1 week, +1 month, and +3 months; also, useful in stratifying patients with radiological SD/PR at +1 month[32]

ctDNA, circulating tumor DNA; OR, odds ratio; ORR, overall response rate; PFS, progression-free survival; PR, partial response; SD, stable disease; VDJ, variable, diversity, and joining gene segments.

NR ($p < 0.0001$) and a median OS of 19 months vs NR ($p = 0.0080$) for those without ctDNA. Moreover, of the patients with radiologically SD/PR at day +28, only 1/10 with concurrently undetectable ctDNA relapsed vs 15/17 with concurrently detectable ctDNA ($p = 0.0001$). Finally, all patients with durable responses had undetectable ctDNA at or before +3 months from infusion. It should be considered that this study used clonoseq assay using VDJ gene rearrangement as liquid biopsy technique, which is possibly not the best method. Other techniques such as PhasED-Seq or CAPP-seq have more potential in this setting.[33,34]

Finally, expansion and persistence of anti-CD19 CAR T-cell showed a mixed effect on survival. In the JULIET study, no effect on outcomes were observed in terms of CAR T-cell expansion and persistence.[2] On the contrary, in the ZUMA-1 study, CAR T-cell expansion during the first 28 days was associated with response ($p < 0.001$) with an area under the curve 5.4 times higher between responders and no responders.[1] Also, in the TRANSCEND study, expansion of lisocabtagene maraleucel was associated with response. In fact, patients who reached a PR/CR had a higher Cmax (3.55-fold, $p < 0.0001$) and area under the curve during the first 28 days (2.72-fold, $p < 0.0001$).[3] Monfrini et al showed that CAR T-cell expansion has a prognostic role also in the real-life setting (only axi-cel and tisa-cel).[26] In their study, patients in PR/CR within +3 months of infusion had a superior CAR T-cell expansion compared to non-responders measured by CAR T-cell concentration at day +7 and +10, with maximum concentration and area under the curve during the first 30 days. When CAR T-cell are used as second-line therapy, no prognostic role of expansions has been observed for axi-cel in terms of overall survival or for tisa-cel in terms of event-free survival.[34,35] The role of anti-CD19 CAR T-cell persistence did not show any prognostic significance in the lymphoma setting.

Post-CAR T-cell relapse prognostic factors

In the setting of relapse after anti-CD19 CAR T-cell was used after 2 or more lines of therapy, there are a few studies that identified prognostic factors able to predict survival outcomes (Table 3). No data are currently available for relapse after anti-CD19 CAR T-cell is used as second-line therapy. When patients relapse after anti-CD19 CAR T-cell is used beyond second-line therapy, median PFS and OS are 3 and 6 months, respectively.[35-40] The treatment landscape in such a scenario is rapidly changing, considering the emergence of newer therapeutic strategies in this setting. One of the first prognostic risk factors identified was having a refractory disease (progression of disease <30 days from infusion). Chow et al showed that the progression of disease within 30 days of infusion has a median OS of 3.75 months vs 9.29 months ($p = 0.042$).[41] The same results were demonstrated by Zurko et al, who recorded a OS of 2.9 months vs 8.0 months ($p<0.01$) for patients with SD or progression of disease at day +30.[36] An elevated LDH at CAR T-cell infusion was confirmed as one of the most important factors in the post-CAR T-cell relapse setting. No prospective clinical trials defined which is the best therapeutic approach in this setting. Data on the use of newer therapies (eg, lenalidomide; Rituximab-polatuzumab-bendamustine) should not be viewed as definitive because of known biases in the selection of patients fit for additional therapy. Enrollment into clinical trials should be always encouraged, considering the dismal outcomes of such patients.

Conclusions

Factors related to patients, anti-CD19 CAR T-cell product, and disease characteristics can be identified before or after infusion or at relapse post-CAR T-cell product infusion. Currently, there is not a personalized risk score with high accuracy that can be

Table 3. Risk factors associated with survival outcomes for lymphoma patients with relapse after anti-CD19 CAR T-cell infusion

Risk factor	Hazard ratio HR (95% CI)
• Response to axi-cel	**OS**: HR 0.45 (95% CI: 0.29–0.71; $p = 0.0005$)[40]
• Progression <30 day	**OS**: HR 2.93 (95% CI: 1.56–5.50; $p = 0.0009$)[37]
• CAR T-cell refractoriness	**OS**: HR 2.33 (95% CI: 1.02–5.29)[38]
• Grade 3–4 CRS	**OS**: HR 5.39 (95% CI: 2.48–11.7; $p = 2.2.\times10^{-5}$)[40]
• Bridging chemotherapy	**OS**: HR 2.11 (95% CI: 1.32–3.39; $p = 0.002$)[40]
• >2 lines of therapy before CAR T-cell infusion	**PFS**: HR 1.89 (95% CI: 1.1–3.5; $p = 0.03$)[36]
• No double hit or double expressor lymphoma subtype	**PFS**: HR 0.42 (95% CI: 0.18–0.89; $p = 0.03$)[36]
• Polatuzumab-bendamustine-rituximab after CAR T-cell failure as salvage treatment	**PFS**: HR 0.097 (95% CI: 0.013–0.57; $p = 0.01$)[36]
• Lenalidomide-based after CAR T-cell failure as salvage treatment	**PFS**: HR 0.15, (95% CI: 0.026–0.76; $p = 0.03$)[36] **OS**: HR 0.42 (95% CI: 0.21.0.82; $p = 0.01$)[37]
• High LDH at infusion	**PFS**: HR 3.42 (95% CI: 1.93–6.05; $p < 0.0001$) **OS**: HR 2.10 (95% CI: 1.16–3.78; $p = 0.01$)[37] **OS**: HR 2.95 (95% CI: 1.61–5.38)[38]
• High ferritin at infusion	**PFS**: HR 1.02 (95% CI: 1.00–1.03; $p = 0.01$)[37]
• High CRP at infusion	**OS**: HR 1.11 (95% CI: 1.04–1.19; $p = 0.003$)[37]
• Bulky disease at apheresis	**OS**: HR 2.27 (95% CI: 1.10–4.72)[38]
• Older age	**OS**: HR 2.65 (95% CI 1.49–4.73)[38]

CRP, C-reactive protein; CRS, cytokine release syndrome; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival.

used in clinical practice to detect high-risk patients. Moreover, disease progression may depend on factors that are not apparent pre-infusion but emerge after therapy, making the risk stratification process more dynamic than static as a means of evaluation. In the future, better pre-infusion risk factor identification could possibly guide therapeutic decisions, such as the use of bridging chemotherapy. The same consideration can be made for the post-infusion setting, where high-risk patients could be considered for consolidation/maintenance therapy.

Conflict-of-interest disclosure

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Off-label drug use

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Management of aggressive lymphoma after CAR T-cell therapy failure

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Several recent advances have affected the treatment landscape of diffuse large B-cell lymphoma. Chimeric antigen receptor (CAR) T-cell therapy has transformed the management of chemorefractory disease. Two randomized studies in early relapse disease have expanded the label to provide access to CAR T-cell therapy as early as second line for some patients. Despite the durable remissions that have been achieved, many patients will experience relapse. There is a growing population of patients previously treated with CAR T-cell therapy facing dismal outcomes. We review the prospective studies that inform treatment options in later lines and highlight the limited data examining outcomes with novel therapies after CAR T-cell failure. The treatment landscape is anticipated to continue to evolve with the emergence of bispecific antibodies that appear to be a promising approach, particularly after CAR T-cell therapy, although little is known about overlapping mechanisms of resistance. Enrichment for patients who have received prior CAR T-cell therapy on prospective trials is a critical unmet need to inform the preferred management for these high-risk patients.

LEARNING OBJECTIVES

- Evaluate the available evidence that may inform treatment strategies for patients after chimeric antigen receptor (CAR) T-cell therapy, including emerging therapies
- Understand potential mechanisms of resistance of CAR T-cell therapy that may inform subsequent treatment

CLINICAL CASE

A 67-year-old woman presents with advanced stage diffuse large B-cell lymphoma (DLBCL), nongerminal center immunophenotype with double expression of MYC and BCL2 and no *MYC* gene rearrangement. She had additional high-risk features, including extranodal involvement (bone and soft tissue) and elevated lactate dehydrogenase, resulting in an International Prognostic Index of 4. She completed 6 cycles of rituximab (R), cyclophosphamide, doxorubicin, vincristine, and prednisone, achieving a complete response (CR) at the end of induction imaging. However, 6 months later, she presents with recurrent bone pain and imaging confirms recurrent lymphoma involving multiple bone lesions, soft tissue, muscle, and adenopathy above and below the diaphragm. Largest mass is 6 cm in diameter. Biopsy confirms recurrent DLBCL. With early-relapse DLBCL, she is evaluated for chimeric antigen receptor (CAR) T-cell therapy and is deemed to be a good candidate. She receives bridging therapy with R and polatuzumab. Positron emission tomography/computed tomography postbridging reveals progressive lymphoma in extranodal sites, including bone and soft tissue. She proceeds with standard-of-care lisocabtagene maraleucel (liso-cel), tolerating it well with no ongoing toxicity at day 30. Imaging reveals she has achieved a CR. She remains in CR for 6 months; however, she returns for follow-up with concerns about recurrent lymphoma.

CAR T-cell therapy has transformed the management of chemorefractory DLBCL based on the pivotal, single-arm phase 2 trials leading to approval of 3 anti-CD19 autologous CAR T-cell therapies for the management of relapsed or refractory DLBCL.[1–3] Objective response rates across these studies were very promising, ranging from 52% to 82%, with approximately 40% maintaining a CR beyond 12 months. In addition to providing novel cellular therapy for patients otherwise facing dismal outcomes, these studies provided the rationale for the randomized trials conducted in second line examining CAR T-cell therapy vs

standard of care for early-relapse DLBCL. ZUMA-7, TRANSFORM, and BELINDA each explored whether an anti-CD19 CAR T-cell therapy would be superior to platinum-based salvage chemotherapy followed by high-dose therapy and autologous stem cell transplant among chemosensitive patients deemed appropriate candidates for intensive therapy.[4–6] Both ZUMA-7 and TRANSFORM met their primary end points of significant improvement in event-free survival, resulting in approval for axicabtagene ciloleucel (axi-cel) and liso-cel as early as second line for patients with primary refractory or early-relapse DLBCL.

Despite the enthusiasm surrounding CAR T-cell therapy in early-relapse DLBCL or for those who have had at least 2 prior lines of therapy, at least 50% of patients will fail to achieve a durable remission. As CAR T-cell therapy moves into earlier lines of treatment, there is an increasing population in need of effective management options after CAR T-cell therapy (Figure 1). There are many challenges these patients will face. The acute toxicity of cellular therapy, including cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome, if severe, can result in debilitation. In addition, real-world analyses report patients treated with standard-of-care CAR T-cell therapy often would not meet the stringent eligibility criteria of prospective trials.[7-9] Late toxicity, including prolonged cytopenia (observed in 20%-40% of patients), and B-cell aplasia increase the risk for opportunistic infections and necessitate the need for prophylactic antimicrobials, growth factors, and/or transfusion support. This undoubtedly affects eligibility for participation in clinical trials. However, pursuit of clinical trials for these patients should be of highest priority. Many patients exhibit high-risk disease from the onset, and progressing after CAR T-cell therapy has been associated with very poor outcomes.[10,11] Not surprisingly, failing to achieve a response to CAR T-cell therapy or progressing within 90 days is associated with the most unfavorable outcomes.

Little is currently known about mechanisms of resistance to CAR T-cell therapy, and few prospective studies provide a hint at strategies to successfully overcome them. Our current understanding of resistance can be summarized as antigen loss, diminished T-cell fitness, and/or a protumor microenvironment. Among patients with lymphoma treated with CD19-directed CAR T-cell therapy, antigen loss in the form of CD19 mutations or decreased CD19 membrane expression is relatively uncommon.[11,12] As the treatment landscape continues to evolve with several CD19-directed therapies emerging, it is advisable to pursue a biopsy if feasible in the post-CD19 CAR T-cell setting to determine the immunophenotype and potentially guide therapy.

It is unclear whether it is necessary to demonstrate maintained expression of CD19 to achieve efficacy from 2 approved CD19-directed therapies in relapsed DLBCL, tafasitamab, an Fc-modified, humanized, anti-CD19 monoclonal antibody, or loncastuximab (lonca) tesirine, an anti-CD19 antibody conjugated to a pyrrolobenzodiazepine dimer. The L-MIND study enrolled patients with relapsed DLBCL who had 1 to 3 prior lines of therapy and were not candidates for high-dose therapy and autologous stem cell transplant, leading to approval of tafasitamab in combination with lenalidomide for relapsed DLBCL (Table 1).[13] This study did not include patients previously treated with CAR T-cell therapy. Limited data suggest tafasitamab is not associated with antigen loss or limitation of CAR-T effector function, which may lessen concerns about sequencing tafasitamab plus lenalidomide prior to CD19-directed CAR-T cell therapy,[13,14] but little is known about the efficacy of this regimen after CAR T-cell therapy. A multicenter, retrospective analysis of standard-of-care tafasitamab plus lenalidomide included 17 patients who had received prior CAR T-cell therapy.[15] Outcomes among these patients were similar to the larger cohort, with a median time of 14.3 months from CAR T-cell therapy to the start of tafasitamab plus lenalidomide. Response rates were slightly higher among patients with relapsed disease

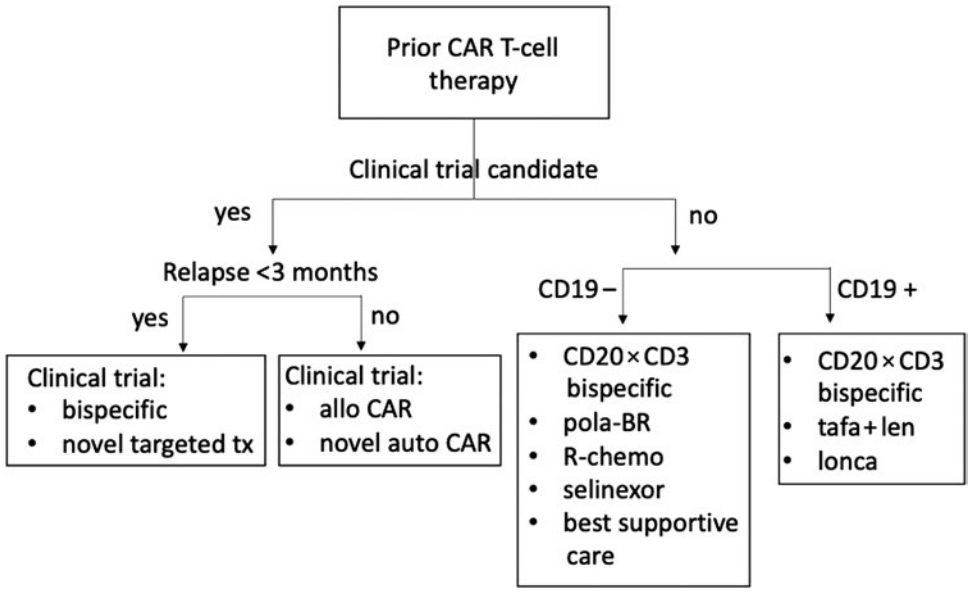

Figure 1. Treatment algorithm, after CAR T-cell therapy management. allo, allogeneic; BR, bendamustine-rituximab; pola, polatuzumab; R-chemo, rituximab plus chemotherapy; tafa+len, tafasitamab plus lenalidomide; tx, treatment.

Table 1. Targeted therapies approved by the US Food and Drug Administration in R/R DLBCL, pivotal trials

Characteristic	Tafasitamab + lenalidomide	Loncastuximab tesirine	Rituximab-bendamustine-polatuzumab vedotin	Selinexor
Trial name	L-MIND[13]	LOTIS-2[16]	R-Benda-Pola[17]	SADAL[19]
Sample size	80	145	80	267
ORR, %	60	48	63	28
Follow-up, mo	17.3	7.3	27	14.7
PFS, mo	12.1	4.9	9.2	2.6
OS, mo	Not reached	9.9	12.4	9.1
DOR, mo	21.7	10.3	10.9	9.3
ORR post-CART, %	36 (n = 42)[15]	46 (n = 13)[28]	44 (n = 57)[29]	—
PFS after CAR T-cell, mo	1.4	1.4	2.5	—

Benda, bendamustine; DOR, duration of response; Pola, polatuzumab vedotin; R/R, relapsed/refractory.

after CAR T-cell therapy as opposed to those with refractory disease (objective response rate [ORR] 36% vs 17%), suggesting a higher chance of success among those who respond to CAR T-cell therapy but then relapse.

Lonca is approved for relapsed DLBCL after 2 lines of therapy based on the LOTIS-2 trial.[16] This heavily pretreated and refractory population achieved an ORR of 48%, including similar responses among 13 (9%) patients who had prior anti-CD19 CAR T-cell therapy (Table 1). Patients were required to have a biopsy demonstrating CD19 expression to be eligible for this prospective trial. One potential advantage of lonca is that it is an intravenous formulation readily available for refractory patients in need of urgent therapy. We might consider this first among those patients with refractory disease.

Among patients with loss of CD19 or prescriber preference for an alternate target, polatuzumab vedotin is an antibody drug conjugate targeting CD79b approved for relapsed DLBCL in combination with R-bendamustine based on a randomized phase 2 study in transplant-ineligible patients. The polatuzumab-containing arm resulted in a significant improvement in progression-free survival (PFS, 9.2 vs 3.7 months) and overall survival (OS, 12.4 vs 4.7 months) (Table 1).[17] The efficacy of the control arm (R-bendamustine) was limited, leading many to question the role of bendamustine in relapsed DLBCL and omission of bendamustine in some retrospective series reporting outcomes with polatuzumab-based approaches after CAR T-cell therapy.[11,18] Responses ranged from 44% to 48% with polatuzumab-based approaches, but responses were not durable in either series.

Selinexor, a selective inhibitor of the nuclear export protein XPO1, is an oral option approved for patients with relapsed or refractory DLBCL who have received at least 2 lines of therapy. The SADAL study was a single-arm phase 2 trial demonstrating an ORR of 28% (12% CR) with single-agent selinexor.[19] With the modest efficacy and common grade 3 to 4 cytopenias, selinexor should be reserved for those without an acceptable alternative option.

Targeting CD20 has been an established approach for B-cell lymphomas for the past 2 decades. Chemotherapy in combination with CD20 monoclonal antibodies after CAR T-cell therapy has not resulted in favorable outcomes.[10,11] This may be due to the prescription of CAR T-cell therapy for patients with chemorefractory disease; therefore, expecting to overcome early signs of chemoresistance is unlikely with retreatment. Can we enhance CD20 targeting and omit chemotherapy after CAR T-cell therapy? An emerging class of bispecific antibodies that target tumor antigen and recruit T cells may provide a new and effective option for the post–CAR T-cell space (Table 2).

Glofitamab is a 2:1 CD20×CD3 bispecific monoclonal antibody with promising efficacy in a phase 1/2 study of patients with relapsed or refractory DLBCL after at least 2 prior lines of therapy.[20] A third of the study population (51 patients) had prior CAR T-cell therapy, including 30% who were refractory to CAR T. The CR rates were similar for this subgroup, 35% vs 39% compared with the overall study population. There were also no reports of increased toxicity among patients receiving a bispecific antibody after CAR T-cell therapy. Epcoritamab, a subcutaneous CD20×CD3 bispecific antibody, was also explored in a phase 1/2 study of patients with relapsed or refractory DLBCL after at least 2 prior lines of therapy.[21] Nearly 40% (61 patients) of this study population included patients with prior CAR T-cell therapy, with 46 (75%) having progressed within 6 months of CAR T-cell therapy. The ORR among patients who received prior CAR T-cell therapy was 54%, 34% achieved a CR, and the median duration of response was 9.7 months. The CR rate was lower among those refractory to prior CAR T-cell therapy, 28% but still meaningful.

Odronextamab, a fully human IgG4-based CD20×CD3 bispecific, included 33 patients with relapsed/refractory DLBCL and prior CAR T-cell therapy in a phase 1/1b study.[22] Among these patients, ORR was 33%; 24% achieved a CR, with an estimated 4.4-month duration of response; and duration of CR was not reached. Mosunetuzumab, a CD20×CD3 bispecific antibody currently approved for the treatment of relapsed follicular lymphoma following at least 2 lines of therapy, included 19 (n=15 DLBCL) patients who had received prior CAR T-cell therapy in the dose escalation study.[23] The responses observed were comparable to the those observed with aggressive lymphoma who had not undergone CAR T-cell therapy. As a class, the CD20-bispecific antibodies appear very promising for patients who have progressed following CAR T-cell therapy. The favorable toxicity profile lends itself to combination approaches that

Table 2. Emerging bispecific antibodies

Characteristic	Epcoritamab[21]	Glofitamab[20]	Mosunetuzumab[23]	Odronextamab[22]
Overall cohort				
Sample size	157	155	129	49*
ORR, %	63	52	34.9	39*
CR, %	39	39	19.4	24*
DOR, mo	12	18.4	7.6	4.4*
PFS, mo	4.4	4.9	1.4	11.5
Follow-up, mo	10.7	12.6	11.9	4.2
Post–CAR T-cell cohort				
Sample size	61	52	19	33
ORR, %	54	Not reported	37	33
CR, %	34	35	26	24
DOR (mos)	9.7	Not reported	Not reported	Not reached

*Responses reported are for patients with DLBCL without prior CAR T-cell therapy.

Table 3. Prospective trials recruiting after CAR T-cell therapy

Trial	Therapy	Clinical trial info
SWOG	Mosunetuzumab±polatuzumab	NCT05633615
ALPHA2	ALLO-501A CD19 allo CAR	NCT04416984
PBCAR0191	CD19 allo CAR	NCT03666000
ANTLER CB-010	CRISPR edited CD19 allo CAR	NCT 04637763
TAK-007	CD19 allo natural killer CAR	NCT05020015
Phase 1/2 CD20 CAR T	CD20-specific CAR T cell	NCT03277729
Phase 1/2 CD19/20 CAR T	CD19/CD20 CAR T cell	NCT04186520
KITE-363, phase 1	CD19/CD20 CAR T cell	NCT04989803
JV-213, phase 1,	CD79b CAR T cell	NCT05773040
Phase 2/3, NKTR-255 vs placebo following CD19 CAR T-cell therapy	NKTR-255 (IL-15 receptor agonist)	NCT05664217

are under investigation (Table 3). Little is known about overlapping mechanisms of resistance such as T-cell exhaustion. Effort should be made to enroll patients on ongoing prospective trials to gain more experience.

Several trials are under way exploring the safety and preliminary activity of allogeneic CAR T-cell therapy, using T cells from healthy donors to overcome limitations of T-cell fitness and the delay necessary to manufacture from an autologous product (Table 3). Many of these trials are recruiting patients who have had prior autologous CAR T-cell therapy given the nature of the experimental treatment. ALLO-501A is an allogeneic anti-CD19 CAR T-cell product with disrupted T-cell receptor (TCR) α gene (reduce graft-versus-host disease risk), and the edited CD52 gene may permit use of ALLO-647 (a humanized anti-CD52 monoclonal antibody) to selectively deplete host T cells to enhance lymphocyte depletion.[24] The ongoing ALPHA2 study allows prior CD19 autologous CAR T-cell therapy if tumors retain CD19 expression. Several other phase 1 studies are actively recruiting, using allogeneic CARs with novel gene editing to knock out the native TCR, eliminate immune checkpoints, or enhance avoiding immune detection, including enhanced lymphocyte-depleting therapy (Table 3). Other sources such as natural killer cells or dual-targeting CARs are also under exploration. A number of studies are targeting the postautologous CAR T-cell failures given the unmet clinical need. Prioritizing trial enrollment is critical.

Radiation can also be an effective treatment option for patients with relapsed disease after CAR T-cell therapy that is localized. In the large multicenter French registry DESCAR-T study, patients with localized disease (n=12) who received radiation therapy had an ORR of 35.7% with a CR of 14.3%, median PFS of 3.7 months, and a median OS of 9.6 months.[25] Another series of 14 patients with localized relapse after CAR T-cell therapy demonstrated sustained benefit with site-specific radiation, including in high-risk patients with double-hit lymphoma and patients refractory to chemotherapy, with a median response rate of 43% and median OS of 10 months. While radiation alone may not achieve durable remissions, radiation therapy can also be

integrated with other novel agents or transplantation to achieve long-lasting remissions.[26]

Allogeneic stem cell transplant (allo-HCT) remains a potential strategy to address post–CAR T-cell relapses in fit patients with suitable donors. A recently published large Center for International Blood & Marrow Transplant Research (CIBMTR) multicenter study evaluating efficacy and toxicities of allo-HCT following CD19 CAR T-cell failure demonstrated 1-year OS, PFS, and graft-versus-host disease-free relapse-free survival rates of 59%, 45%, and 39%, respectively. One-year nonrelapse mortality and progression/relapse were 22% and 33%, respectively. These data suggest that allo-HCT can provide durable disease control in a proportion of transplant-eligible, fit patients.[27]

CLINICAL CASE (continued)

Given our patient had a 6-month response following CAR T-cell therapy, she was deemed an excellent trial candidate given full count recovery and no evidence of significant comorbidities. She enrolled on a novel phase 1 trial with a lenalidomide-based approach. Unfortunately, she experienced disease progression. She then went on to receive standard-of-care lonca and achieved a CR that lasted 9 months. With recent progression, we are exploring clinical trial options or a CD20-bispecific antibody.

Conclusions

CAR T-cell therapy has transformed our approach to chemorefractory DLBCL. However, many of these patients are expected to experience disease relapse, and with CAR T-cell therapy moving into earlier lines of treatment, there is a great unmet need for understanding the optimal management of patients in the post–CAR T-cell space. These patients can have persistent toxicity in the form of prolonged cytopenias, comorbidities, and risk of infection. However, prioritizing pursuit of novel therapeutic trials is critical given the promising therapies currently under investigation and the gap in knowledge about how to best serve these patients. In our practice, we will pursue a clinical trial as our preferred approach for patients experiencing disease progression after CAR T-cell therapy. However, for patients where a clinical trial is not feasible, we consider patient- and disease-specific characteristics to navigate the available standard-of-care options. Like many in the field, we are optimistic about the CD20×CD3 bispecific antibodies that will be our preferred option after CAR T-cell therapy, but many questions remain, including the potential for overlapping mechanisms of failure such as T-cell exhaustion and potential risk for cytopenias or infection. We must continue to explore real-world evidence as these novel therapies enter the treatment landscape to inform treatment selection as the treatment landscape continues to evolve.

Conflict-of-interest disclosure

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Off-label drug use

Loretta J. Nastoupil: There is nothing to disclose.
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Selection of bispecific antibody therapies or CAR-T cell therapy in relapsed lymphomas

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Patients with relapsed and refractory (R/R) aggressive B-cell non-Hodgkin lymphomas have historically poor survival outcomes, with chimeric antigen receptor T-cell (CAR-T) therapy now presenting a curative option for a subset of those patients. However, with the approval of several novel bispecific monoclonal antibody (BsAb) therapies with considerable activity in R/R aggressive large B-cell lymphomas (LBCL), patients and oncologists will be faced with decisions regarding how to sequence CAR-T and BsAb therapies based on patient- and disease-related factors. In this review, we compare CAR-T and BsAb therapies for R/R LBCL, highlighting data on the efficacy and toxicity of each treatment paradigm, and provide a roadmap for sequencing these highly effective therapies.

LEARNING OBJECTIVES

- Understand the efficacy and safety of chimeric antigen receptor T-cell (CAR-T) and bispecific antibody therapies for aggressive large B-cell lymphoma in the third-line setting
- Examine the real-world efficacy and safety of CAR-T therapies in patients who were not eligible for the initial registrational trials
- Compare advantages and disadvantages of initial sequencing of CAR-T first vs bispecific antibodies first in relapsed and refractory large B-cell lymphomas

Introduction

Survival of patients with relapsed and refractory (R/R) aggressive large B-cell lymphomas (LBCLs) was historically dismal in the chemotherapy era. However, the regulatory approval of chimeric antigen receptor T-cell (CAR-T) therapy has not only improved outcomes for heavily pretreated patients with R/R LBCL but also heralded a new epoch of immunotherapeutic trials in non-Hodgkin lymphomas (NHLs). Leading these trials are CD20-directed bispecific monoclonal antibody (BsAb) therapies, which have shown excellent efficacy and safety in R/R LBCL. With new regulatory approval of several BsAb agents, it is imperative to conceptualize a roadmap for sequencing these agents with CAR-T therapy in the treatment paradigm of aggressive LBCL. In this review, we will compare CAR-T and BsAb therapies for R/R LBCL and propose a treatment algorithm to guide practicing clinicians, highlighting data and arguments for the use of CAR-T first vs BsAb first in R/R LBCL.

CLINICAL CASE

A 70-year-old man with a good performance status and history of atrial fibrillation was diagnosed with stage IV diffuse large B-cell lymphoma (DLBCL) (germinal center cell of origin, MYC-amplified) 3 years ago and received 6 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone with a complete response (CR) at the end of treatment. He relapsed 15 months later and subsequently received second-line chemotherapy followed by autologous stem cell transplantation (ASCT). He did well for 11 months when he unfortunately developed a biopsy-proven relapse of DLBCL.

Third-line CAR-T therapy for aggressive LBCL

There are 3 CAR-T constructs that are approved by the US Food and Drug Administration (FDA) in the third-line setting for LBCL: axicabtagene ciloleucel (axi-cel) in 2017, tisagenlecleucel (tisa-cel) in 2018, and lisocabtagene maraleucel

(liso-cel) in 2021. These FDA approvals were based on the ZUMA-1, JULIET, and TRANSCEND trials (Table 1). The long-term curative potential for CAR-T therapy has been demonstrated with axi-cel, with 5-year follow-up data demonstrating a progression-free survival (PFS) plateau of 32% and a disease-specific survival of 51%,[1] as well as with CTL019 (tisa-cel) with a 5-year PFS of 31%.[2] Although clearly with curative potential, CAR-T therapy can be associated with unique toxicities, with cytokine release syndrome (CRS) and neurotoxicity noted with all CAR-T constructs, as well as prolonged cytopenias in up to 45% of cases which can impact post–CAR-T outcomes.[3] The TRANSCEND trial included more broad inclusion criteria than the other trials, including patients with older age, secondary central nervous system (CNS) involvement, and mild renal and cardiac insufficiency, and with no minimum absolute lymphocyte count requirement for apheresis. The efficacy and toxicity of CAR-T products in the third-line setting have not been directly compared. Indirect comparisons between the ZUMA-1 and TRANSCEND trials have demonstrated comparable efficacy between axi-cel and liso-cel with lower rates of toxicities with liso-cel, underscoring differing functions of the constructs' costimulatory domains (CD28 vs 4-1BB).[4]

Second-line CAR-T therapy for aggressive LBCL

ASCT is curative in less than 30% of patients with transplant-eligible R/R LBCL in the second-line setting, and its efficacy is particularly poor for patients with primary refractory or early-relapsed LBCL.[15] Given the considerable activity of CAR-T therapy in LBCL in the third-line setting, especially for patients with relapse after ASCT, it was intuitive to assess the efficacy of CAR-T in the second-line setting in patients with high-risk R/R LBCL. There have been 3 large randomized controlled trials of CAR-T vs standard of care (SOC) in the second-line setting, with SOC consisting of second-line chemoimmunotherapy followed by ASCT in transplant-eligible high-risk primary refractory or early-relapsed LBCL within 12 months of initial therapy: ZUMA-7, BELINDA, and TRANFORM. ZUMA-7 and TRANSFORM met their primary end points of event-free survival, while BELINDA did not. In ZUMA-7, 359 patients with high-risk R/R LBCL were randomized to axi-cel vs SOC.[16] Median event-free survival (EFS) was significantly higher with axi-cel at 8.3 months compared to 2 months for standard care, with 2-year EFS of 41% and 16% for axi-cel and standard care, respectively. Improved outcomes with axi-cel were also observed in patients aged ≥65 years.[17] Although there was no significant difference in overall survival (OS) initially reported, updated statistically significant OS results have now been reported in favor of axi-cel.[18] The TRANSFORM trial of 184 patients randomized to liso-cel vs SOC utilized a similar trial design as ZUMA-7, although crossover to the liso-cel arm was allowed per protocol. Similarly, median EFS was superior with liso-cel (not reached vs 2.4 months) with 18-month EFS of 53% and 21% for liso-cel and standard care, respectively.[19] In a prespecified OS analysis adjusting for crossover, there was a trend toward improvement in OS in the liso-cel arm, with 18-month OS rates of 73% for liso-cel and 54% for SOC. In both ZUMA-7 and TRANSFORM, there were no deaths due to CRS or neurotoxicity.

Given the favorable efficacy and toxicity profile of liso-cel in transplant-eligible R/R LBCL, PILOT was a phase 2 single-arm trial that investigated the efficacy of liso-cel in 74 transplant-ineligible patients with R/R LBCL in the second-line setting.[20] The overall response rate (ORR) was 80% (CR 54%) with a median PFS and OS of 9 months and not reached, respectively; for patients who achieved a CR, median PFS and OS were 23 months and not reached, respectively.

Based on the positive results of the ZUMA-7, TRANSFORM, and PILOT trials, the FDA approved both axi-cel and liso-cel for patients with high-risk LBCL in first relapse, as well as liso-cel in transplant-ineligible patients after failure of 1 line of therapy.

Third-line CD20-directed bispecific antibody therapy for aggressive LBCL

BsAb therapies cotarget tumor antigens and endogenous immune effector cells to induce tumor killing by recruiting and activating peripheral and intratumoral T cells, natural killer cells, and/or macrophages to the tumor microenvironment. In B-cell lymphomas, several off-the-shelf IgG-type BsAb agents have been developed that cotarget CD20 and CD3 for T-cell–mediated cytotoxicity—namely, mosunetuzumab, glofitamab, epcoritamab, and odronextamab (Table 1). Both glofitamab and epcoritamab have been studied in third-line LBCL and received FDA approval in mid-2023. In a phase 2 study of 154 patients with LBCL who received up to 12 cycles of fixed-duration intravenous glofitamab after at least 2 prior lines of therapy, ORR was 52% (CR 39%) with 1-year PFS and OS of 37% and 50%, respectively.[8] Importantly, the 33% of patients in the study who had previously received CAR-T had a similar CR rate of 35%. Most patients who achieved a CR had durable remissions, with a median duration of CR not reached at 18.1 months of follow-up.[9] Although CRS was common (63%), only 4% were high-grade events and none were grade 5; any-grade neurotoxicity occurred in 8% of patients with only 3% of high grade.

In a phase 1/2 study of 157 patients with LBCL after at least 2 lines of previous therapy who received subcutaneous epcoritamab until disease progression or unacceptable toxicity, ORR was 63% (CR 39%) with a median duration of response (DOR) of 12 months and 12-month PFS of 38%.[10,11] Thirty-nine percent of patients had previously received CAR-T with an ORR of 54% (CR 34%) with median DOR of 9.7 months. Similar to glofitamab, CRs were durable after epcoritamab with median duration of CR not reached at 10.7 months of follow-up, including in post–CAR-T patients. CRS was also common with epcoritamab (50%), and although only 2.5% of events were high-grade CRS with no grade 5 events, there was 1 treatment-related death due to neurotoxicity in the study.

Although mosunetuzumab was FDA approved in December 2022 for R/R follicular lymphoma, activity was significantly lower in LBCL in a phase 1 study, with ORR 35% (CR 19%) as compared to ORR 66% (CR 49%) in indolent NHL, with a median PFS of 1.4 months in aggressive NHL.[12] Follow-up phase 2 data from this study in 88 patients with R/R LBCL demonstrated ORR 42% (CR 24%) with a median PFS of 3.2 months, with a lower CR rate of 12% in patients who had previously received CAR-T.[13] Combinations of other agents with mosunetuzumab have been investigated to improve efficacy, including polatuzumab vedotin with mosunetuzumab in R/R LBCL, which has demonstrated an ORR of 72% (CR 56%) in older patients who may not be candidates for CAR-T or who are relapsed after CAR-T.[21]

The durable remissions achieved in patients with heavily pretreated and poor-prognosis LBCL, including after CAR-T and with fixed-duration regimens such as glofitamab, have generated considerable interest in BsAb therapies, particularly given the low incidence of high-grade CRS and neurotoxicity.

Table 1. Clinical trials of CAR T-cell and bispecific antibodies therapies in the third-line setting for aggressive LBCL

Clinical trial	Construct	Patients	Histologies	Response rates	Survival outcomes	Longest median follow-up	Duration of response	Rates of CRS	Rates of neurotoxicity	Treatment-related mortality	FDA approved
CAR-T therapies for third-line LBCL											
ZUMA-1[1,5]	Axi-cel	101	DLBCL, PMBCL, tFL	ORR 82% (CR 54%)	mPFS 5.9 mo 12 mo: PFS 44%, OS 59% 5y: PFS 32%, OS 43%	63.1 mo	mDOR 11.1 mo mDOCR 62.2 mo	93% (13% grade 3+)	64% (28% grade 3+)	3 patients (3%), 1 death due to CRS, 1 death due to HLH	Yes
JULIET[2,6]	tisa-cel	115	DLBCL, tFL, HGBL	ORR 53% (CR 39%)	mPFS 2.9 mo 12 mo: RFS 65%, OS 49% 40 mo: PFS 38%, OS 39% 5y: PFS 31%	60.7 mo	mDOR NR	58% (22% grade 3+)	21% (12% grade 3+)	0 patients	Yes
TRANSCEND[7]	Liso-cel	256	DLBCL, tFL, HGBL, PMBCL, FL 3B	ORR 73% (CR 53%)	mPFS 6.8 mo 1y: PFS 44%, OS 58%	18.8 mo	mDOR 17 mo	42% (2% grade 3+)	30% (10% grade 3+)	7 patients (3%)	Yes
Bispecific antibody therapies for third-line LBCL											
NCT03075696[8,9]	Glofitamab	154 51 (33%) with prior CAR-T exposure	DLBCL, tFL, HGBL, PMBCL	ORR 52% (CR 39%) Prior CAR-T: CR 35%	mPFS 4.9 mo 1y: PFS 37%, OS 50%	12.6 mo	mDOR 18.4 mo mDOCR NR (74% remained in CR at median of 18.1 mo of follow-up)	63% (4% grade 3+)	8% (3% grade 3+)	8 patients (5%)	Yes (June 2023) for LBCL after at least 2 lines of therapy
EPCORE NHL-1 (NCT03625037)[10,11]	Epcoritamab (dose escalation)	68 46 with LBCL	Any relapsed or refractory CD20+ mature B-cell NHL	ORR 68% (CR 45%) for LBCL	mPFS for LBCL: 9.1 mo	9.3 mo	75% of LBCL responders in ongoing remission at 6 mo	59% (no grade 3+)	6% (3% grade 3+)	0 patients	Yes (May 2023) for LBCL after at least 2 lines of therapy
	Epcoritamab (dose expansion)	157 61 (39%) with prior CAR-T exposure	DLBCL, PMBCL, HGBL, FL 3B	ORR 63% (CR 39%) Prior CAR-T: ORR 54% (CR 34%)	mPFS 4.4 mo 6 mo: PFS 44% mPFS NR among patients with CR	10.7 mo	mDOR 12 mo (9.7 mo if prior CAR-T) mDOCR NR (NR if prior CAR-T)	50% (2.5% grade 3+)	6% (0.6% grade 3+)	9 patients (6%), 1 death due to ICANS	

Table 1. Clinical trials of CAR T-cell and bispecific antibodies therapies in the third-line setting for aggressive LBCL (*Continued*)

Clinical trial	Construct	Patients	Histologies	Response rates	Survival outcomes	Longest median follow-up	Duration of response	Rates of CRS	Rates of neurotoxicity	Treatment-related mortality	FDA approved
NCT02500407[12,13]	Mosunetuzumab (phase I data)	197 129 with aggressive NHL	Any relapsed or refractory B-cell NHL	ORR 35% (CR 19%)	mPFS 1.4 mo	11.9 mo	mDOR 7.6 mo mDOCR 23 mo	27% (1% grade 3+)	10–18% (4% grade 3+)	3 patients (1.5%)	Yes, for relapsed or refractory FL
	Mosunetuzumab (phase II data)	88	DLBCL, tFL, HGBL	ORR 42% (CR 24%) Prior CAR-T: ORR 23% (CR 12%)	mPFS 3.2 mo mOS 11.5 mo Prior CAR-T: mPFS 1.4 mo	10.1 mo	mDOR 7.0 mo mDOCR NR	26% (2.3% grade 3+)	1% (no grade 3+)	3 patients (3.4%)	
ELM-1 (NCT02290951)[14]	Odronextamab	85 with LBCL 33 (39%) of LBCL with prior CAR-T exposure	Any relapsed or refractory B-cell NHL	No prior CAR-T: ORR 53% (CR 53%) Prior CAR-T: ORR 33% (CR 27%)	No prior CAR-T: mPFS 11.5 mo Prior CAR-T: mPFS 2.0 mo	4.2 mo	No prior CAR-T: mDOR NR Prior CAR-T: mDOR NR	54% (7% grade 3+)	12% (3% grade 3+)	7 patients (5%)	No

FL 3B, grade 3B follicular lymphoma; HGBL, high-grade B-cell lymphoma; HLH, hemophagocytic lymphohistiocytosis; ICANS, immune effector cell-associated neurotoxicity syndrome; mDOCR, median duration of complete response; mDOR, median duration of response; mOS, median overall survival; mPFS, median progression-free survival; NR, not reached; PMBCL, primary mediastinal large B-cell lymphoma; tFL, transformed follicular lymphoma.

Further, both glofitamab and epcoritamab demonstrated a relatively fast time to response of 1.4 months, which is particularly important for an off-the-shelf therapeutic that does not require manufacturing. Other novel CD20xCD3 BsAb agents are under development, including odronextamab and plamotamab with ORR 33% to 53% (CR 27%-53%) and ORR 47% (CR 26%) in LBCL, respectively.[14,22] Given the encouraging results of these therapies in heavily pretreated LBCL, several trials with BsAb therapies are ongoing in earlier lines of therapy either as single agents or in combination with chemotherapy. These results are awaited to clarify how BsAb therapies may be best sequenced after first-line therapy.[23]

Case for CAR-T therapy first

The primary advantage of sequencing CAR-T therapy before BsAb therapy in the third-line LBCL setting is the potential for cure with CAR-T in approximately one-third of patients at 5 years of follow-up, which has not yet been demonstrated with BsAb agents. However, given the higher risk of toxicities with CAR-T compared to BsAb therapies, particularly CRS and neurotoxicity (Table 1) as well as prolonged cytopenias and infections, there is concern that administering CAR-T to patients who did not meet the inclusion criteria of the initial trials may incur excess toxicity and mortality. Several retrospective, "real-world" studies of CAR-T outcomes have demonstrated comparable survival and safety outcomes when CAR-T was administered to patients who would have been ineligible for the original clinical trials due to advanced age, comorbidities, or performance status (Table 2). These real-world studies also demonstrated durable remissions in patients who achieved CR, suggesting the possibility for cure in trial-ineligible patients.[24,25] Toxicities also appear to be comparable outside of the trial setting, with preserved survival rates despite higher use of tocilizumab and steroids in the real-world setting.[24] Longitudinal improvements in quality of life and patient-reported outcomes after CAR-T have been demonstrated in both clinical trials and real-world studies,[26,27] further supporting the tolerability of CAR-T outside of the trial setting.

When considering BsAb vs CAR-T therapy in the third-line setting, cross-trial comparisons (Table 1) suggest lower response rates and PFS with BsAb therapies with shorter follow-up. In particular, patients with refractory disease have lower CR rates to BsAb therapies as compared to CAR-T: for glofitamab, CR rates are 34% with refractory as compared to 70% with nonrefractory disease,[8] and for epcoritamab, CR rates are 30% with refractory vs 53% with nonrefractory disease.[11] The lack of a costimulatory domain on current CD20xCD3 BsAb constructs may account for this observation, as cytotoxicity of BsAb therapy is dependent on innate immunity, whereas CAR-T constructs contain 4-1BB or CD28 costimulatory domains, which promote T-cell proliferation and persistence. From a logistic perspective, although CAR-T therapy requires significant upfront resources surrounding apheresis, CAR production, and intensive monitoring and toxicity management, current BsAb therapy strategies require ongoing dosing every several weeks for up to 12 cycles (glofitamab) or indefinitely until progression (epcoritamab), which may incur additional time and resource costs for patients as well as centers. Although CAR-T is considered cost-effective compared to other chemoimmunotherapy regimens and transplantation,[39] cost-effectiveness

Table 2. Real-world CAR-T outcomes in third-line LBCL

Real-world study design	Constructs	Patients	Percent of patients ineligible for CAR-T clinical trials	Response rates	Survival outcomes	Median duration of response	Rates of CRS	Rates of neurotoxicity	Treatment-related mortality	Conclusions
Locke et al.[28] Postapproval safety observational study	Axi-cel	1343	38% aged ≥65, 4% with ECOG PS 2+, 13% cardiac comorbidities, 2% hepatic comorbidities, 2% renal comorbidities, 15% double/triple-hit lymphoma, 66% refractory disease	ORR 74% (CR 56%) ORR 78% (CR 62%) for patients aged ≥65 ORR 57% (CR 29%) for hepatic comorbidities ORR 70% (CR 43%) for renal comorbidities ORR 47% (CR 20%) for ECOG 2–3	18 mo: PFS 42%, OS 52%	18 mo: DOR 61%	83%	55%	Not assessed	Patients aged ≥65 with moderate to severe pulmonary disease and ECOG 2–3 had inferior ORR. Age ≥65 was not associated with inferior survival, but was associated with higher rates of CRS and ICANS. ECOG PS significantly affected all efficacy outcomes.
Jacobson et al.[25] Postauthorization safety study through the CIBMTR registry	Axi-cel	1297	57% were ineligible for ZUMA-1	ORR 73% (CR 56%)	mPFS 8.6 mo mOS 21.8 mo	mDOR NR 24-mo DOR 57%	83% (8% grade 3+)	55% (24% grade 3+)	3%, 2% died from CRS, 1% died from ICANS	Real-world response rates were similar to ZUMA-1, with similar DOR in patients who were and were not eligible for ZUMA-1. High-grade CRS and ICANS were lower in the real-world cohort. ECOG PS of 2 or greater was associated with inferior response rates, PFS, and OS. Patients age ≥65 had a higher ORR despite higher risk of CRS and ICANS as compared to younger patients.
Landsburg et al.[29] Observational study through the CIBMTR registry	Tisa-cel	1159	31% ineligible for JULIET	ORR 60%	2y: PFS 28%, OS 44%	2y: DOR 53%	58% (6% grade 3+)	23% (7% grade 3+)	Not assessed	Real-world outcomes were similar to JULIET. Patients with comorbidities (who were not eligible for JULIET) had similar efficacy outcomes. Patients with ECOG PS 2–4 had higher rates of high-grade CRS and ICANS but similar efficacy outcomes.

Table 2. Real-world CAR-T outcomes in third-line LBCL (*Continued*)

Real-world study design	Constructs	Patients	Percent of patients ineligible for CAR-T clinical trials	Response rates	Survival outcomes	Median duration of response	Rates of CRS	Rates of neurotoxicity	Treatment-related mortality	Conclusions
Bachy et al.[30] Retrospective French DESCAR-T registry study with propensity score matching between axi-cel and tisa-cel	Axi-cel Tisa-cel	452 axi-cel 277 tisa-cel	Not assessed	Axi-cel: ORR 80% (CR 60%) Tisa-cel: ORR 66% (CR 42%)	1y: PFS 47% axi-cel, 33% tisa-cel OS 64% axi-cel, 49% tisa-cel	1y: DOR 54% axi-cel, 42% tisa-cel	Axi-cel: 86% (5% grade 3+) Tisa-cel: 76% (9% grade 3+)	Axi-cel: 49% (14% grade 3+) Tisa-cel: 22% (3% grade 3+)	Axi-cel: no grade 5 CRS, 1 patient grade 5 ICANS Tisa-cel: 2 grade 5 CRS, no grade 5 ICANS No other treatment-related grade 5 AEs	Real-world response and survival rates were similar to clinical trials. Real-world ORR, CR, PFS, and OS better with axi-cel compared to tisa-cel, although axi-cel with more toxicity.
Chihara et al.[31] Retrospective cohort from Medicare claims data	All CAR-T	551	31% of patients in this cohort were age ≥75	Not assessed	Age ≥75: mPFS 160 d mOS 403 d Age 70–74: mPFS 379 d mOS 603 d Age 65–69: mPFS 194 mOS 518 1y PFS: Age ≥75 34% Age 70–74 52% Age 65–69 43%	Not assessed	Not assessed	Not assessed	Not assessed	Older patients, particularly those age ≥75, had significantly worse PFS and OS compared to younger patients as well as compared to clinical trials.
Nastoupil et al.[32] Retrospective cohort from the US Lymphoma CAR-T Consortium	Axi-cel	298	43% were ineligible for ZUMA-1 due to comorbidities	ORR 82% (CR 64%)	1y: PFS 47% OS 68% PFS 34% for ZUMA-1 ineligible patients	mDOR NR (median follow-up of 12.9 mo)	91% (7% grade 3+)	69% (31% grade 3+	4.4%, 1 death due to HLH, 1 death due to ICANS	Real-world outcomes and safety were comparable to ZUMA-1, although ECOG PS 2–4 had worse PFS, OS, and toxicities. Patients ineligible for ZUMA-1 had inferior PFS and OS.
Sano et al.[33] Retrospective cohort from the US Lymphoma CAR-T Consortium	Axi-cel	272 30% were aged ≥65	Not assessed	Age ≥65 ORR 84% (CR 71%) Age ≤65 ORR 82% (CR 51%)	Age ≥65 mPFS 9.2 mo, mOS not assessable Age ≤65 mPFS 7.4 mo, mOS 18.7 mo	Not assessed	Age ≥65 92% (7% grade 3+) Age ≤65 91% (7% grade 3+)	Age ≥65 78% (35% grade 3+) Age ≤65 65% (31% grade 3+)	2 deaths (1 in age ≥65 and 1 age <65)	Rate of complete response was higher in patients aged ≥65 compared to age <65. There was no difference in PFS, OS, and toxicities between patients aged <65 and ≥65.

Table 2. Real-world CAR-T outcomes in third-line LBCL (*Continued*)

Real-world study design	Constructs	Patients	Percent of patients ineligible for CAR-T clinical trials	Response rates	Survival outcomes	Median duration of response	Rates of CRS	Rates of neurotoxicity	Treatment-related mortality	Conclusions
Bethge et al.[34] Retrospective cohort from the German Registry for Stem Cell Transplantation	Axi-cel Tisa-cel	173 axi-cel 183 tisa-cel	13% eligible for ZUMA-1 89% eligible for JULIET	Axi-cel: ORR 74% (CR 42%) Tisa-cel: ORR 53% (CR 32%)	Axi-cel: 1y PFS 35%, OS 55% Tisa-cel: 1y PFS 24%, OS 53%	Not assessed	Axi-cel: 81% (10% grade 3+) Tisa-cel: 65% (13% grade 3+)	Axi-cel: 44% (16% grade 3+) Tisa-cel: 22% (7% grade 3+)	Axi-cel: 2y 10.4% Tisa-cel: 2y 3.5%	Real-world ORR, CR, and OS were comparable to clinical trials, but real-world PFS was worse. Higher rates of delayed infection-related NRM in real-world cohort.
Riedell et al.[35] Retrospective cohort of 8 US centers	Axi-cel Tisa-cel	168 axi-cel 92 tisa-cel	61% axi-cel ineligible for ZUMA-1 43% tisa-cel ineligible for JULIET	Axi-cel: ORR 52% (CR 44%) Tisa-cel: ORR 41% (CR 35%)	Axi-cel: 1y PFS 42%, OS 62% Tisa-cel: 1y PFS 32%, OS 59%	Axi-cel: 1y DOR 70% Tisa-cel: 1y DOR 75%	Axi-cel: 85% (9% grade 3+) Tisa-cel: 39% (1% grade 3+) mDOR NR for either cohort	Axi-cel: 56% (38% grade 3+) Tisa-cel: 11% (1% grade 3+)	Axi-cel: 9%, 4 deaths from ICANS Tisa-cel: 7%	Real-world outcomes were slightly lower compared to ZUMA-1 and JULIET, although safety outcomes were comparable. Axi-cel and tisa-cel had comparable efficacy, although less CRS and ICANS with tisa-cel.
Pasquini et al.[36] Postauthorization safety study through the CIBMTR registry	Tisa-cel	155	Not assessed	ORR 62% (CR 40%)	1y: EFS 52% OS 77%	1y DOR 61%	45% (4.5% grade 3+)	18% (5.1% grade 3+)	1.2%	Similar efficacy and improved safety compared to JULIET.
Kittai et al.[37] Retrospective cohort from 4 centers	Axi-cel Tisa-cel	94 axi-cel 36 tisa-cel	57% of patients had high comorbidity based on CIRS score	ORR 68% (CR 42%)	mPFS 6.7 mo mOS NR 1y OS 60%	Not assessed	79%	58%	12.3%, 2 deaths due to CRS, 1 death due to ICANS	Worse ECOG PS and comorbidities by CIRS score were associated with worse survival. Presence of more comorbidities was not associated with worse CRS or ICANS.
Cook et al.[38] Meta-analysis of prospective and retrospective studies of CAR-T in primary and secondary CNS lymphoma	Multiple CAR-T products	30 patients with primary CNS lymphoma (PCNSL) 98 patients with secondary CNS lymphoma (SCNSL)	Not assessed	PCNSL: ORR 64% (CR 56%) SCNSL: ORR 57% (CR 47%)	PCNSL: 6-mo PFS 37% SCNSL: 6-mo PFS 37%	PCNSL: mDOR 9 mo SCNSL: mDOR 4.6 mo	PCNSL: 70% (13% grade 3+) SCNSL: 72% (11% grade 3+)	PCNSL: 53% (18% grade 3+) SCNSL: 48% (26% grade 3+)	Not assessed	Incidence of CRS and ICANS in real-world cohort of PCNSL and SCNSL is comparable to clinical trials. Approximately one-third of patients with PCNSL and SCNSL had 6-mo complete responses, which could be durable with longer follow-up.

Table 2. Real-world CAR-T outcomes in third-line LBCL (*Continued*)

Real-world study design	Constructs	Patients	Percent of patients ineligible for CAR-T clinical trials	Response rates	Survival outcomes	Median duration of response	Rates of CRS	Rates of neurotoxicity	Treatment-related mortality	Conclusions
Lin et al.[50] Single-center retrospective cohort of older adults who did and did not receive CAR-T	Axi-cel and tisa-cel	24 older adults received CAR-T 18 older adults did not receive CAR-T 25 younger adults (age <65) received CAR-T	Not assessed	100-d CR 51% (for 49 patients who received CAR-T)	6-mo PFS 48%, OS 71% (for 49 patients who received CAR-T) Older adults who received CAR-T had a significantly lower risk of death (HR 0.31) compared to older adults who did not receive CAR-T No difference in PFS or OS between older or younger adults who received CAR-T by chronological age, functional limitations, cognitive impairment, or comorbidity burden	Not assessed	83% (8% grade 2±)	54% (25% grade 2±)	2 deaths in older adults after CAR-T	Older adults who receive CAR-T have better postrelapse OS compared to patients who instead receive chemotherapy and/or supportive care. PFS, OS, and rates of CRS and ICANS are not different between older and younger adults who receive CAR-T.

AE, adverse event; CIBMTR, Center for International Blood and Marrow Transplant Research; ECOG PS, Eastern Cooperative Oncology Group Performance Status; NRM, nonrelapse mortality.

studies between CAR-T and BsAb in the third-line setting are warranted. An additional advantage to sequencing CAR-T first is the demonstrated efficacy of BsAb therapies in patients with previous CAR-T exposure, whereas limited data have demonstrated efficacy of the converse strategy, with 1-year PFS and OS of 37% and 54%, respectively, in a registry cohort of 28 patients who received CAR-T after BsAb therapies.[51] Further, there is theoretical concern about T-cell exhaustion post-BsAb therapy, which could limit the ability to apherese and manufacture an efficacious CAR T-cell product.[40]

CAR-T eligibility

Given the curative potential of CAR-T among diverse patient cohorts, there is not a uniform definition for CAR-T eligibility, specifically for patients who may be considered too frail for CAR-T. Automatic exclusion of patients based on chronological age, presence of comorbidities, or cognitive and/or functional impairments is not advised as these characteristics do not consistently affect post–CAR-T outcomes.[28,33,41,42] Further, transplant eligibility is not the same as CAR-T eligibility, as demonstrated in the PILOT trial of liso-cel in transplant-ineligible patients.[20,43]

Based on patient cohorts with inferior outcomes after CAR-T (Table 2), we suggest that the following patients be referred to a multidisciplinary clinic with involvement by geriatrics, physical medicine and rehabilitation, physical therapy, occupational therapy, social work, and nutrition for comprehensive frailty assessment: age >70 to 75,[31] Eastern Cooperative Oncology Group (ECOG) Performance Status of 2 or greater,[25,28,29,32,37] multiple comorbidities per the Cumulative Illness Rating Scale (CIRS)-derived "Severe4" comorbidity index validated in CAR-T (CIRS score of 3 or higher in any of the respiratory, upper gastrointestinal, renal, or hepatic organ systems),[37,44] significant weight loss and/or poor nutrition, and secondary CNS involvement and/or baseline cognitive impairment.[41]

Although these characteristics have not yet been validated for determining CAR-T patient eligibility in routine clinical care, extrapolated evidence from the transplantation literature suggests that comprehensive frailty assessments in a dedicated multidisciplinary clinic may improve outcomes.[45,46] Planning for functional optimization of at-risk patients before ("prehab") and after ("rehab") CAR-T, as well as discussion of bridging therapies and prophylactic steroids and/or anticytokine therapies prior to CAR-T infusion, should also occur.[41]

CLINICAL CASE (continued)

After discussion with the patient, the decision was made to proceed with liso-cel CAR-T therapy, which was complicated by grade 2 CRS and grade 2 neurotoxicity, both of which completely resolved. One year after CAR-T therapy, he developed relapsed DLBCL in a single cervical lymph node, which was treated with radiation therapy resulting in a CR. Six months later, he developed progressive DLBCL.

Case for bispecific antibody therapy first

Although CAR-T has the potential for cure in LBCL, two-thirds of patients will eventually relapse after CAR-T, and outcomes for these patients are dismal, with a median OS of only 5.2 months.[47] There are not currently any data to inform which patients may preferentially benefit from BsAb first rather than CAR-T therapy to maximize survival. For the aforementioned patients who are known to have inferior survival after CAR-T, the potential benefits of BsAb therapies over CAR-T (Table 3) should be discussed, particularly the availability of off-the-shelf BsAb products and the generally lower rates of high-grade toxicities. These benefits should be placed in context of the unknown long-term curative potential of BsAb therapies, as well as the known activity of BsAb therapies in the post–CAR-T setting.

It is critically important to assess patient preferences when deciding on sequencing of BsAb vs CAR-T therapies, as there are significant logistic disadvantages to CAR-T administration that may be burdensome to patients. CAR-T therapies are generally only available at large specialized cancer centers, which may require patients to travel great distances for consultation and apheresis, followed by prolonged stays away from home for CAR-T infusion and postinfusion monitoring.[48] Further, although there are some centers that administer CAR-T therapies in the outpatient setting, most centers pursue inpatient administration for several weeks, which may be undesirable for patients and may lead to progressive frailty in older adults. BsAb therapies, on the other hand, will likely be available in the community setting and require much shorter inpatient stays for initial dose escalation, with formal studies ongoing to assess the safety of outpatient administration with premedication.[8] A significant advantage of BsAb therapies is their off-the-shelf availability and capability for immediate treatment initiation, without requirement for apheresis and manufacturing, which may take several weeks for CAR-T therapies. This is particularly important for patients with florid and rapidly progressive LBCL, as interruptions in bridging therapies surrounding necessary washout periods before CAR-T apheresis and lymphodepleting chemotherapy may lead to significant disease progression during the manufacturing period and worse post–CAR-T outcomes and toxicities. In this vein, although CAR-T therapies have a time to best response of approximately 1 month, the time to best response of 1.4 months of glofitamab and epcoritamab may be preferable for patients with aggressive disease who may not be able to tolerate the additional several weeks of CAR-T manufacturing time. Finally, BsAb clinical trials in R/R LBCL included significant proportions of older patients (19% were age ≥75 years for epcoritamab and 54% were age ≥65 years for glofitamab),[8,11] demonstrating efficacy and safety in this patient cohort. However, it is important to note that a subgroup analysis of patients aged ≥65 years in ZUMA-1 did not find any age-related differences in the efficacy or safety of axi-cel,[49] which has been confirmed in real-world cohorts (Table 2). These data underscore the importance of a nuanced discussion with patients and their caregivers regarding the risks and benefits of CAR-T vs BsAb therapies.

CLINICAL CASE (continued)

The patient elected to enroll on a clinical trial of BsAb therapy mosunetuzumab in combination with polatuzumab vedotin. He received 8 cycles of combination therapy on trial and achieved a complete metabolic response, with his treatment course complicated by grade 1 CRS during the first 2 cycles and grade 3 neutropenia after cycle 5. His serial positron emission

Table 3. Pros and cons of CAR-T vs bispecific antibody therapies in third-line LBCL

Characteristic	CAR-T therapy	Bispecific antibody therapy
Curative potential	Confirmed at 5 years of follow-up	Data not yet mature; complete responses appear to be durable with short follow-up
Administration	Single infusion	Repeated infusions required
Off-the-shelf availability	Not currently	Yes
Time to treatment initiation	Several weeks required for product manufacturing	Treatment can be started immediately
Bridging therapy	Optional, but may be needed in patients with aggressive disease	Not needed
Geographic availability	Only at specialized, accredited centers	Community settings
Upfront commitment	Substantial: hospitalization often required for administration	Less than CAR-T: initial hospitalization required for dose escalation, although emerging data regarding safety of outpatient initiation
Caregiver support required	Substantial	Less than CAR-T
Time to best response	~1 month	~1.4 months
T-cell requirements	Dependent on T-cell health (manufacturing failures, bendamustine exposure)	No costimulatory domain, so relies on innate immunity, which may be impaired by T-cell exhaustion
Activity in CNS involvement	CAR-T has demonstrated activity and comparable safety in primary and secondary CNS lymphoma	Not yet studied
Real-world data	Comparable efficacy and safety in real-world cohorts as compared to clinical trial cohorts	No real-world data regarding safety or efficacy
Patient-reported outcomes	Clinically meaningful longitudinal improvements in quality of life in the second- and third-line settings	Data not yet published
Cost-effectiveness	Cost effective in the second and third lines	Not yet studied
Existing sequencing data	No data for CAR-T after BsAb therapy	Known efficacy and safety of BsAb therapy after CAR-T

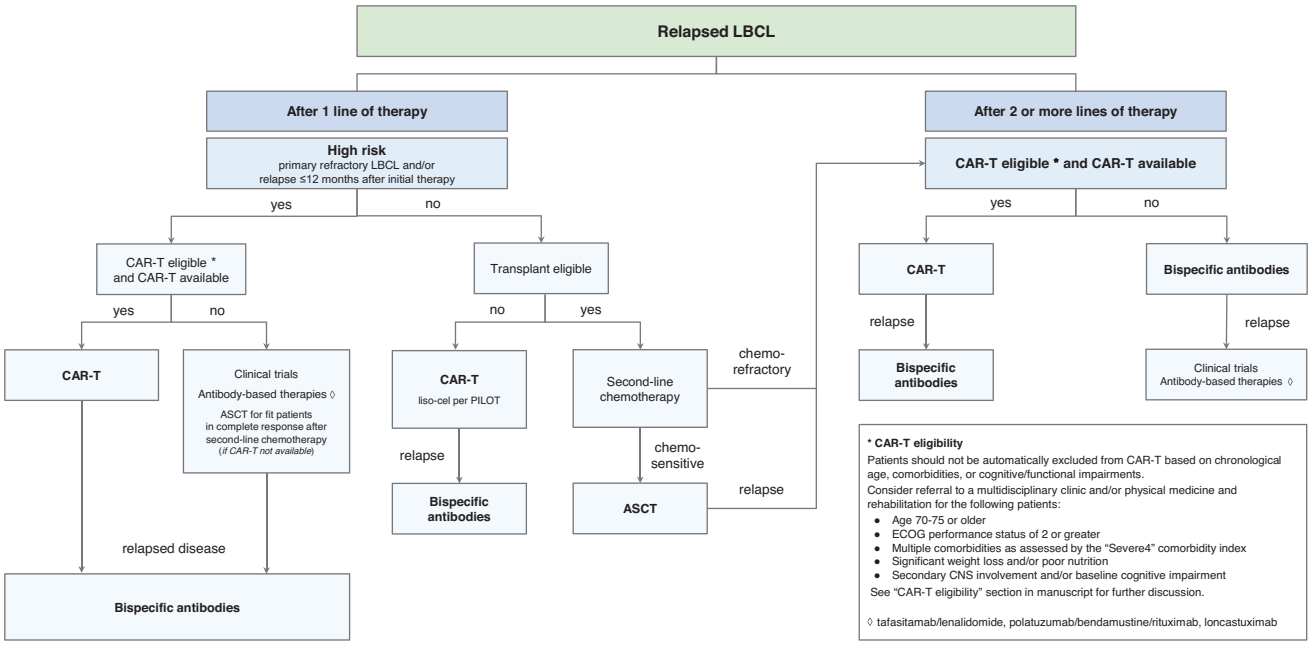

Figure 1. Our approach to sequencing CAR T-cell and bispecific antibody therapies in relapsed large B-cell lymphomas.

tomography scans at 12, 18, and 24 months after study enrollment continued to demonstrate ongoing remissions.

Our sequencing approach for relapsed/refractory LBCL and conclusions

We propose a treatment algorithm for R/R LBCL that prioritizes CAR-T administration in all eligible patients in the second- and third-line settings given the curative potential and manageable toxicities of CAR-T in real-world cohorts (Figure 1).

However, discussions with patients regarding their treatment preferences will be paramount as more long-term data on BsAb efficacy emerge in the literature. We recognize the limitations of cross-trial comparisons between CAR-T and BsAb therapies; prospective trials that compare the 2 strategies will be critical to treatment decision-making, particularly with risk stratification schema to study which patient cohorts may most benefit from CAR-T or BsAb therapies as the first in sequence. Ongoing trials studying specific CAR-T and BsAb sequencing strategies, such as SWOG 2114 and NCT04889716, which investigate various BsAb therapies as consolidation after commercial CAR-T, will also define optimal sequencing paradigms.[52] Finally, efforts to increase the accessibility and safety of CAR-T delivery in community settings that are closer in proximity to patients and their caregivers will be vital in improving patient-centered outcomes.

Conflict-of-interest disclosure

Ajay Major: no competing financial interests to declare.
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Off-label drug use

Ajay Major: Nothing to disclose.
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EVIDENCE-BASED MINIREVIEW

Barriers to accessing cellular therapy for patients receiving care in community practices

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LEARNING OBJECTIVES

- Recognize common barriers limiting access to cellular therapies for patients with large B-cell lymphoma
- Explore strategies for improving access to cellular therapy among patients treated in community settings

CLINICAL CASE

A 56-year-old Hispanic male without health insurance coverage was diagnosed with stage IV nonbulky diffuse large B-cell lymphoma, not otherwise specified (MYC, BCL2, BCL6 nonrearranged; Ki-67 70%). He received county health coverage and initial treatment at a local county hospital. Treatment with R-CHOP initially achieved complete response (CR), but unfortunately the lymphoma relapsed with bulky adenopathy. His cancer care system does not perform cellular therapies, but he is motivated to get the best available treatment.

Prior to 2022, the standard of care (SOC) approach for patients with relapsed or refractory large B-cell lymphoma (LBCL) consisted of salvage immunochemotherapy to achieve response followed by consolidation with high-dose therapy and autologous hematopoietic stem cell transplantation (auto-HSCT). However, only approximately 30% to 40% of patients with relapsed/refractory LBCL respond and proceed to auto-HSCT.[1] Among patients receiving curative intent auto-HSCT, approximately 50% to 60% ultimately relapse.[2] The prognosis for patients with primary refractory LBCL or LBCL that relapses in ≤12 months historically has been particularly poor.[1]

Utilization of CD19-directed chimeric antigen receptor T-cell (CAR T) therapy transformed the treatment landscape for patients with early-relapsed LBCL. CAR T-cell therapy first demonstrated significant efficacy in the third-line setting with the results of JULIET, TRANSCEND, and ZUMA-1 studies establishing these products as SOC options.[3] In 2022, 2 multicenter, randomized phase 3 trials, ZUMA-7 and TRANSFORM, demonstrated superior efficacy of axicabtagene ciloleucel (axi-cel) and lisocabtagene maraleucel (liso-cel) compared with SOC in fit patients relapsing within 12 months of fronte therapy.[4,5] A recent update showed that axi-cel significantly improved overall survival compared to SOC.[5] The BELINDA trial, however, failed to demonstrate improved efficacy with tisagenlecleucel (tisa-cel) compared with SOC in the second-line setting.

HSCT and CAR T therapy are technologically sophisticated, resource-intense, and costly procedures requiring specialized care at specially certified centers; they also require complex interactions between patients, caregivers, providers, and health care systems. Several sociodemographic and geographical factors can lead to disparities in access to these therapies, including insurance coverage, affordability, distance to centers, other geographic considerations, and referral patterns. In addition to barriers patients face when seeking HSCT therapy, access to CAR T therapy is limited by additional barriers, including manufacturing delays, limited availability, and provider familiarity. These barriers are particularly relevant for patients receiving treatment in community settings (Table 1).

Manufacturing delays and limited availability

There are various steps involved in using autologous CAR T-cell products, including leukapheresis, manufacturing, quality checks, transportation to and from the manufacturer, and ultimately, CAR T administration. The median time from leukapheresis to product delivery or infusion was 17 days for axi-cel,[5] 36 days for liso-cel,[4] and 54 days for tisa-cel.[6] Given the potential for rapid progression of lymphoma, especially with refractory disease, early referral of patients to a center that offers cellular therapies is essential to maximize eligibility for this potentially life-saving therapy. Long delays in time to CAR T infusion can result in increased tumor bulk, worsened organ function,

Table 1. Clinical outcomes and time to receipt of CART product in key CAR-T cell therapy clinical trials

	Axi-cel	SOC	Tisa-cel	SOC	Liso-cel	SOC
Second-line therapy	**ZUMA-7**		**Belinda**		**Transform**	
Received bridging chemotherapy (%)	0	—	83	—	63	—
Median time to CAR T-cell infusion (days)	29 (27–34)*	—	52 (31–135)†	—	NR	—
Received intended ASCT (%)	—	36	—	32.5	—	45.6
ORR	83	50	46	43	86	48
CR rate	65	32	28	28	66	39
EFS, median (months)	8.3	2	3	3	10.1	2.3
EFS HR (95% CI)	0.4 (0.31–0.51)		1.07 (0.82–1.4)		0.35 (0.23–0.53)	
PFS, median (months)	14.7	3.7	NR	NR	14.8	5.7
OS, median (months)	NE	25.7	16.9	15.3	NE	16.4
Third-line therapy (single-arm trials)	**ZUMA-1**		**JULIET**		**TRANSCEND**	
Follow-up, median (months)	63.1	—	40.3		23.0	—
Received bridging chemotherapy (%)	0	—	90	—	59	—
Received intended CAR T cell (%)	91	—		—		—
Median time to CAR T-cell infusion (days)	29 (27–34)*	—	54	—	37 (27–224)†	—
ORR	83%		53%	—	73%	—
CR rate	58%	—	39%	—	53%	—
Median DOR	11.1	—	3	—	23.1	—
Median duration of PR (months)	1.9	—		—	—	—
Duration of CR (months)	62.2	—	0.35 (0.23–0.53)	—	26.1	—
EFS, median (months)	5.7	—	NR			—
PFS, median (months)	5.9	—	—		6.8	—
OS, median (months)	NR		11.1		27.3	—

*interquartile range.

†range.

ASCT, autologous stem cell transplant; CAR, chimeric antigen receptor; CR, complete response; DOR, duration of remission; EFS, event-free survival; HR, high risk; NE, neutrophil elastase; NR, not reached; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; SOC, standard of care.

and potentially loss of eligibility for CAR T due to decline in clinical status. These delays are particularly relevant for patients receiving care in the community who require referrals to certified centers. Several real-world studies at academic centers report CAR T administration rates after leukapheresis ranging from 90% to 93%.[7] However, community-based practices have reported lower CAR T completion rates with 1 large community-based practice noting that 47% of patients referred for CAR T evaluation ultimately received CAR T therapy, with a median time from referral to CAR T infusion of 143 days.[8] The primary reasons for inability to receive CAR T were disease-related issues such as disease progression and decline in clinical status. Unlike auto-HSCT, patients receiving CAR T therapy do not require demonstration of chemosensitive lymphoma at the time of infusion, though their disease should not be rapidly progressing to allow time for leukapheresis, manufacturing, and infusion of the CAR T cells. Early recognition of patients eligible for cellular therapies is important to decrease delays.

Additionally, use of effective bridging therapy is particularly important to improve disease control while undergoing evaluation for CAR T therapy. Numerous patients considered for CAR T-cell therapy have lymphoma with some degree of chemotherapy resistance. Novel targeted agents such as antibody drug conjugates and bispecific antibodies have shown promising efficacy for disease control and can improve eligibility for CAR T-cell therapy. Notably, these therapies also are expensive, and providers may encounter difficulties in obtaining access depending on insurance coverage and approval. Thus, early initiation of this process is crucial.

Cost and insurance coverage

Current list pricing for the 5 FDA-approved CAR T-cell therapies ranges between $373000 and $475000 per one-time infusion. However, the average total cost of care for patients was >$700000, and 12% of patients had costs exceeding $1 million.[9] The high cost relates to combined costs of the CAR T-cell product, patient preparation (eg, leukapheresis and lymphodepletion), product infusion, pre- and post-infusion patient management, and monitoring for side effects. Despite the high cost, CAR T-cell therapy products are significantly efficacious and

cost-effective in the second-line (axi-cel and liso-cel) or third-line setting (axi -cel, liso-cel and tisacel).[10,11]

Insurance coverage is critical, and prior studies have shown that patients who were uninsured or Medicare insured were less likely to receive CAR T compared with commercially insured patients.[12] Despite the proven efficacy and cost-effectiveness of CAR T-cell therapy, based on current Medicare reimbursement structure, hospitals can lose up to $304000 on each inpatient administration of CAR T for Medicare beneficiaries, which disincentivizes appropriate use of these potentially curative therapies.[13]

In the era prior to CAR T, patients with LBCL who were Asian/Pacific Islander, American Indian/Alaska Native, Black/African American, from rural neighborhoods, Medicaid insured, and/or uninsured had worse survival.[14-16] Patients from disadvantaged socioeconomic status groups and racial/ethnic minority groups have been historically underinsured and are thus at increased risk of a major barrier to accessing CAR T-cell therapy due to insurance. Although data addressing the relationships between insurance coverage and access to salvage therapy and CAR T-cell therapy are lacking, data regarding stem cell transplantation suggest that expansion of coverage for uninsured or underinsured since the Affordable Care Act was enacted in 2014 led to increased access, with approximately 40% of HSCT procedures performed in the United States now reimbursed by governmental payers.[17] Prior to expansion, this therapy that was routinely only offered to relatively young, otherwise healthy patients who likely had commercial, employer-based insurance coverage.[17] Addressing inequities in access to CAR T therapy will likely require similar policy changes. Expanding access to insurance and decreasing the cost of specialized care can be accomplished through collaborative efforts across stakeholders, including health care system, payers/insurers, and pharmaceutical manufacturers.

Site of care and geographic limitations to access

Unlike cytotoxic chemotherapy regimens that are readily available at most centers that care for cancer patients, access to cellular therapies is currently limited to specific certified centers meeting the requirements set up by manufacturers and regulatory agencies. CAR T-cell administration is generally limited to well-resourced academic medical centers with HSCT experience and Foundation for the Accreditation of Cellular Therapy (FACT) accreditation.[12,18] At the time of this article, there are approximately 307 FACT-accredited centers across the United States.[19] As such, there is a geographic limitation to where CAR T therapy is available. Additionally, because of the unique toxicities of CAR T-cell therapies, patients are required to be monitored closely for the first week after cell administration and then required to stay within 2 hours of the facility for up to 30 days with a dedicated caregiver.[12] One study found that one-third of patients lived >120 minutes of driving from where CAR T-cell therapy was administered.[12] Geographically restricted access may disproportionately impact patients in rural locations and patients from socioeconomically disadvantaged backgrounds (such as those living below the federal poverty line). Distance can increase other out-of-pocket expenses not covered by insurance, including the costs of travel and lodging. Patients and caregivers may also experience financial consequences from short-term loss of income due to required relocation. A study using quantitative and qualitative methods identified that the financial impact on patients receiving CAR T-cell therapy extends beyond the cost of treatment. Expanding access to care through site-of-care planning could help address regional, rural-urban, and sociodemographic equity in the geographic allocation of CAR T-cell therapy.[20]

Conclusions

Auto-HSCT remains an important option for patients experiencing late relapse of LBCL who remain chemosensitive. CAR T-cell therapies revolutionized the treatment of LBCL in the second line and beyond. The wide adoption of these therapies presents challenges, particularly for patients who receive care in community centers and remote areas. Improving provider awareness of CAR T-cell products, uses, and toxicities is essential to improving familiarity with these products and expediting referrals to centers that offer these therapies. Understanding the impacts of insurance coverage and the full cost of CAR T for patients is important to expand access. This also must consider costs of travel, housing, medications, and other unseen costs like lost wages. We recommend early involvement of social workers and case management to aid patients in mitigating challenges. Engagement of disease-specific patient advocacy groups such as the Leukemia and Lymphoma Society (www.lls.org) and the Lymphoma Research Foundation (www.lymphoma.org) can offer additional guidance and support services to patients with lymphoma and their caregivers. These services may include financial support for socioeconomically disadvantaged patients. In the future, decreasing manufacturing time for CAR T products and expanding manufacturing capacity will be essential to increasing the ability to treat more patients with cellular therapies. We recommend timely considerations of effective bridging therapies (such as novel targeted therapies) where clinically indicated to optimize disease control and avoid clinical decline that could make patients ineligible while awaiting production of CAR T cells. Together these efforts can aid in wider application of CAR T therapy where clinically appropriate and improve outcomes for patients with LBCL.

Conflict-of-interest disclosure

Chijioke Nze: no competing financial interests to declare.
Christopher R. Flowers: consultant: AbbVie, AstraZeneca, Bayer, BeiGene, Bio Ascend, Bristol Myers Squibb, Celgene, Denovo Biopharma, Foresight Diagnostics, Genentech/Roche, Genmab, Gilead, Karyopharm, N-Power, Pharmacyclics/Janssen, Seagen, Spectrum; stock/stock options private company: Foresight Diagnostics, N-Power; researcher: 4D, AbbVie, Acerta, Adaptimmune, Allogene, Amgen, Bayer, Celgene, Cellectis, EMD Serono, Genentech/Roche, Gilead, Guardant, Iovance, Janssen, Kite, MorphoSys, Nektar, Novartis, Pfizer, Pharmacyclics, Sanofi, Takeda, TG Therapeutics, Xencor, Ziopharm.

Off-label drug use

Chijioke Nze: nothing to disclose.
Christopher R. Flowers: nothing to disclose.

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Langerhans cell histiocytosis: promises and caveats of targeted therapies in high-risk and CNS disease

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Langerhans cell histiocytosis (LCH) is a rare myeloid neoplasm driven by activating mutations in the MAPK pathway, most commonly *BRAF-V600E* and *MAP2K1*. It affects children and adults, with a wide spectrum of clinical presentations ranging from self-limited to multisystem (MS) life-threatening forms. LCH is defined by the accumulation of CD1a$^+$/CD207$^+$ cells in different organs, and patients with liver, spleen, or hematopoietic system involvement have a higher risk of mortality. Patients with neurodegeneration (ND) have devastating outcomes and are resistant to systemic therapies. MS-LCH is treated with risk-adapted therapy, but many patients require multiple salvage regimens that are myelosuppressive and expensive. MAPK inhibitors are increasingly being used, but most patients relapse upon discontinuation of therapy. Here, we review the management of central nervous system disease and how novel cerebrospinal fluid biomarkers might predict patients at high risk of ND who could benefit from early MAPK inhibition. Further, we discuss treatment strategies for refractory/relapsed (R/R) LCH, with a focus on MAPK inhibitors' efficacy and challenges (ie, the unknown): long-term toxicity in children, optimal duration, if they are curative, whether it is safe to combine them with chemotherapy, and their high price tag. Lastly, emerging strategies, such as the new panRAF inhibitor (Day 101) in patients with R/R LCH, *ERK1/2* or *CSF1R* inhibition in patients with MEK1/2 inhibitor resistance, and targeting the microenvironment (checkpoint plus MEK inhibition) or senescent cells (*mTOR* or *BCL-XL* inhibitors) in R/R patients, are also examined.

LEARNING OBJECTIVES

- Discuss the treatment approach to patients with neurodegeneration and the potential role of novel cerebrospinal fluid biomarkers
- Learn how comprehensive genomic profiling at the diagnosis of Langerhans cell histiocytosis can add prognostic information and positively impact clinical care
- Describe the promises and caveats of MAP kinase targeted therapies in refractory/relapsed high-risk Langerhans cell histiocytosis

CLINICAL CASE

Federico, a 2-year-old boy, presented with scalp rash (Figure 1A), polyuria, polydipsia, hepatosplenomegaly, pancytopenia, and liver dysfunction. Skin biopsy revealed CD1a$^+$/CD207$^+$ histiocytes (Figure 1B, C), compatible with Langerhans cell histiocytosis (LCH); *BRAF-V600E* was positive (Figure 1D). Brain magnetic resonance imaging (MRI) showed pituitary stalk thickening, absent posterior bright spot (Figure 1E). Vinblastine/prednisone for 6 weeks were given with no response. Salvage with cladribine/cytarabine followed by oral mercaptopurine (6-MP)/methotrexate led to complete remission (CR) for 8 years.

Introduction

LCH is a rare disorder characterized by expansion of myeloid precursors that differentiate into CD1a$^+$/CD207$^+$ lesions. It affects children and adults with a wide spectrum of clinical manifestations, ranging from single-system (SS) to multisystem (MS) disease. LCH is an inflammatory myeloid neoplasm due to the presence of MAPK-ERK pathway mutations in most cases, beyond *BRAF-V600E*.[1-3] Next-generation sequencing (NGS) showed that *BRAF* wild-type LCH harbors other mutations, like *MAP2K1* (in 15%), *KRAS*, *NRAS*, *ARAF*, *MAP3K1*, *ERBB3*,[3] kinase fusions (*BRAF*, *NTRK1*, *ALK*), *BRAF* duplications, insertions, deletions, and *PI3K-AKT-mTOR* pathway/receptor-colony stimulating factor 1 receptor (*CSF1R*) mutations[3,4] (Figure 2A). LCH incidence ranges

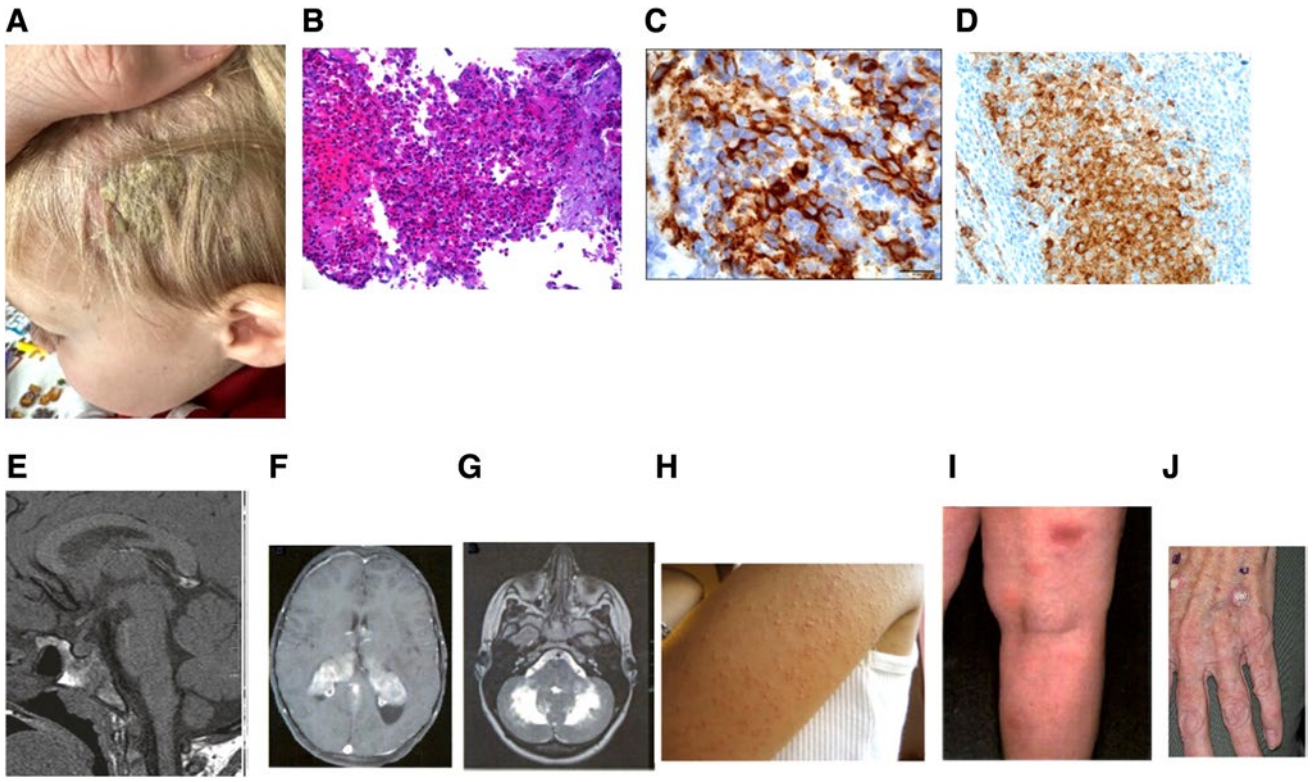

Figure 1. Clinical, pathological, and radiographic features of LCH with cutaneous toxicities of MAPK inhibitors. (A) Scaly scalp rash in a 2-year-old boy. (B) LCH with a rich inflammatory background including osteoclast-like giant cells, eosinophils, and neutrophils (hematoxylin and eosin). (C) Immunostain showing surface CD1a expression. (D) Mutant-specific BRAF-VE1 immunostain with dark granular cytoplasmic staining in the lesional histiocytes (immunostain 400×). (E) Granulomatous CNS-LCH: brain MRI sagittal T1W image lacking posterior pituitary bright spot. (F) Brain MRI in a patient with granulomatous CNS-LCH: axial contrast-enhanced T1W image showing extensive bilateral lesions in the choroid plexus. (G) ND-LCH: axial T2-weighted image showing extensive dentate nucleus and white matter cerebellar neurodegeneration. (H) Skin hyperkeratosis pilaris of the left arm in a patient treated with a BRAF inhibitor. (I) Lobular panniculitis of the right leg in a patient on a MEK inhibitor. (J) Cutaneous squamous cell carcinoma in a adult treated with a BRAF inhibitor.

from 2.6 to 8.9 cases/million children/year[5] and 1-2 cases/million/year in adults.[6]

The classification of LCH in children is based on number of involved sites (SS, MS/unifocal or multifocal) and if risk organs (ROs; risk of mortality) like liver, spleen, or the hematopoietic system are involved (Table 1).[7] SS/MS disease account for 50% of patients each, and 15% of MS cases are RO$^+$. Bone involvement occurs in 80%; skull is the most common.[7] Skin is the second most common, especially in infants, more frequently in the scalp as seborrheic eczema. "Skin-only" LCH has a 60% chance of spontaneous resolution; however, progression to MS disease occurs in 40%.[8]

Adult LCH usually presents with MS disease. Clinical classification does not differentiate RO separately, due to lack of data on prognostic implications in the targeted therapies era (Table 1). Pulmonary LCH (PLCH) occurs usually in isolation, mostly associated with smoking, and usually responds to smoking cessation.[9]

Central nervous system LCH

Central nervous system (CNS) LCH can be granulomatous or neurodegenerative. Granulomatous or tumorous lesions account for 10% to 15% of all LCH cases and tend to occur early in the course of the disease. Typical neuroimaging findings include pituitary stalk thickening (Figure 1E), absent posterior pituitary spot, enlargement of the pineal gland, thickening and enhancement of the choroid plexus (1F), or intraparenchymal masses. Depending on the location of the lesions, patients present more frequently with diabetes insipidus (DI), focal seizures, or symptoms of increased intracranial pressure. DI has been reported to occur in up to 24% of patients with LCH and in half of those with MS disease. The diagnosis of DI usually precedes or is concurrent with the diagnosis of LCH in one-third of cases, while in the remaining two-thirds, it is diagnosed as a late sequela.[10] Patients with LCH and coexisting DI are prone to developing anterior pituitary dysfunction, with growth hormone deficiency (in up to 50%), precocious or delayed puberty, hypothyroidism, hypogonadism, hypocortisolism, or panhypopituitarism.[10]

Neurodegenerative LCH (ND-LCH) can present as LCH-associated abnormal CNS imaging (LACI), which includes asymptomatic patients with radiographic findings in up to 24% of all children with LCH, and LCH-associated abnormal CNS symptoms (LACS), which includes patients with abnormal neurocognitive findings.[11] Both forms have increased T2-weighted MRI signals in the dentate nuclei of the cerebellum (Figure 1G), basal ganglia, and pons. Long-term neurodegeneration incidence is 1.9% to 11% and is higher in patients with MS disease, DI, previous CNS-risk

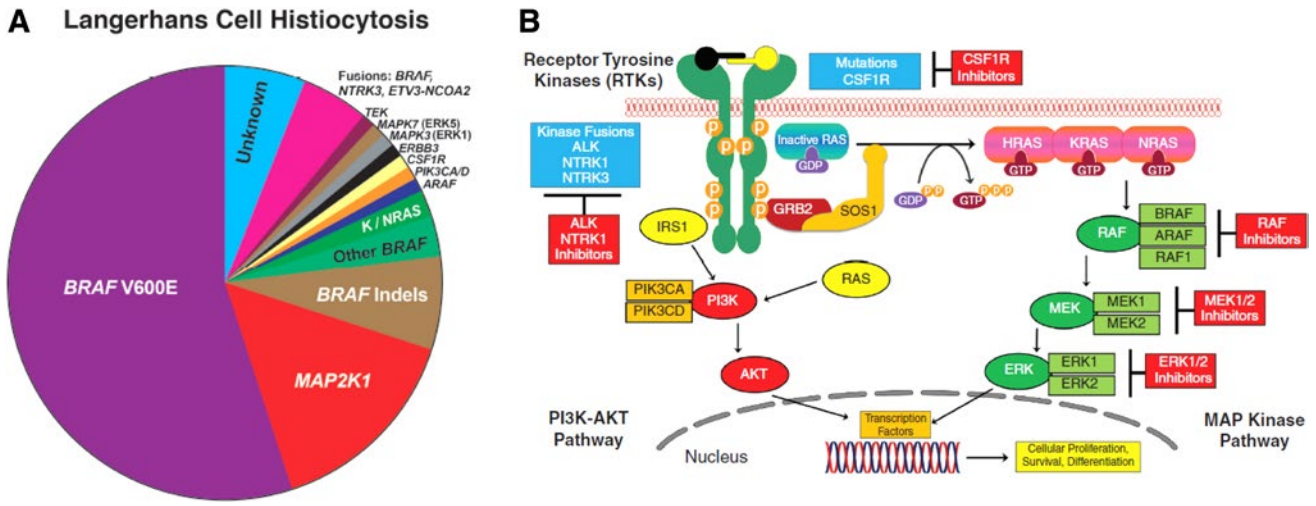

Figure 2. (A) Summary of diverse kinase alterations discovered by next-generation sequencing techniques in LCH in the past 13 years: pie chart illustrating a composite of the diverse kinase alterations driving LCH, many of which are targetable. **(B) Diagram of the MAPK and PI3K-AKT signaling pathways:** description of the activation of the RAS proteins with annotation of the signaling proteins affected by genetic alterations in the histiocytic neoplasms.

Table 1. Clinical classification of LCH

Children	
Clinical group	**Description**
Multisystem	Two or more systems involved
With risk organ involvement	Involvement of liver, spleen, or bone marrow
Without risk organ involvement	Without involvement of liver, spleen, or bone marrow
Single system	Only 1 system involved
Single site	Skin, bone, lymph node, thyroid, thymus
Multiple sites	Multifocal bone disease
Special site	Skull-base lesion with intracranial extension or vertebral lesion with intraspinal soft tissue extension
Pulmonary LCH	Isolated lung disease
CNS-LCH	Tumorous lesions
	Neurodegenerative disease LACI LACS
Adults	
Unifocal	Solitary lesion involving any organ
Single-system pulmonary	Isolated lung involvement, predominantly smoking related
Single-system multifocal	≥1 lesion involving any organ
Multisystem	≥2 organ/system involvement

bone lesions, or *BRAF-V600E*–mutated LCH. The onset of radiographic or clinical findings can occur with the initial diagnosis of LCH, although it more commonly occurs years (up to 10) after the resolution of LCH. LACS is a neurodegenerative syndrome of variable severity and course. Symptoms in children may initially include ataxia, dysarthria, tremors, behavioral changes, and learning or psychiatric problems. Some patients may develop a progressive cerebellar syndrome, spastic tetraparesis, pseudobulbar palsy, and cognitive deterioration.[10,11] Adult patients usually present with cerebellar deficits.[9]

Whether ND represents active disease or a sequela of prior active disease is not clear. A recent study identified CD1a-negative *BRAF-V600E*$^+$ myeloid precursors in brain biopsies of patients with ND-LCH. Further, *BRAF-V600E*$^+$ cells were identified in the blood of patients with ND who had no systemic findings of active LCH[12]; responses to BRAF inhibitors further support this notion.[13] Cerebrospinal fluid (CSF) biomarkers could be a promising tool for patients with ND-LCH. *BRAF-V600E* mutation can be detected in CSF cell-free DNA in only 10% of cases. CSF osteopontin[12] and neurofilament light protein (NFL) are elevated in patients with ND[14]; indeed, CSF NFL levels normalized within 9 months in children receiving MAPK inhibitors, with clinical/radiologic improvement.[14] In summary, novel CSF biomarkers might predict patients at high risk of ND, who could potentially benefit from early MAPK inhibition.

Surveillance

A clinical assessment by a neurologist with standardized tests, such as International Cooperative Ataxia Rating Scale (ICARS) and neuropsychological testing, should be performed initially (as soon as radiologic changes suggestive of ND appear on brain MRI) and at regular intervals for better longitudinal assessment and therapeutic decision-making.[11] An ICARS score increase of 5 points indicates clinical deterioration, which, together with radiologic progression on MRI, should merit initiation of therapy.[11] Patients with symptoms suggestive of hormonal deficits should be assessed and monitored by an endocrinologist for appropriate testing and possible hormonal replacement.[11]

Treatment of CNS-LCH

There is no standard therapy for CNS-LCH. For children with granulomatous lesions and new-onset DI, systemic treatment with a standard LCH regimen, such as vinblastine/prednisone,

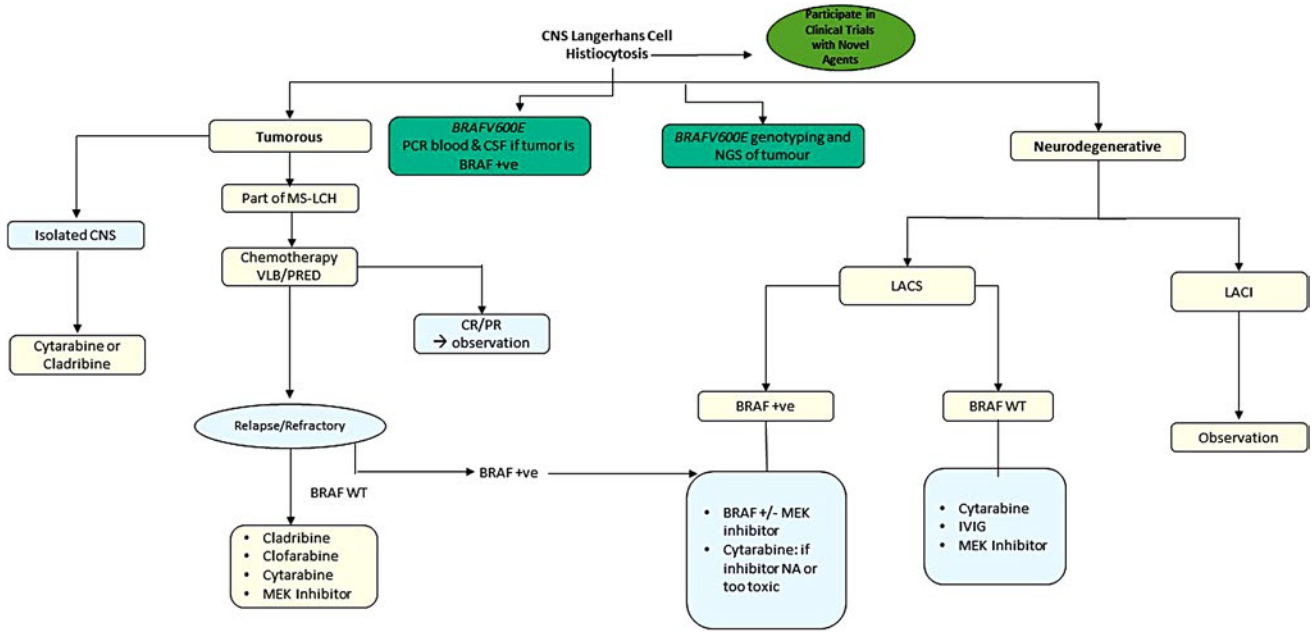

Figure 3. Algorithm for the management of CNS-LCH.

is advocated with the goal of preventing ND disease and anterior pituitary dysfunction. Reversal of DI has been achieved only in anecdotal cases, and almost all patients require lifelong replacement therapy with desmopressin. Parenchymal mass lesions of the brain due to LCH may respond to vinblastine/prednisone, cytarabine, cladribine, or clofarabine.[15] For adult granulomatous lesions, cladribine, higher doses of cytarabine, or MAPK inhibitors are preferred.[9]

Treatment of LACS is challenging and less defined; early treatment in patients with worsening symptoms is recommended. Clinical and radiologic stabilization was previously reported with cytarabine, intravenous immunoglobulin, infliximab, or rituximab.[16] A retrospective study of children with LCH on BRAF or BRAF/MEK inhibitor combinations showed favorable clinical and radiologic responses in 12 of 13 children with LACS.[13] Patients with radiographic ND lesions without symptoms (LACI) do not require any treatment, as there is no proof that starting treatment would prevent progression to clinical ND (LACS).

In summary, the prevention and management of LACS is quite challenging and requires a multidisciplinary approach. Early start of therapy with MAPK inhibitors, with a close neurologic and neuropsychologic monitoring, is recommended (Figure 3). Novel inhibitors with better CNS penetration are urgently warranted (see section on Day 101). In case of intolerance to, or unavailability of, inhibitor therapy, then treatment with cytarabine or immunoglobulin should be considered.

Quality of life and survivorship

Overall morbidity can be significant, resulting in disability in more than half of survivors of MS-LCH. Health-related quality of life, which assesses the patient's perspective of disease burden, has been studied in these patients and was found to correlate closely with morbidity, as measured by professionals. Health-related quality of life parameters were particularly affected by the presence of CNS and lung disease, with an inability to lead independent lives in the most severely affected patients.[17]

Neurologic problems such as cerebellar ataxia, learning difficulties, and psychological symptoms can develop concurrently or, more often, several years after the diagnosis of LCH. Cerebellar damage may be seen in up to 12% of all patients with LCH, but this increases to 60% in patients with recognized CNS involvement. Neuropsychological sequelae of LCH include intellectual loss, learning deficits, poor school performance, problems with immediate auditory verbal memory, and emotional disturbances.[11]

Molecular analysis for somatic MAPK-ERK mutations

Immunohistochemical staining/molecular testing for *BRAF-V600E* should be performed at diagnosis. Quantitative polymerase chain reaction (PCR) or droplet digital PCR is more sensitive than immunohistochemistry or NGS.[18] In LCH lesions without *BRAF-V600E*, NGS for other MAPK mutations is recommended. Peripheral blood (PB) cell-free DNA testing is a good alternative in cases with insufficient lesional tissue, but its correlation with tissue NGS is higher for *BRAF-V600E* than other mutations.[18,19]

Clinical implications of BRAF and other MAPK mutations

BRAF-V600E correlated with high-risk (HR) LCH, frontline therapy resistance, DI, ND, and relapse in 315 children.[19] However, a clinicogenomic study (377 children) questioned the independent prognostic value of lesional *BRAF-V600E*, which was associated with HR-MS and skin involvement, CNS-risk bones, and gastrointestinal involvement[20] (additive unfavorable prognostic factor in HR-LCH[21]), whereas *MAP2K1* mutations associated with SS-bone/skin and less MS disease and *BRAF* exon 12 deletions correlated with lung involvement[20] (in accordance with adult PLCH[9]). Although *BRAF-V600E* correlated with reduced event-free survival (EFS) in all patients, neither *BRAF-V600E* nor *MAP2K1*

mutations were associated with EFS when patients were stratified by disease extent. Furthermore, lesional *BRAF-V600E* status did not affect outcomes in adult LCH.[22]

BRAF-V600E allele detection in circulating cell-free (ccf) DNA using digital droplet PCR was investigated in children with *BRAF-V600E*–mutated LCH. After vinblastine-steroid induction, 7 of 7 nonresponders remained positive for ccf *BRAF-V600E* compared to 2 of 4 partial responders and 0 of 4 complete responders. Thus, ccf *BRAF-V600E* is a promising biomarker for monitoring response to therapy for children LCH resistant to frontline chemotherapy.[23] More recent data, however, showed that *BRAF-V600E*$^+$ cells persisted in PB after *MAPK* inhibitor treatment but were not correlated with clinical response.[13] In summary, while *BRAF-V600E* measurements have been helpful in assessing patient clinical responses, they are not considered independent determinants of LCH outcome.

Frontline therapy of MS-LCH

Children

Risk-adapted treatment led to improved survival for childhood LCH. Current therapy for multifocal and MS-LCH is based on the LCH-III trial, including vinblastine/prednisone for 1 year. Long-term overall survival (OS) for HR (RO$^+$) patients is 85%, while OS in low-risk (LR) disease is >95%.[24] Nevertheless, 50% of patients will be refractory or develop reactivations, mostly within 2 years. Despite their excellent survival, LR patients with refractory/relapsed (R/R) LCH have morbidities, including chronic pain, hearing loss, sclerosing cholangitis, pituitary dysfunction, growth retardation, and progressive ND; all of these late sequelae are also very common in HR (RO$^+$) patients. The Histiocyte Society LCH-IV trial (NCT02205762) is trying to optimize frontline therapy outcomes by testing prolonged (12 vs 24 months) and intensifying (± 6-MP) treatment for HR patients and comparing 6- vs 12-month treatment for patients with multifocal bone disease.

Adults

The significant increase in survival of patients with MS-LCH seems to have favored children, with 5-year survival rates of only 70% in adults. Similar treatment guidelines are recommended for adults and children, with some modifications.[9] For MS disease, vinblastine-based regimens are effective but cause more neuropathies in adults; thus, cytarabine or cladribine/clofarabine is preferred, although *BRAF/MEK* inhibitors are increasingly being used. For PLCH, smoking cessation is essential for symptom improvement, followed by observation. Patients with severe disease benefit from cladribine, although *BRAF/MEK* inhibitors can be considered.[9]

Treatment of refractory/relapsed LCH

Optimal therapy for patients with R/R LCH is undefined. For LR reactivation, less toxic regimens are effective, including cytarabine/prednisone/vincristine, 6-MP, methotrexate, indomethacin, bisphosphonates, or hydroxyurea[7,9] (Figure 4). HR (RO$^+$) R/R patients respond to acute myeloid leukemia–like therapies, including cytarabine, cladribine, and clofarabine. Cladribine (5 mg/m^2/d×5 days) yielded responses in 22% of R/R HR and 62% of LR patients, but only 4% had CR by 6 months.[25] However, cladribine is associated with long-term myelosuppression and secondary acute myeloid leukemia.[9] Cytarabine (100-170 mg/m^2/d) yielded 41% 3-year EFS in children with first relapse or greater.[26] High doses of cytarabine (1 g/m^2/d) and cladribine (9 mg/m^2/d) salvage yielded 5-year OS of 85% in 27 HR-RO$^+$ R/R children but was associated with high treatment-related toxicity.[27]

Clofarabine (25 mg/m^2/d, 5 days/cycle, for 6 cycles) is also promising, with 1-year PFS of 76% in 11 multiply refractory patients and minimal toxicity.[15] Results of a North American Consortium for Histiocytosis prospective study (NCT02425904) testing clofarabine in R/R histiocytoses are pending. Furthermore, patients with multiply refractory HR-RO$^+$ disease were historically treated

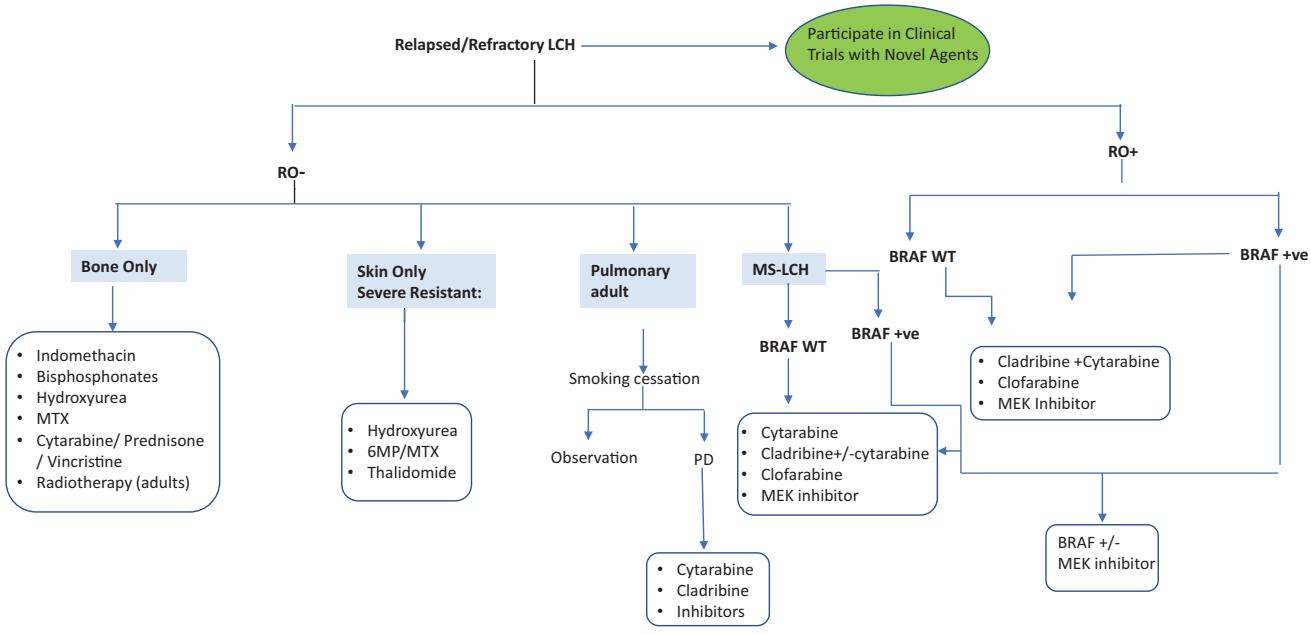

Figure 4. Algorithm for the management of relapsed/refractory LCH.

with hematopoietic stem cell transplant, achieving better outcomes with reduced-intensity conditioning.[28] However, since the introduction of targeted therapies, the role of hematopoietic stem cell transplant in LCH has been unclear.[9] A summary of the different salvage therapies is shown in Figure 4.

CLINICAL CASE (continued)

Federico's annual brain MRI after 8 years showed new cerebellar white matter lesions (Figure 1G). He was neurologically intact; therefore, he underwent close observation. After 1 year, he developed ataxia/dysarthria with an ICARS score increase of 5 points, and brain MRI showed worsening cerebellar white matter lesions. His symptoms were affecting his quality of life with frequent falls and learning problems at school. Neuropsychologic testing showed a clear cognitive decline. Therefore, he was started on dabrafenib (5.25 mg/kg/d in divided doses) and trametinib (0.025 mg/kg/d) with rapid clinical and radiographic improvement within 2 months.

Rationale for targeted therapies in LCH

Although LCH can be almost universally cured with chemotherapy, major challenges remain. High rates of treatment failure in patients with MS disease, high toxicity of salvage therapies, increased risks of death in HR patients, and increased risk of long-term morbidity for all LCH patients with R/R disease are among those challenges. Thus, more effective and less toxic treatment options are warranted. While chemotherapy drugs are cytotoxic to all rapidly dividing normal and cancerous cells, targeted therapies are cytostatic (block tumor cell proliferation) by acting on specific molecular targets associated with cancer (molecular on/off switch).

Treatment with MAPK inhibitors

Vemurafenib is a BRAF inhibitor that has been approved by the US Food and Drug Administration (FDA) for adults with *BRAF-V600E*–mutated Erdheim-Chester disease (ECD) and was the first reported targeted therapy to treat refractory MS-LCH in an 8-month old infant.[29] Subsequently, an overall response rate (ORR) of 100% was observed in patients with *BRAF-V600E*–mutated MS and refractory LCH treated with vemurafenib monotherapy in an observational study of 54 children[30] and in a small cohort of 4 adults enrolled in a phase 2 basket study (Table 2).[31] Dabrafenib, another BRAF inhibitor, given as monotherapy yielded an ORR of 65% in a retrospective study of 20 children with *BRAF-V600E*–mutated LCH.[32] A recently published phase 1/2 study in pediatric patients with R/R *BRAF-V600E*–mutated LCH showed an ORR of 76.9% (10/13 patients) with dabrafenib monotherapy (NCT01677741), while dabrafenib plus an MEK inhibitor, trametinib (NCT02124772), demonstrated an ORR of 58.3% (7/12) (Table 2).[33] Another study of 21 children with MAPK-mutated LCH also reported an ORR of 86% with BRAF or MEK inhibitor monotherapy or combinations.[13] MAPK inhibitors have also shown effectiveness in patients with ND-LCH, and patients with early-onset ND achieve better outcomes (Table 2).[13,34] Cobimetinib, an MEK1/2 inhibitor, was recently FDA approved for the treatment of adults with histiocytic neoplasms, based on a phase 2 trial in which 20 of 26 (76.9%) patients with different histiocytic neoplasms, including mutations in BRAF, N/KRAS, and MEK1/2, showed a CR or a partial response by fluorodeoxyglucose-positron emission tomography (FDG-PET)[35] (Figure 2B).

Data on frontline inhibitor therapy in LCH are emerging in adults and children.[36,37] Eighteen patients (ages 0.2-45 years) with histiocytoses, including LCH, received frontline MAPK inhibitors. All had favorable responses with a median treatment duration of 2 years. Inhibitors were well tolerated; 5 patients with SS-LCH discontinued therapy and remain in CR off therapy.[37]

Whether combination therapy with BRAF/MEK inhibitors has greater benefits in patients with *BRAF-V600E*–mutated LCH compared with BRAF inhibitor monotherapy remains unknown.[33] However, combination therapy seems to reduce resistance/toxicity and improves outcomes in adults and pediatric patients with different types of *BRAF-V600E* mutant disease.[33]

CLINICAL CASE (continued)

At 2 years after starting inhibitor therapy, Federico comes back with a 4-week history of bilateral leg pain, myalgias, and frequent falls. Recurrent LCH was ruled out with positron emission tomography/computed tomography. Serum creatine kinase (CK) was high, compatible with mild trametinib-induced rhabdomyolysis. Therefore, trametinib was held and dabrafenib monotherapy continued. After 1 month, his muscular symptoms disappeared and serum CK normalized, and thus trametinib was restarted at two-thirds of his previous dose. Since then, he has been tolerating well his double inhibitors without major side effects.

Caveats of MAPK-targeted therapies

MAPK inhibition in patients with histiocytoses is usually well tolerated. The most common toxicities of BRAF inhibitors include dermatologic (hyperkeratosis pilaris [Figure 1H], migratory panniculitis [Figure 1I], and photosensitivity), fever, vomiting, cough, renal/liver dysfunction, fatigue, constipation, prolonged QT, and joint pain[33]; uveitis was reported in adults. Most adverse events are grade 1 or 2. Toxicities from MEK inhibitors include fever, fatigue, nausea, diarrhea, decreased neutrophil/platelet counts, skin rash/infections, and rhabdomyolysis; in adults, these include retinopathy, retinal vein occlusion, and decreased ejection fraction.[38] Thyroid cancer and leukemia have been reported in adult LCH treated with vemurafenib.[31] Further, cutaneous spinocellular carcinoma/squamous cell carcinoma (Figure 1J)/basal cell carcinoma and myeloproliferative syndromes were seen in adult patients with ECD after MAPK inhibitors.[38] There are no pediatric reports of second malignancies with MAPK inhibitors, and their long-term toxicities in this population are unknown.

Day 101 (tovorafenib) is a type II panRAF inhibitor that does not cause the severe dermatologic, cardiac, or ophthalmologic toxicities of other RAF/MEK inhibitors. It results in potent inhibition of *BRAF-V600E* mutations and, in contrast to type I RAF inhibitors (vemurafenib, dabrafenib), inhibits both wild-type BRAF and CRAF/RAFI kinase, as well as hyperactivated signaling resulting from *BRAF* fusions, including *KIAA1549:BRAF* fusion.[39] In vitro studies demonstrated that Day 101 inhibits phosphorylated ERK signaling and cell proliferation in cell lines with high

Table 2. MAPK-targeted therapies in patients with LCH and other histiocytic disorders

Disease	Drug name (target)	Dose	N	Age, y	Pathogenic variants	Disease characteristics	Response	Response after DC	Reference
LCH	Vemurafenib* (BRAF)	8.5–33.8 mg/kg/d	1	0.7	*BRAF-V600E*	RR-MS	CR 1/1	Relapse 1/1	[29]
LCH	Vemurafenib* (BRAF)	20 mg/kg/d	54	0.2–14	*BRAF-V600E*	RR-MS	CR 38/54 PR 16/54	Relapse 14/30	[30]
LCH	Dabrafenib* (BRAF) Vemurafenib* (BRAF) Trametinib* (BRAF)	NA	21	0.4–21	*BRAF-V600E*	RR-MS CNS-ND (13)	CR 4/21 PR 14/21 SD 2/21 PD 1/21	NA	[13]
LCH	Dabrafenib* (BRAF) Trametinib* (BRAF)	D: 5 mg/kg/d T: NA	4	0.1–36	*BRAF-V600E* *BRAF* indel	RR-MS CNS-ND (1)	CR 3/4 PR 1/4	NA	[34]
LCH	Dabrafenib* (BRAF)	4 mg/kg/d	20	0.6–6	*BRAF-V600E*	RR-MS	CR 0/20 PR 14/20 SD 2/20 PD 4/20	NA	[32]
LCH	Dabrafenib* (BRAF) Trametinib* (BRAF)	D: 4.5-5.25 mg/kg/d T: 0.025-0.032 mg/kg/d	25 D: 13 D+T: 12	1–13	*BRAF-V600E*	RR-MS (24) CNS-ND (1)	ORR: D: 76.9% D+T: 58.3%	NA	[33]
ECD, LCH	Vemurafenib† (BRAF)	960 mg bid	26	51–74	*BRAF-V600E*	Refractory (17/26) CNS (11/26)	CR 2/26 PR 14/26 SD 9/26	NA	[31]
ECD, LCH, RDD, mixed	Cobimetinib‡ (MEK)	60 mg daily	18	18–80	*BRAF-V600E* *BRAF* *MEK1* *ARAF* *MEK2* *NRAS* WT *BRAF*	Refractory and/or multisystem and/or brain and/or cardiac involvement	CR 13/18 PR 3/18 SD 1/18 ND 1/18	NA	[35]

D, dabrafenib; NA, not available; ND, neurodegeneration; PD, progressive disease; PR, partial response; RDD, Rosai-Dorfman disease; SD, stable disease; T, trametinib; WT, wild-type.

*Drug is not FDA approved for this indication.

†Drug is FDA approved for ECD with *BRAF-V600E* mutation.

‡Drug is FDA approved for adults with histiocytic neoplasms.

MAPK activity. Interestingly, Day 101 does not cause paradoxical activation of wild-type RAF kinase dimers in cells with moderate RAS activity; thus, there is less risk of skin rash/cancer.[39] An upcoming Children's Oncology Group trial (NCT05287295) will test the efficacy/safety of Day 101 in children, adolescents, and young adults with R/R LCH.

Higher grades (3 or 4) are reversible with dose reduction or inhibitor discontinuation. Successful rechallenge of inhibitor combination was reported in an adult patient with melanoma after trametinib-induced rhabdomyolysis.[40] It is common practice in adults, in case of toxicity, to reduce the dose or have intermittent dosing/treatment holidays.[9] Patients on BRAF inhibitors need dermatology follow-up to monitor for skin rash/cancer, while patients on MEK inhibitors need, in addition to dermatology, echocardiogram/electrocardiogram and ophthalmology follow-up.

Not all mutations respond to inhibition; thus, evaluating the mutational landscape of patients with LCH before inhibitor therapy is essential. *BRAF*$^{N486\_P490indel}$ mutation is resistant to dabrafenib/vemurafenib but responds to trametinib[34] or sorafenib.[41] Further, RAF-dependent *MAP2K1* mutations respond to RAF inhibitors,[42] but RAF-independent mutations (*MAP2K1*$^{p.L98\_K104>Q}$) are resistant to trametinib.[43] Ulixertinib (oral *ERK1/2* inhibitor) recently led to CR/partial response in 3 of 4 adults with histiocytic neoplasms (including LCH) with class 3 *MAP2K1* mutation (*exon 3 p.E102-1103 in-frame deletion*) who progressed after MEK inhibition[44] (see Figure 2B).

MAPK inhibitors modulate the differentiation and function of LCH cells rather than eradicate precursors, like chemotherapy does; thus, they are not curative. The *BRAF-V600E*$^{+}$ cells persist in PB after *MAPK* inhibition, although this was not correlated with clinical responses.[13,34,45,46] Eight of 9 infants receiving salvage vemurafenib/chemotherapy responded without toxicity. Nevertheless, 5 of 8 patients relapsed after discontinuing vemurafenib and required vemurafenib maintenance.[46]

Table 3. Price summary of MAPK inhibitors with regimens/doses

Drug	Regimen-dose/cost: USD/month	
	Adults	Children (weight=20kg)
Vemurafenib	480–960 mg bid/$6500-$12 500	20 mg/kg/d div bid/$2600 (200 mg bid)
Dabrafenib	75–150 mg bid/$8500-$16 500	5.25 mg/kg/d div bid/$5500 (50 mg bid)
Trametinib	1–2 mg qd/$8400-$16 800	0.025 mg/k/d qd/$4200 (0.5 mg/d)
Cobimetinib	20–60 mg qd × 21 of 28-day cycle/$10 000-$30 000	1 mg/kg/d $10 000-$13 000 (20–25 mg/d)

BRAF/MEK inhibitors are substrates of P-glycoproteins, and their efflux by the blood-brain barrier leads to limited drug levels within the CNS.[47] Patients with unfavorable CNS responses to BRAF inhibitor monotherapy in *BRAF-V600E*–mutated ECD respond to combined BRAF/MEK inhibition.[48] In contrast to all approved MEK/BRAF inhibitors, Day 101 has greater CNS penetration.[39]

Optimal duration of MAPK inhibitors is unknown, and most patients (75%) will relapse upon treatment discontinuation,[30,38] but inhibitor reintroduction is usually effective. Lastly, although effective, MAPK inhibitors carry a high price tag. Data are lacking on their cost-effectiveness in patients with histiocytoses (Table 3).

In summary, MAPK inhibitors are quite effective in high-risk LCH patients (ie, those with RO$^+$ MS disease who are refractory or multiply relapsed and had failed more than 1 salvage chemotherapy regimen). Low-risk patients (multifocal bone or MS-RO$^-$) with R/R disease respond to chemotherapy or other agents (Figure 4) and do not require inhibitor therapy. This is due to the unknown optimal duration of these drugs and the risk of indefinite and unnecessary treatment for mild disease. In addition, these inhibitors are not indicated in SS-LCH that can resolve spontaneously, or in LR-LCH (MS-RO$^-$) or MS-LCH-RO$^+$ and rapid early responders, in whom OS rates are at 85% with conventional chemotherapy.[24]

Emerging therapies

Beyond MAPK pathway inhibitors

Alpelisib, a PI3K inhibitor, led to complete metabolic (PET) and clinical response in an adult patients with LCH who was harboring the *M1043V-PI3K* mutation.[49] A patient with refractory ECD carrying *CSF1R*$^{R549\text{-}E554delinsQ}$ had a sustained CR after treatment with pexidartinib, a *CSF1R* inhibitor[50]; clinical trials in LCH are warranted.

Combination therapy with PD-1/PD-L1 inhibitors

The inflammatory background of the LCH lesion could be another treatment target. Treating *BRAF-V600E*$^+$ mice with anti–PD-1 antibodies significantly reduced the disease burden, while MEK inhibitors combined with anti–PD-1 treatment were synergistic, causing a decrease in infiltrating myeloid/lymphoid cells and a restored function of CD8$^+$ T cells[51]; human trials are clearly needed.

Targeting senescent cells

In vitro inhibition of the *mTOR* pathway with rapamycin (sirolimus) reduced inflammatory cytokines and the differential potential toward mononuclear phagocytes in bone marrow *BRAF-V600E*$^+$ CD34$^+$cells. It also improved organomegaly and inflammatory infiltration of involved organs, but the apoptosis of *BRAF-V600E*$^+$ cells did not increase.[52] Sirolimus (+ prednisone) induced objective responses in adults with refractory ECD[53] and was effective as monotherapy in children with refractory Rosai-Dorfman disease (another non-LCH disorder).[54] Prospective trials of sirolimus in patients with LCH is worth consideration, particularly as an exit strategy after inhibitor discontinuation. Lastly, direct targeting at senescence by treating the LCH cells with ABT-263, a *BCL-XL* inhibitor, could increase their apoptosis and be another promising strtategy.[55]

Summary

Targeted therapy with MAPK inhibitors is particularly helpful for high-risk LCH that is refractory to treatment or progressive (and possibly fatal). With the current state of knowledge, this remains as the main indication for these drugs. The use of inhibitors in ND-LCH is promising but needs more validation in rigorous clinical trials. The risks associated with these inhibitors are the inability to discontinue treatment, high cost, and almost fully unknown potential for long-term side effects, especially in children. Prospective trials testing novel inhibitors are under way. International collaboration is required to harmonize LCH response criteria during the inhibitors era. Funding agencies and drug companies should support LCH research efforts to improve outcomes for patients, particularly in countries with limited resources.

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Conflict-of-interest disclosure

Oussama Abla: no competing financial interests to declare.

Off-label drug use

Oussama Abla: Dabrafenib, vemurafenib and trametinib are not FDA approved for LCH.

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Mastocytosis demystified

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Mastocytosis is a rare, clinically heterogenous clonal hematological neoplasm. Over 95% of patients harbor the driver *KIT* D816V mutation resulting in mast cell (MC) accumulation and proliferation in various organs, leading to variable symptom manifestations that result from MC mediator release in patients with systemic mastocytosis (SM) and end-organ damage in those with advanced SM. The accurate diagnostic and clinical classification of patients with SM is vital to underpin appropriate treatment options and personalize therapy. This review evaluates the current diagnostic criteria, clinical classification, risk stratification, and therapeutic options available for adult patients with nonadvanced and advanced SM.

LEARNING OBJECTIVES

- Review the diagnostic criteria and classification of mastocytosis
- Summarize the current treatment options for patients with SM
- Discuss the impact of potent TKIs in the current and future management landscape for patients with SM

CLINICAL CASE

A 45-year-old man with a 20-year history of biopsy-proven *KIT D816V* mutation-positive urticaria pigmentosa developed flushing and gastrointestinal symptoms. He subsequently had a life-threatening anaphylactic episode precipitated by aspirin, requiring intensive care. The following year he was treated for an acute gastrointestinal hemorrhage due to severe gastritis and multiple gastro-duodenal erosions. CT imaging showed enlarged retroperitoneal and mediastinal lymph nodes and hepatosplenomegaly. Serum tryptase was 525 µg/L and alkaline phosphatase 315 IU/L. A bone marrow biopsy revealed 50% round and spindle-shaped mast cells (MCs) with no evidence of associated myeloid neoplasm (AMN). Immunohistochemistry showed strong expression for MC tryptase, CD117, CD25, CD2, and CD30. G-banding chromosomal analysis was normal, and next-generation sequencing did not detect any additional myeloid mutations. His enlarging spleen with progressive lymphadenopathy and raised alkaline phosphatase (ALP) (>2.5×ULN) were considered "C-findings" clinically to classify him as having aggressive systemic mastocytosis, and he was started on midostaurin 100 mg twice daily with an initial good clinical response. After 18 months, serum tryptase plateaued at approximately 250 µg/L with no change in the MC burden in the bone marrow. He was enrolled in the PATHFINDER study and received avapritinib 200 mg daily. There was resolution of palpable hepatomegaly and splenomegaly, and the serum tryptase had normalized to 11 µg/L within 2 months. Repeat bone marrow assessment showed no clonal MCs with normalization of all C findings and blood counts, indicating he had achieved a complete remission. Avapritinib was reduced to 100 mg daily and at his 2.5-year follow-up, he remains in a complete remission.

This case illustrates a patient who would have been diagnosed with MC in the skin and then confirmed as smoldering systemic mastocytosis (SSM) when he had a bone marrow biopsy with high MC disease burden (MC disease burden 50% and tryptase >200 µg/L) and mild organomegaly when he was referred and developed significant mediator-related symptoms. He progressed to aggressive systemic mastocytosis (SM) (organomegaly and evidence of end-organ damage) 2 decades after his initial presentation to the dermatologist and responded to first- and second-line tyrosine kinase inhibitor (TKI) therapy. Most patients with indolent systemic mastocytosis, however, do not progress to advanced disease, and those patients with high disease burden such as those with smoldering mastocytosis should be monitored closely.

Figure 1 montage summarizes the case with a descriptive legend.

Introduction

Mastocytosis is a rare clonal MC disease, driven by a somatic mutation in the *KIT* gene (D816V) in more than 90% of adults with the disease, resulting in the expansion and accumulation of neoplastic MCs in various tissues, including skin, bone marrow, gastrointestinal tract, liver, and/or spleen.[1,2] The symptoms that can affect mastocytosis patients[3] are shown in Figure 2.

Each patient is unique in how mastocytosis affects them. This can present diagnostic challenges as patients may initially present to several clinicians before a formal diagnosis is made.[4]

Mastocytosis is classified into 3 subtypes: cutaneous mastocytosis (CM), SM, and MC sarcoma (MCS). CM is characterized by atypical MCs limited to the skin only and is more common in children than adults. SM is characterized by the infiltration of clonal MCs in the bone marrow and other extracutaneous organs. Diagnosis of SM is usually confirmed by a bone marrow biopsy. There are 2 subcategories of SM: nonadvanced SM and advanced SM (AdvSM).[1,2,5] Up to 80% of SM patients have indolent disease with a variable symptom burden but a normal life expectancy. Patients with AdvSM have a poor prognosis as a result of MC infiltration leading to end-organ damage with overall survival (OS) ranging from 2 to 6 months for patients with MC leukemia (MCL) and 2 to 4 years for patients with SM and an associated myeloid neoplasm (SM-AMN).[6,7]

Classification

The World Health Organization (WHO) 2022 classification of myeloid neoplasms[2] recognizes 6 SM subtypes: bone marrow mastocytosis (BMM), indolent SM (ISM), smoldering SM (SSM), aggressive SM (ASM), MC leukemia (MCL), and SM with an associated hematologic neoplasm (SM-AHN). BMM, ISM, and SMM are regarded as nonadvanced while ASM, MCL, and SM-AHN are regarded as advanced SM. BMM was added as a new SM subtype characterized by the absence of skin lesions and B-findings and a serum tryptase below 125 µg/L, whereas the ICC[1] regards it as a subtype of ISM.

The International Consensus Classification 2022 (ICC) classifies ISM and SSM in the nonadvanced SM category, reflecting that there is no end-organ damage in normal hematological and biochemical parameters and no significant organomegaly.

ICC has modified the subtype of SM-AHN to SM with an associated myeloid neoplasm (SM-AMN) to reflect that the associated neoplasm typically shares a *KIT* mutation and/or other clonal genetic abnormalities is typically myeloid. To diagnosis SM-AMN, the patient must meet the diagnostic criteria for SM and for an associated myeloid neoplasm (e.g. chronic myelomonocytic leukaemia or other myelodysplastic/myeloproliferative neoplasms, myelodysplasia, myeloproliferative neoplasms, acute myeloid leukaemia, or other myeloid neoplasm), and the associated myeloid neoplasm should be fully classified according to established criteria (Table 1).

B-findings reflect high MC disease burden, and C-findings indicate organ damage caused by MC infiltration and are used to distinguish between ISM, SMM, and ASM. Some minor refinements have been made in the ICC, namely the presence of cytopenias not meeting the criteria for C-findings. In the WHO classification, *KIT* D816V mutation with a variant allele frequency (VAF) >10% now qualifies as a B-finding. Highly sensitive polymerase chain reaction (PCR) assays, eg, digital droplet PCR or allele-specific PCR, should be used to detect and quantify the *KIT* VAF. Next-generation sequencing to identify the presence of high-risk mutations *SRSF2*, *ASXL1*, and *RUNX1* (*S/A/R* panel) enables prognostic risk stratification and presence of myeloid mutations, eg, *TET2*, *JAK2*, *DNMT3A*, *NRAS,CBL*, and *EZHZ*, which may be detected in patients who have an AHN/AMN.

The current 2 histological classifications, WHO and ICC, are not aligned and therefore can cause confusion.

Diagnostic criteria

The ICC and WHO have introduced some refinements to the diagnostic criteria for SM (Table 2). Demonstration of tryptase and *KIT* (CD117) positivity by immunohistochemistry have been added to enable proper identification of MCs in stained sections. The presence of CD30 and of any activating *KIT* mutation have been added as minor diagnostic criteria. An adjustment method for tryptase levels has been proposed for patients proven to have hereditary α tryptasemia.

Risk stratification

Schwab et al. demonstrated that 89% of patients with AdvSM harbored additional somatic aberrations, the most commonly affected genes being *TET2*, *SRSF2*, *ASXL1*, *RUNX1*, and *CBL*.8 Mutated genes in the *S/A/R* panel are associated with inferior OS in SM patients and are considered high-risk mutations. Jawar et al. analyzed 70 SM patients and demonstrated a clear difference in 3-year OS between patients with 0 mutations (90% OS), 1 mutation (73% OS), and ≥2 mutations (42% OS) in the *S/A/R* gene panel.[9,10] Patients with additional somatic mutations detected were in the SM-AMN group, and associated myeloid neoplasm was noted.

Prognostic scoring systems integrating clinical and molecular characteristics in patients with SM have evolved: the International Prognostic Scoring System for Mastocytosis,[7] the Mayo Alliance Prognostic System,[11] the Mutation-Adjusted Risk Score,[12] and the Global Prognostic Score for Mastocytosis.[13] The validated Mutation-Adjusted Risk Score scoring model for patients with AdvSM (*n*=383) identified from multivariate analysis that inferior OS was associated with age >60 years, anemia (Hb <100 g/L), thrombocytopenia (platelet count <100×10^9/L), and the presence of 1 or ≥2 high-risk mutations (*S/A/R* panel). Low-risk, intermediate-risk, and high-risk groups were devised based on these parameters, with respective OS times not reached, 4.3 years and 1.9 years.

Well-differentiated systemic mastocytosis

In the rare (<5%) separate morphological variant of SM known as well-differentiated systemic mastocytosis, MCs are usually round, hypergranulated, and lacking CD25 and CD2 expression but frequently expressing CD30. Mutations in extracellular, juxtamembrane, and transmembrane domains in exons 8 through 10 may be seen, eg, *K509I* and *F522C*, which are imatinib sensitive, while *KIT* is usually wild type with *KIT* D816V or exon 17 mutations not detected.[14]

Approach to diagnosis

Diagnosis requires a multidisciplinary, integrated approach. Ideally, patients should be referred to a specialist center. Patients with a suspected diagnosis of SM need a comprehensive symptom review and clinical examination. Most adult patients will have CM. A minority of patients present with

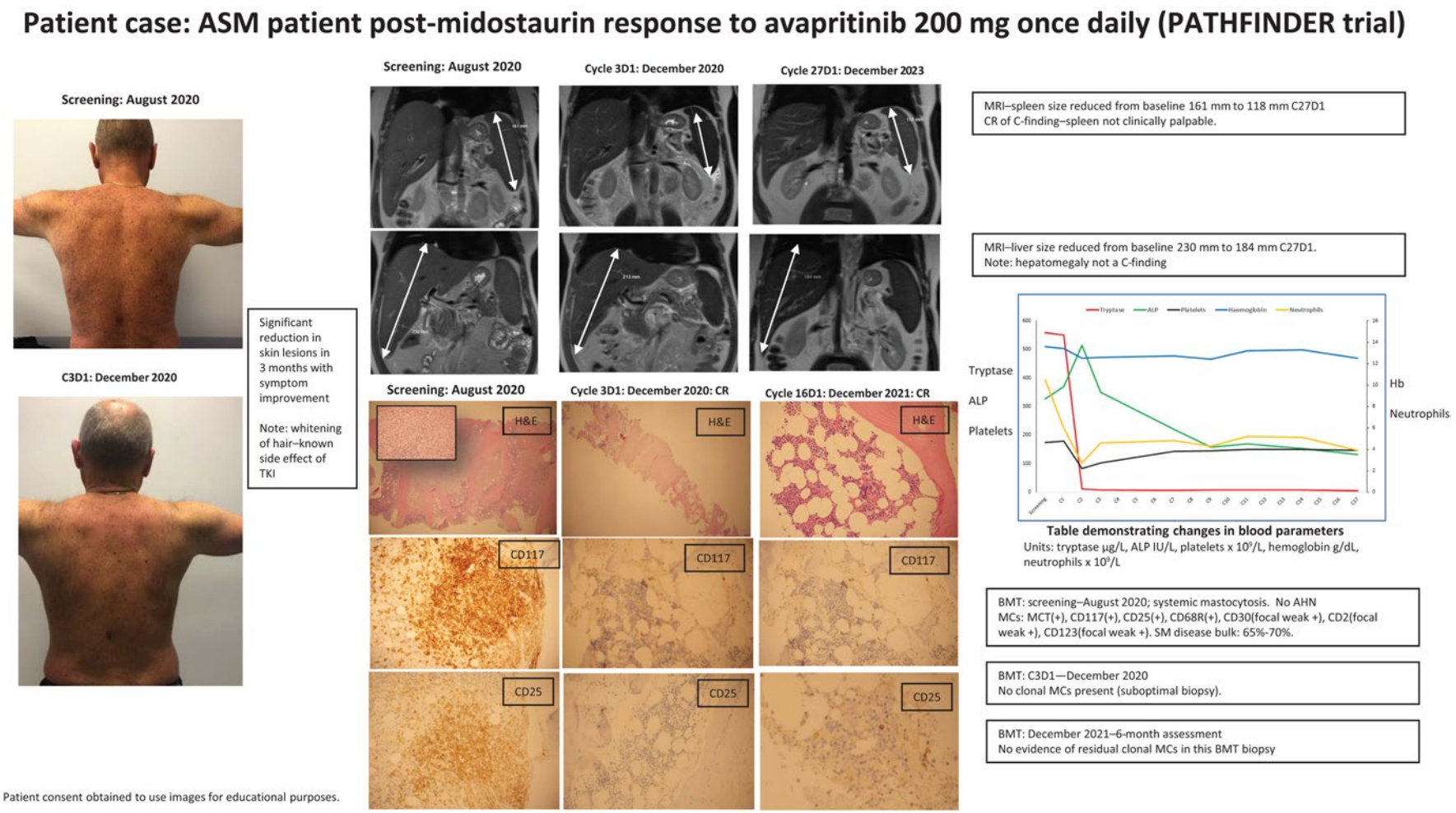

Figure 1. Clinical case summary of ASM patient's response to second-line TKI treatment. In patients with pure MCL and ASM, treatment with midostaurin can result in a PR as our case illustrates. This patient had a diagnosis of c-KIT–positive ASM with AMN nor any additional myeloid mutations. He was initially treated with midostaurin 100mg twice a day. His symptoms and MC disease burden improved, as reflected by reduced spleen size, improvement of skin rash, and decreased tryptase levels. After 18 months his symptoms recurred, including fatigue, sweats, worsening rash, and increased splenomegaly and tryptase levels. He had lost his response to midostaurin. He subsequently was enrolled in the PATHFINDER trial and received avapritinib 200mg once daily initially. As seen in Figure 1, he had an excellent response, achieving a CR within 3 months of treatment, which has been maintained. He experienced the expected side effects of mild periorbital edema, whitening of his hair, and an "ALP flare," which is seen with initiation of TKI treatment and normalizes in 4 to 6 weeks. As reported in PATHFINDER, patients with pure ASM achieve a deep, fast response despite previous exposure to another TKI—midostaurin, in his case. Should he have an allogenic bone marrow transplant? The current view is that because he has no AMN and only harbors the c-KIT mutation, he should continue on a reduced dose of avapritinib 100 mg once daily. It will be interesting to see if this dose could be reduced or interrupted to see if he maintains a CR in the future. BMT, bone marrow trephine; H&E, hematoxylin and eosin stain; MRI, magnetic resonance imaging.

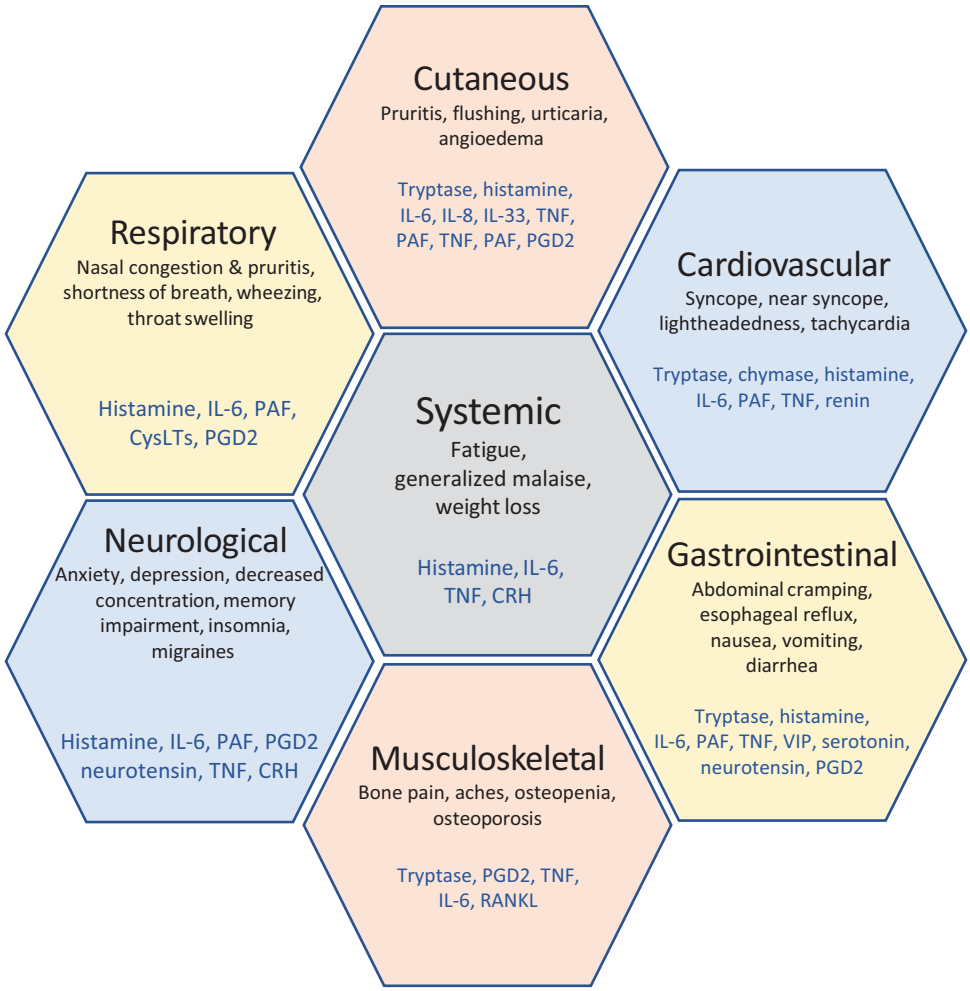

Figure 2. MC mediator release with biological and clinical consequences. CRH, corticotropin-releasing hormone; CysLTs, cysteinyl leukotrienes; IL-6/8/33, interleukin 6/8/33; PAF, platelet-activating factor; PGD2, prostaglandin D2; RANKL, receptor activator of nuclear factor kβ ligand; TNF, tumor necrosis factor; VIP, vasoactive intestinal peptide. Adapted from Theoharides et al.[3]

osteoporosis, idiopathic anaphylaxis, or significant mediator symptoms lacking skin lesions and may have BMM. The presence of lymphadenopathy/organomegaly points to a possible diagnosis of AdvSM. Patients with idiopathic anaphylaxis and BMM may have tryptase levels lower than 20 µg/L, and applying the REMA score or the European Competence Network on Mastocytosis/Fuchs score may be helpful when considering whether a bone marrow biopsy is warranted.[15]

Baseline blood tests include a full blood count with differential as the latter may subtly indicate the presence of an AMN, eg, monocytosis/eosinophilia. A blood film is informative in AdvSM cases; morphological signs of dysplasia, atypical monocytes suggestive of a myelodysplastic or myelomonocytic component, leukoerythroblastic blood film, leukocytosis, or thrombocytosis may point to a myeloproliferative disorder or myeloid leukemia. Circulating MCs confer a diagnosis of MCL; in acute MCL these are medium/large atypical blastic MCs with coarse metachromatic granules with pleomorphic nuclei and nucleoli. In chronic MCL they are round, hypergranular forms with inconspicuous nuclei. Serum tryptase, liver, and renal profiles and lactate dehydrogenase are useful. Bone marrow biopsy can confirm SM diagnosis. In clinical practice it is vital to review any histology looking for an occult associated myeloid-neoplasm, if somatic mutations in addition to *KIT* D816V are detected. The reverse applies when patients with the most commonly associated myeloid neoplasm, CMML, also have a *KIT* D816V mutation detected, then clinician should look for clonal mast cells.

A DEXA scan is required in all adult patients at diagnosis and on follow-up for assessment of T scores to evaluate for osteoporosis or osteopenia. Generally patients with ISM osteoporosis need treating while osteosclerosis is an expected finding in patients with AdvSM. The need for additional radiological investigation, such as skeletal survey for lytic lesions or magnetic resonance imaging for sclerotic lesions, should be guided by clinical presentation.

Treatment options

Nonadvanced SM

The mainstay of nonadvanced SM management in most patients is symptom control with a combination of antimediator medications[16] (Figure 3). Identified triggers exacerbating symptoms

Table 1. ICC and WHO 2022 classification for the diagnosis of mastocytosis

	WHO	ICC
	CM Urticaria pigmentosa/maculopapular cutaneous mastocytosis • Monomorphic variant • Polymorphic variant Diffuse cutaneous mastocytosis Cutaneous mastocytoma • Isolated mastocytoma • Multilocalized mastocytoma	**CM** Urticaria pigmentosa/maculopapular cutaneous mastocytosis Diffuse cutaneous mastocytosis Mastocytoma of skin
	SM BMM* ISM SSM ASM SM-AHN MCL **MC sarcoma**	**SM** ISM (includes BMM) SSM ASM SM-AMN MCL **MC sarcoma**
B-findings	>30% BM cellularity by MCs in histology **and** serum tryptase >200 ng/ml **and/or** KIT D816V VAF >10% in BM or PB leukocytes	>30% of BM cellularity by MC aggregates (assessed on BM biopsy) **and** serum tryptase >200 ng/mL
	Hypercellular BM with loss of fat cells and prominent myelopoiesis±left shift and eosinophilia±leukocytosis and eosinophilia and/or discrete signs of myelodysplasia (<10% neutrophils, erythrocytes, and megakaryocytes)	Cytopenia (not meeting criteria for C-findings) or cytosis. Reactive causes are excluded, and criteria for other myeloid neoplasms are not met.
	Palpable hepatomegaly without ascites or other signs of organ damage **and/or** palpable splenomegaly without hypersplenism and without weight loss **and/or** lymphadenopathy (>2 cm)	Hepatomegaly without impairment of liver function **and/or** splenomegaly without features of hypersplenism including thrombocytopenia **and/or** lymphadenopathy (>1 cm size) on palpation or imaging
	BMM: no B-findings, absence of skin lesions a basal serum tryptase <125 mg ***ISM <2 B-findings*** ***SSM ≥2 B-findings***	***ISM <2 B-findings*** ***SSM ≥2 B-findings***
C-findings	Cytopenias: ANC <1×10⁹/L and/or Hb <100 g/L and/or platelets <100×10⁹/L	
	Hepatopathy: ascites and elevated liver enzymes±hepatomegaly or cirrhotic liver±portal hypertension	
	Spleen: palpable splenomegaly with hypersplenism±weight loss±hypoalbuminemia	
	GI tract: malabsorption with hypoalbuminemia±weight loss	
	Bone: large-sized osteolysis (≥2 cm) with pathologic fracture±bone pain **ASM 1 or more C-findings/SM-AHM/AMN and MCL may not have C-findings**	

BM, bone marrow; PB, peripheral blood.

should be avoided. Antihistamine medications (both anti H1 and anti H2)[17,18] in combination with MC-stabilizing agents are effective, the latter with gastrointestinal symptoms.[19,20] A short course of corticosteroids helps minimize "flares" of severe symptoms. In a small cohort of patients, leukotriene receptor inhibitors (Montelukast) or cyclo-oxygenase inhibitors (Aspirin) may be of use.[21,22] Neuropathic symptoms may be managed with tricyclic antidepressants and anticonvulsants (eg, gabapentin). Medications will need adjustment due to fluctuating patient symptoms, with higher doses required than recommended in national formularies. Immune tolerance therapy is recommended for patients with BMM or ISM presenting with anaphylaxis due to bee/wasp venom allergies.[23,24] Omalizumab has shown benefit in this subset of patients.[25-27] Some patients experience significant symptoms despite combinations of high doses of antimediator treatments and have a poor quality of life (QoL). These refractory patients should be considered for cytoreductive therapy with cladribine (2CdA)[28,29] or targeted TKI agents if available. Avapritinib, elenastinib (BLU-263/HARBOR), masitinib, and bezuclastinib (CGT9486/SUMMIT) are currently being evaluated within trials.

In May 2023 avapritinib was approved by the Food and Drug Administration for the treatment of symptomatic ISM patients upon meeting the primary end points in the PIONEER trial (Blueprint Medicines Corporation; ClinicalTrials.gov number NCT03731260).[30] This double-blind, placebo-controlled, phase 2 trial randomized patients with moderate to severe ISM (total symptom score [TSS] ≥28) 2:1 to avapritinib 25 mg once daily (n=141) or placebo (n=71), both with BSC. The primary end point was mean change in TSS (range 0-110) based on the 14-day average of patient-reported severity of 11 symptoms. Secondary end points included ≥50% and ≥30% reduction in TSS; ≥50% reductions in serum tryptase, blood *KIT* D816V VAF, and bone marrow MCs; and QoL measures. Primary and key secondary end points were assessed from baseline to week 24. Avapritinib significantly improved TSS (mean change −15.6 vs −9.2; P=0.003) and the likelihood of achieving a ≥50% TSS (25% vs 10%; P=0.005) and ≥30%

Table 2. ICC and WHO 22 diagnostic criteria for the diagnosis of SM

ICC **Presence of the major criterion is sufficient for diagnosis. In the absence of the major criterion, at least 3 of the 4 minor criteria must be present.**
Major criterion Multifocal dense infiltrates of tryptase- and/or CD117-positive MCs (≥15 MCs in aggregates) detected in sections of BM and/or other extracutaneous organ(s)
Minor criteria a. In BM biopsy or in section of other extracutaneous organs >25% of MCs are spindle-shaped or have an atypical immature morphology b. MCs in BM, PB, or other extracutaneous organs express CD25, CD2, and/or CD30, in addition to MC markers c. *KIT* D816V mutation or other activating *KIT* mutation detected in BM, PB, or other extracutaneous organs d. Elevated serum tryptase level, persistently >20 ng/mL In cases of SM-AMN, an elevated tryptase does not count as an SM minor criterion.
WHO **Requires at least 1 major criterion and 1 minor or 3 minor criteria.**
Major criterion Multifocal dense infiltrates of MCs (≥15 MCs in aggregates) in BM biopsies and/or in sections of other extracutaneous organ(s)
Minor criteria a. >25% of all MCs are atypical cells (type I or type II) on BM smears or are spindle-shaped in MC infiltrates detected on sections of visceral organs b. KIT point mutation at codon 816 in the BM or another extracutaneous organ c. MCs in BM, blood, or another extracutaneous organ exhibit CD2 and/or CD25 d. Baseline serum tryptase level >20 ng/mL In case of an unrelated myeloid neoplasm, item d is not valid as an SM criterion.

BM, bone marrow; PB, peripheral blood.

TSS reduction (45% vs 30%; *P*=0.009) compared with placebo. A greater proportion of avapritinib-treated patients achieved ≥50% reductions in serum tryptase, *KIT* D816V VAF, and bone marrow MC burden (all *P*<0.0001). QoL scores showed up to 4.1-fold greater improvement with avapritinib versus placebo. Safety profiles were similar between treatment groups with few discontinuations due to adverse events (AEs). Avapritinib 25 mg once daily appeared to be well tolerated by most patients.

Advanced systemic mastocytosis

Over the last decade, TKIs have moved to the forefront of treatment in the limited licensed therapeutic options available for AdvSM patients. Patients usually presenting with end-organ damage due to MC infiltration should be considered for cytoreductive or targeted treatment with TKIs (Figure 4).

Treatment options

TKIs

Imatinib. Twose et al. reported on the efficacy of imatinib in *KIT* D816V–negative well-differentiated systemic mastocytosis patients treated with 300 or 400 mg daily for 12 months[31,32] with an overall response rate (ORR) of 50%. Imatinib is also effective in patients with SM and a coexisting chronic eosinophilic leukemia associated with the PDGFR-α/β rearrangements. The lack of activity against the common *KIT* D816V mutation excludes it as a treatment in most SM patients. Imatinib was approved in 2006 for the treatment of adult ASM patients with wild-type KIT or unknown mutational status.

Midostaurin has in vitro activity against kinase domain *KIT* D816V and D816Y mutations and FMS-related tyrosine kinase 3, PDGRF α/β, and vascular endothelial growth receptor 2 mutations. Midostaurin gained approval in 2016 following results from a multicenter international phase 2, single-arm study of 116 patients demonstrating a favorable safety and efficacy profile.[33] Patients received midostaurin 100 mg twice daily until disease progression or unacceptable toxicity. Results from 89 patients in the primary efficacy population of the study (16 ASM; 16 MCL; 57 SM-AHN) with a median follow-up of 26 months (range 12-54 months) demonstrated an ORR of 60% (45% major response and 15% partial response) as per the modified Valent and modified Cheson criteria. The median duration of response was 24.1 months, median OS 28.7 months, and median progression-free survival (PFS) 14.1 months. Response rates reported were ASM, 75%; SM-AHN, 58%; and MCL 50%, regardless of prior therapy, AHN status, or *KIT* D816V status. Significant decreases were seen in tryptase levels and bone marrow MC burden in >50% and spleen size in 77% of patients with improvements in C-findings. Objective improvements in disease-related symptoms and skin lesions were noted. Eighty-two percent of patients reported mild gastrointestinal AEs at all grades, with 6% to 8% experiencing grade 3-4 symptoms. Nausea and vomiting (related to the capsule) were the main adverse symptoms, and most patients tolerated midostaurin with adjunctive antiemetic medication (ondansetron + domperidone). Myelosuppression seen in patients with cytopenias (neutropenia [24%], anemia [41%], and thrombocytopenia [29%]) was managed with dose reduction and growth factor support.

Additional analysis of 38 patients treated with midostaurin (in the global trial or the compassionate-use program) reported poorer outcomes and survival of patients who harbored additional high-risk mutations (*S/A/R*) and did not achieve a >25% reduction in their *KIT* VAF.[34] The development of additional mutations and an increase in the VAF of non–*KIT* D816V mutations (*K/N -RAS*, *RUNXI*, *IDH2*, and *NPM1*) was seen in these patients with disease. No acquired resistance mutations were noted on midostaurin treatment.

Avapritinib is a potent and selective oral type 1 multikinase inhibitor with activity against *KIT* D816V, targeting active kinase formation and preventing it from binding to its substrates. Avapritinib was approved in patients with AdvSM

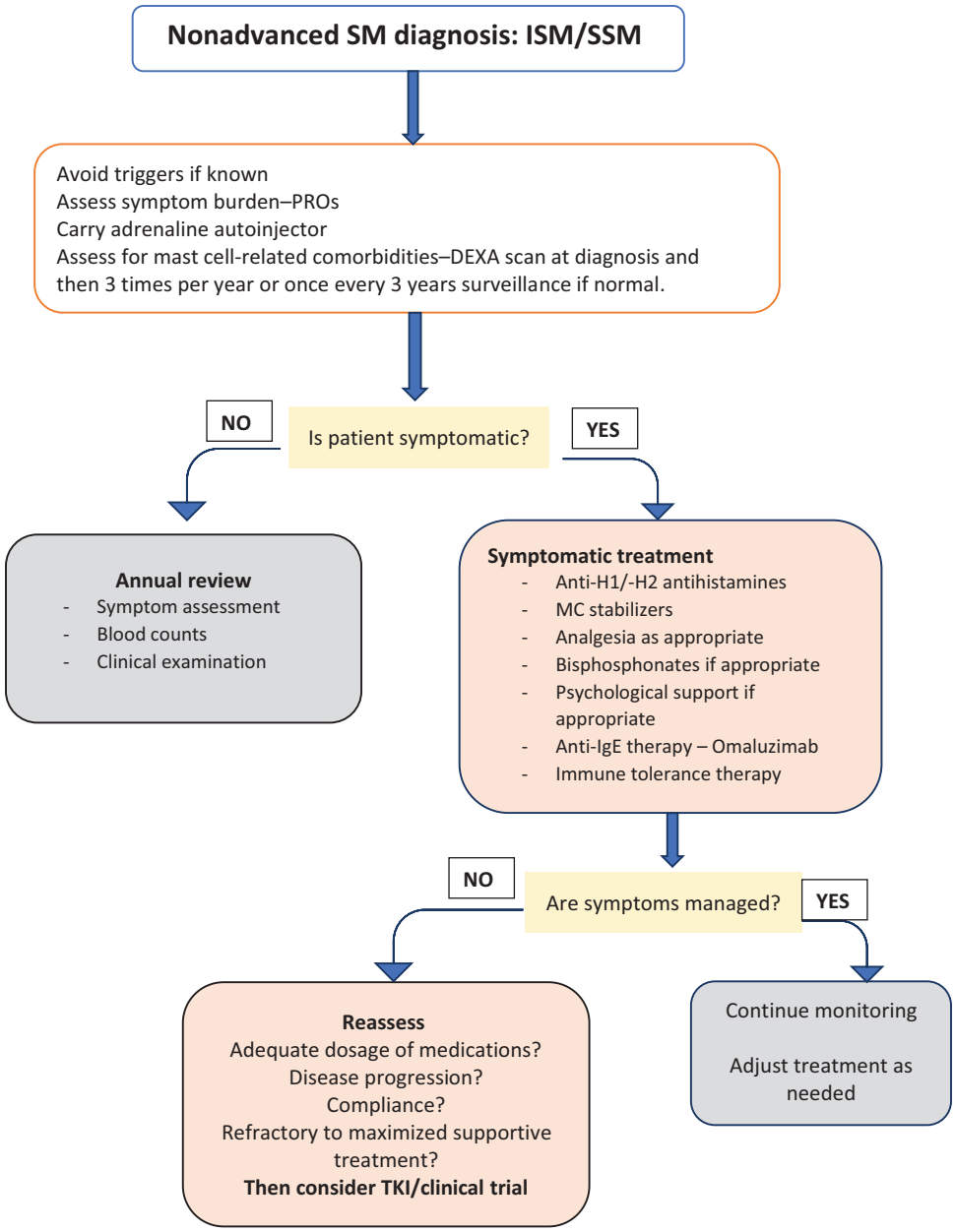

Figure 3. Management of nonadvanced SM. PROs, patient-reported outcomes. Adapted from Gerds AT, Gotlib J, Ali H, et al. Systemic mastocytosis, version 1.2022, https://www.nccn.org/guidelines. Clinical Practice in Oncology (NCCN Guidelines): National Comprehensive Cancer Network; 2022.

with a platelet count >50×109/L by the Food and Drug Administration in June 2021 and European Medicines Agency in March 2022 following data reported from the registrational PATHFINDER study. This single-arm, phase 2 study demonstrated the efficacy and safety of avapritinib with a starting dose of 200 mg once daily in patients with AdvSM, excluding patients with SM-AML and high-risk MDS.[35] A prespecified interim analysis was carried out in 32 response-evaluable patients within the PATHFINDER study using modified IWG-MRT-ECNM criteria. The median follow-up was 10.4 months with a confirmed ORR (complete remission [CR], CR with partial hematologic recovery [CRh], partial remission [PR], and CR with incomplete count recovery [CI]) of 75% (n=24). A modified response criteria CRh was developed for patients who achieved a complete pathological response to the SM component (CR demonstrated in the bone marrow with loss of MC aggregates, normalization of tryptase, and spleen size) but had partial hematological recovery (hemoglobin of >80 but <100 g/L, platelet counts between 50-100×10^9/L, and neutrophil count between 0.5-1.0×10^9/L). The myelosuppressive effect of avapritinib and/or the presence of a concomitant AMN may be responsible for the partial hematological recovery. A CRh was reported in 6 patients (19%), PR in 10 patients (31%), and CI in 8 patients (25%). Responses were

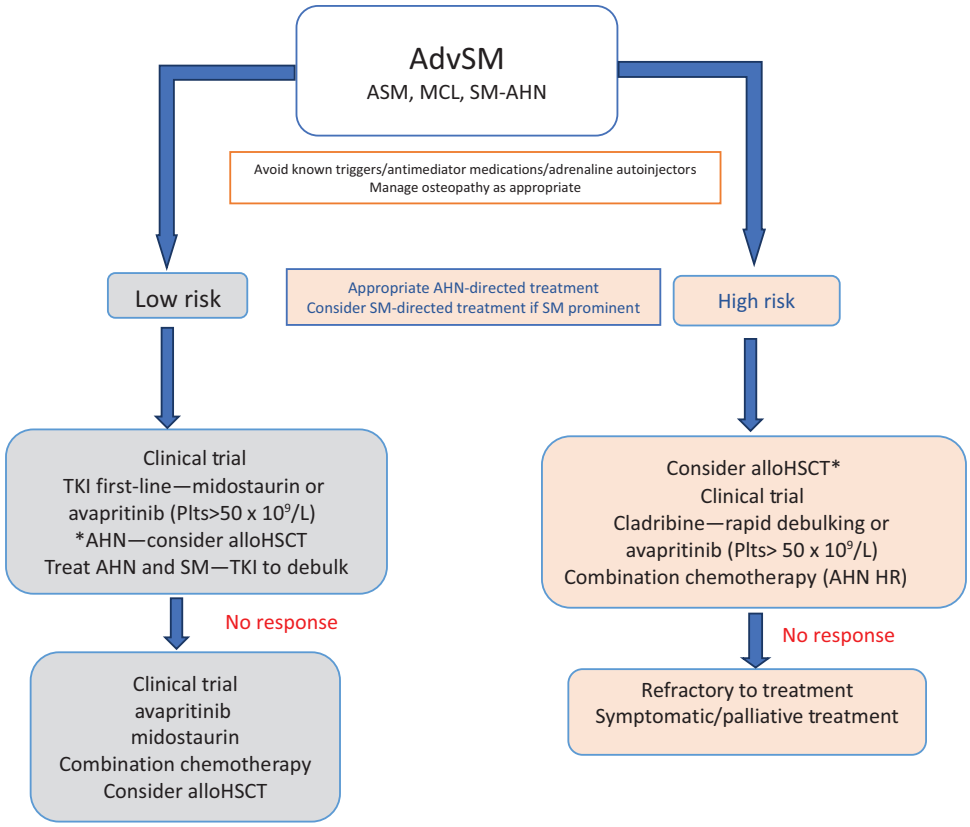

Figure 4. Management options for AdvSM. HR, high risk; Plts, platelets. *****If AHN is the major component contributing to patient symptoms. Reproduced with permission from Radia DH, Moonim MT. Update on diagnostic approaches and therapeutic strategies in systemic mastocytosis. *Best Pract Res Clin Haematol*. 2022;35(2):101380. doi:10.1016/j.beha.2022.101380.

seen regardless of prior therapy and presence of mutations in the *S/A/R* panel. Significant reductions in tryptase, bone marrow MC burden, and *KIT* D816V VAF of at least 50% from the baseline were seen in 93%, 88% and 60% of the patients, respectively. The most frequently reported AEs were peripheral and periorbital oedema (50% and 48%), mainly at grade 1-2 and managed with diuretics and dose reductions. Grade 3 neutropenia, thrombocytopenia, and anemia was seen in 24%, 16%, and 16% of patients, respectively. Gastrointestinal AEs were predominately grade 1-2 with diarrhea (23%), nausea (18%), and vomiting (18%). Cognitive impairment presenting as mild memory impairment was reported in 6 patients (grade 1-2). One patient had a subdural hematoma prior to protocol amendment, which excluded patients with a platelet count of $<50\times10^9$/L. No treatment-related deaths occurred.

Most patients' C-findings improved from baseline. At the time of data cutoff, the median PFS and median OS in the safety population (n=62) had not been reached. The estimated 12-month PFS and OS rates were 79% and 86%, respectively with 52 patients (84%) still on treatment, with a median follow-up of 7 months (range 5.6-8.1 months).

In a subanalysis from the phase 1 EXPLORER study, which looked at the mutational landscape in 69 patients, with a median follow-up of 23 months, 20% (14 patients) progressed on treatment, with progression driven by *KIT* D816V–negative AMN clones in most cases.[36,37] In these patients the *KIT* D816V VAF remained low, suggesting that treatment directed to the AMN component was appropriate.

There are no head-to-head comparisons in trials of avapritinib to midostaurin or cladribine. A global multicenter retrospective review of patient charts was carried out in 6 study sites in the US, Europe, and the UK collecting data from AdvSM patients who received best available therapy (BAT) in routine clinical practice using inclusion and exclusion criteria similar to those for the EXPLORER and PATHFINDER trials.[38] The study population included 176 avapritinib patients and 141 BAT patients, contributing to 222 lines of treatment. Patients treated with BAT contributed data on multiple lines of therapy, and these data were compared with pooled patient data from both trials. In the BAT arm, 50% of the patients had been exposed to midostaurin and 25% to cladribine. Results showed longer treatment (avapritinib 30.6 months vs BAT 5.5 months), a greater reduction in tryptase level (avapritinib 86.6% vs BAT 9.2%), and significantly longer OS (inverse probability treatment weighting–adjusted median OS was avapritinib 49 months vs BAT 26.8 months). This real-world study showed the improved efficacy of avapritinib compared with other available therapies for AdvSM used in clinical practice.

Bezuclastinib is an oral highly selective TKI with potent activity against *KIT* D816V. It has demonstrated preliminary clinical activity and a tolerable safety profile in a phase 1/2 study of patients with advanced solid tumors, including gastrointestinal

stromal tumor. A reduction in *KIT* exon 17 mutational burden was noted in patients treated with bezuclastinib.[38] Bezuclastinib has been designed to avoid activity against other related kinases, eg, PDGFRα, PDGFRβ, wild-type *KIT*, VEGFR2 (KDR), and CSF1R (FMS), in order to avoid related AEs. APEX, a multicenter, phase 2, open-label, 2-part clinical study to evaluate the safety, efficacy, pharmacokinetics, and pharmacodynamics of bezuclastinib in subjects with AdvSM, is currently recruiting patients, with promising preliminary data.[39]

Cytoreductive therapy

Cladribine is a cytoreductive agent delivered at a dose of 0.14 mg/kg intravenous or subcutaneous infusions over 5 days, repeated every 4 to 12 weeks. Barete et al. reported 32 patients with AdvSM treated with cladribine; the median number of courses was 3.7 (range 1 to 9) with a 50% ORR (major remission/PR) and median duration of response of 2.47 years (range 0.5-8.6 years) in this cohort. Myelosuppression led to the most common grade 3/4 serious AEs due to lymphopenia (82%), neutropenia (47%), and opportunistic infections (13%).[29] In current clinical practice cladribine is considered in patients with rapidly progressive AdvSM where fast debulking of disease is required to stabilize the patient and potentially introduce targeted TKI treatment, or in those who cannot be treated with TKI.[28,29]

SM-AHN

The current treatment algorithm for patients with SM-AMN directs treatment to each of the 2 components as if the other were not present. Because most studies suggest that AMN progression is likely, the AMN component is treated invariably first. In some patients with ISM-AHN (eg, ISM- CMML1), an option may be to monitor until one or the other component progresses. It is not always easy to ascertain which of the 2 neoplasms is contributing to a patient's clinical symptoms and organopathy. The patient's mediator symptom profile with diagnostic tests (FBC, ALP, bone marrow biopsy, *KIT* VAF, and next-generation sequencing for myeloid mutations) will provide an overall picture to inform management decisions (Figure 5).

Allogeneic hematopoietic stem cell transplantation

Ustin et al. reported on retrospective outcome data in 201440 from a large multicenter cohort of 57 patients with SM who had an allogeneic bone marrow transplant (38 SM-AHN, 7 ASM, and 12 MCL). The response rate was 70%, with a 16% CR. Of the 30% of patients who did not have a response, 21% had stable disease and 9% had primary refractory disease. Although CR was achieved in all 38 patients with SM-AHN within the AHN component, 10 patients progressed with a relapse of the AHN, and 50% of these patients did not survive. Median 3-year OS for the cohort was 57% (74% SM-AHN, 43% ASM, and 17% MCL). Treatment-related mortality at 6 months and 1 year was 11% and 20%, respectively, and was highest for MCL patients. A diagnosis of MCL and use of reduced-intensity allogeneic transplant was associated with poor OS. Parameters affecting OS or PFS were patient and donor age, donor type, graft source, *KIT* mutation status, karyotype, and conditioning with or without total-body irradiation. The challenge with allogeneic hematopoietic stem cell transplant (alloHSCT) is for patients to have minimal disease, ideally CR in both SM and AMN components to optimize outcomes. The new potent TKIs, eg, avapritinib, that may result in SM CR/CRh moves alloHSCT up the treatment algorithm for eligible patients.[40-42]

Conclusion

Continued education is necessary for health care professionals and patients to recognize the heterogenous presentation of patients with mastocytosis and therefore reduce the time to a formal diagnosis of systemic mastocytosis. Unfortunately, the disharmony of complex diagnostic and classification systems leads to confusion in classifying patients. Health care professionals

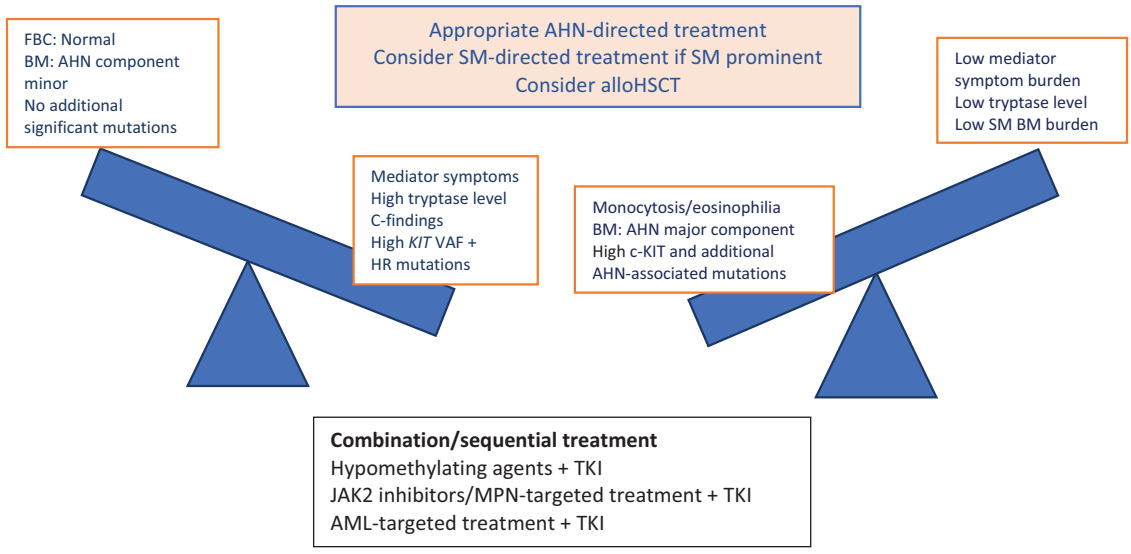

Figure 5. Considerations in the treatment of patients with SM-AHN. BM, bone marrow; FBC, full blood count; HR, high risk; MPN, myeloproliferative neoplasms. Reproduced with permission from Radia DH, Moonim MT. Update on diagnostic approaches and therapeutic strategies in systemic mastocytosis. *Best Pract Res Clin Haematol*. 2022;35(2):101380. doi:10.1016/j.beha.2022.101380.

must work together internationally to ensure that this is not detrimental to patient care and outcomes.

TKIs have heralded a new era in the management of patients with SM. Midostaurin paved the way in demonstrating disease modification that leads to MR/PR with improvement in QoL. Avapritinib has demonstrated improved QoL with a reduction in symptom burdens in patients with debilitating ISM and significant improvement in OS and QoL in AdvSM patients. This enables eligible patients to be considered for a curative alloHSCT, although several questions need to be addressed to optimize outcomes—eg, timing of transplant, when to stop TKI, and monitoring of SM components pre- and posttransplant. The exciting next step is investigating the impact of sequential or combined treatment for patients with SM-AMN through international collaborative trials. Further research to evaluate new diagnostic markers and therapeutic strategies (eg, CD30, PD L1) is ongoing. The European Competence Network on Mastocytosis and the American Initiative in Mast Cell Diseases have proposed a new set of response criteria which include pathologic, molecular, cytogenetic, clinical, and symptom/QoL response within a 4-tiered schema.[43] Validation of better clinical scoring systems for both prognosis and response assessments are exciting avenues to continue improving outcomes for mastocytosis patients.

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Conflict-of-interest disclosure

Scott Veitch: no competing financial interests to declare.
Deepti H. Radia: Clinical advisory board/study steering group member (EXPLORER, PATHFINDER, AZURE); research support and educational events/Speaker for Blueprint Medicines Corporation. Consultant and Study Steering Group member (APEX) Cogent Biosciences educational events/speaker: Novartis. Royalties: Fast Facts Systemic Mastocytosis coauthor, Karger.

Off-label drug use

Scott Veitch: Nothing to disclose.
Deepti H. Radia: Nothing to disclose.

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Amyloid consults do not have to be vexing

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Diagnosing amyloidosis can be challenging due to its clinical heterogeneity, need for multiple specialists to make a diagnosis, and lack of a single diagnostic test for the disease. Patients are often diagnosed late, in advanced stage, and after exhibiting multiple symptoms and signs for a long period. It is important to develop a clinical suspicion of amyloidosis, particularly in those with multisystemic symptoms and high-risk patient populations such as those with precursor hematologic conditions. A systematic approach to the workup of suspected amyloidosis is key, including a comprehensive clinical assessment, laboratory tests to assess organ involvement, advanced imaging studies, screening for plasma cell disorder, and tissue biopsy when necessary. After making a diagnosis of amyloidosis, accurate typing of amyloid deposits, differentiating between localized and systemic amyloidosis, and appropriately staging the disease is important. Early diagnosis is crucial for improving patient outcomes and quality of life in light chain amyloidosis.

LEARNING OBJECTIVES

- Identify concerning symptoms and signs that should trigger a suspicion of amyloidosis
- Describe a systems approach to the diagnosis of amyloidosis
- Outline key steps after a diagnosis of amyloidosis is made

Introduction

Diagnosing amyloidosis can be challenging. Amyloidosis encompasses a group of diseases characterized by abnormal protein accumulation in organs and tissues, leading to heterogeneous and diverse clinical manifestations. There are different presentations in amyloidosis, including hereditary versus acquired, localized versus systemic, and primary versus secondary. Delayed diagnosis is unfortunately all too common in amyloidosis, with patients experiencing symptoms for several months to years and consulting multiple specialist physicians before amyloidosis is considered and confirmed.[1] There are multiple subtypes of systemic amyloidosis, with light chain (AL), transthyretin (ATTR), and secondary (AA) being the most common (Table 1).

Amyloid consults are challenging due to the nonspecific nature of symptoms, which can easily be mistaken for symptoms of other diseases or attributed to normal aging[2]; a need for tissue biopsy to make a definitive diagnosis (we will review a clinical scenario where this is no longer needed); multiple types of amyloidosis,[3] each affecting different organ systems and possibly warranting a different workup; and finally a perception that given limited treatment options, making a diagnosis may not change patient outcomes. While this article will not delve into treatment of amyloidosis, it is no longer true that treatment options for amyloidosis are limited—significant progress has been made in recent years. The US Food and Drug Administration approved patisiran and inotersen for treatment of polyneuropathy associated with hereditary transthyretin amyloidosis (ATTRv) in 2018, tafamidis and tafamidis meglumine for ATTR cardiomyopathy in 2019, daratumumab combined with cyclophosphamide, bortezomib, and dexamethasone for newly diagnosed light chain (AL) amyloidosis in 2021, and vutrisiran for ATTRv polyneuropathy in 2022. New treatments continue to emerge. The effectiveness of treatment depends on the subtype and, in AL amyloidosis, stage of disease. With prompt diagnosis and appropriate management, we can help improve patient outcomes and quality of life.

Hematologists are frequently consulted when amyloidosis is suspected. Furthermore, they may already be involved in the care of patients with precursor hematologic conditions that can progress to systemic AL amyloidosis. In these situations, having a primer for efficient and accurate workup toward early detection is crucial. Expert reviews and consensus recommendations offer excellent algorithmic approaches to suspected amyloidosis,[4-6] which are not reiterated in this article.

Developing a clinical suspicion of amyloidosis

The clinical manifestations of amyloidosis are myriad and often nonspecific; indeed it is one of the medical conditions

Table 1. Summary of common systemic amyloid types

Amyloid type	Precursor protein	Cause	Organ pattern	Primary treatment*
AL	Immunoglobulin light chain	Secondary to a clonal plasma cell or lymphoproliferative disorders	Heart Kidneys Liver PNS ANS Soft tissue	Chemotherapy, autologous stem cell transplantation to control underlying clonal disorder
ATTRwt	Wild-type transthyretin	Aging	Heart Soft tissue	Tafamidis
ATTRv	Mutated transthyretin	Hereditary	Heart PNS ANS GI tract	Patisiran, inotersen, vutrisiran for neuropathy Tafamidis for cardiomyopathy
AA	Serum amyloid A protein	Chronic inflammation	Kidneys Liver Heart ANS	Suppress inflammation (eg, colchicine for FMF, anakinra for periodic fever syndromes, canakinumab for CAPS, biologics for autoimmune diseases)

*Refers to controlling amyloidogenic precursor. Treatment of amyloidosis additionally requires strong supportive care management.
ANS, autonomic nervous system; CAPS, cryopyrin-associated periodic syndrome; FMF, familial Mediterranean fever; GI, gastrointestinal; PNS, peripheral nervous system.

earning the nickname of the great imitator or the great masquerader.[7] The symptoms and presentation of amyloidosis depend on the organs involved and specific amyloid syndromes that occur. Common syndromes include cardiomyopathy with heart failure with preserved ejection fraction, nephrotic range proteinuria, organomegaly due to amyloid deposition (eg, hepatomegaly, splenomegaly, lymphadenopathy, macroglossia, salivary gland enlargement, pseudohypertrophy of muscles), neuropathy—both peripheral and autonomic, gastrointestinal manifestations, purpura, and constitutional symptoms (fatigue, weight loss). Many patients go for several months to years with a growing constellation of symptoms and seeing multiple physicians and health care providers, often misinterpreting and misattributing symptoms to aging.[1,2,8] While scans can now identify cardiac amyloidosis at early stages, such as bone scintigraphy (PYP scan) and cardiac magnetic resonance imaging, and monoclonal gammopathy of undetermined significance (MGUS) can also be readily identified with blood tests, the cost-effectiveness of screening populations is unknown. The likelihood of harboring systemic amyloidosis increases incrementally in the presence of multiple organ symptomatology, and a patient with multisystemic symptoms warrants active investigation for amyloidosis. Nearly universally, patients with a diagnosis of AL amyloidosis harbor multisystemic symptoms and diagnoses preceding the AL diagnosis.[9,10]

High-risk patient populations

Hematologists are aware of and well-versed in screening patients with MGUS and smoldering myeloma for development of active multiple myeloma. It is equally important to consider that these patients are also at risk for developing AL amyloidosis. Some lymphoproliferative disorders such as Waldenström macroglobulinemia, chronic lymphocytic leukemia, and marginal zone lymphoma can also lead to AL amyloidosis, and hematologists caring for patients with these diseases should consider systemic amyloidosis in their differential diagnosis during follow-up. A Mayo Clinic study showed that even with a known antecedent hematologic condition, the median time from diagnosis of the clonal precursor condition to an AL amyloidosis diagnosis was 11 months.[11] An N-terminal pro-brain natriuretic peptide (NT-proBNP), screening for albuminuria (urine albumin to creatinine ratio or 24-hour urine protein study), and alkaline phosphatase can serve as good screening tests for systemic amyloidosis in following these patients.[12] These tests must be supplanted with a good history for symptoms and a physical examination that considers amyloid organ sequelae (Table 2). Family history is helpful to screen for hereditary ATTR amyloidosis or syndromes associated with systemic inflammation that increase the risk for AA amyloidosis. Older African Americans are a unique high-risk group, given a 3% to 4% prevalence of Val122Ile mutation of the *TTR* gene as well as a two to three times higher prevalence of MGUS.

Workup of suspected amyloidosis: a systems approach is needed

CLINICAL CASE

Mr. Smith, a 62-year-old male, is admitted to the medical floor with heart failure. He presented with a 6-month history of progressive fatigue, swelling in his legs, and a 30-pound weight loss. His medical history is notable for hypertension and type 2 diabetes. He was diagnosed with bilateral carpal tunnel syndrome for which he had a right carpal tunnel release 3 years ago and the left one a year ago. His primary care physician attributed these symptoms to his long work hours at the computer. A year ago, his primary care physician also identified microalbuminuria on his urinalysis with a slight elevation in his creatinine and referred him to a nephrologist for further management of chronic kidney disease. He sees a cardiologist for management of his hypertension and has had a transthoracic echocardiogram within the last year, which showed grade 2 diastolic dysfunction, moderate left ventricular hypertrophy, and an interventricular septum thickness of 1.5 cm. In the last

Table 2. Multisystemic symptoms, signs, and clues for amyloidosis

	Clinical manifestations	Key laboratory and imaging findings	Definition of organ involvement
Kidney	Peripheral edema, periorbital puffiness, anasarca, nephrotic syndrome	Albuminuria ≥0.5 g/24h	Biomarker staging (refer to Table 3)
Heart	Dyspnea, jugular venous distension, peripheral edema, arrhythmia, HFpEF	Elevated cardiac biomarkers ECG—low voltage in limb leads, pseudoinfarct pattern, arrhythmia TEE—ventricular hypertrophy, thickened IVS, abnormal GLS	Biomarker staging (refer to Table 3) Echo wall thickness >1.5 cm
Liver	Hepatomegaly with cholestasis Abdominal distension	ALP elevation, elevated bilirubin in advanced infiltration Enlarged liver on imaging	Elevated ALP >1.5 times upper limit of normal Liver span >15 cm in the absence of heart failure
Nerves	Ascending length-dependent symmetric small-fiber peripheral neuropathy, autonomic neuropathy (orthostatic hypotension, early satiety, gastroparesis, irregular bowel movements, voiding difficulty, erectile dysfunction)	EMG/NCS may be helpful	N/A
GI tract	Dysphagia, early satiety, gastroparesis, GI bleeding, irregular bowel movements, weight loss, malabsorption	Anemia, hypoalbuminemia, low vitamin D	N/A
Spleen	Splenomegaly, functional asplenia Abdominal distension	Enlarged spleen on imaging, Howell-Jolly bodies on peripheral smear	N/A
Lung	Dyspnea, cough, hemoptysis	CT imaging—interstitial pattern or nodular cystic lesions	N/A
Skin	Cutaneous plaques, purpura		N/A
Other	Periorbital purpura Macroglossia Enlarged salivary glands Hoarseness Arthropathy Pseudohypertrophy of muscles Jaw claudication Carpal tunnel syndrome	Low factor X activity	N/A

N/A: No specific definition for organ involvement but rather based on symptoms and key laboratory and/or imaging findings.
ALP, alkaline phosphatase; CT, computerized tomography; ECG, electrocardiogram; EMG, ; GI, gastrointestinal; GLS, ; HFpEF, heart failure with preserved ejection fraction; IVS, ; NCS, ; TEE, transthoracic echocardiogram.

6 months, his cardiologist has need to stop, one by one, his 3 anti-hypertensives due to low blood pressure (BP). His BP has remained low, and he sometimes feels dizzy upon standing. Mr. Smith attributes many of his symptoms to aging, but he worries that something might be wrong. He has brought his concerns to his many doctors, and they have done additional testing pertinent to their specialty and reassured him. On physical examination, his BP is 110/70 mm Hg supine and drops to 87/54 mm Hg on standing. He has an enlarged tongue that shows lateral scalloping, and multiple bruises are seen on his arms and face, including purpura on his right eyelid. He exhibits signs of heart failure, including elevated jugular venous pressure, bilateral basal crackles on lung auscultation, and lower extremity edema. Suspecting cardiac amyloidosis, the medical team employs a systems approach to confirm the diagnosis.

Multisystemic assessment

Many symptoms and signs in this patient point to a multisystemic disease. A comprehensive assessment of his medical history and physical examination findings point to general and nonspecific symptoms—fatigue, weight loss; renal disease with proteinuria and elevated creatinine; cardiac disease with ventricular hypertrophy; coagulopathy, and orthostatic hypotension. Seemingly unrelated, but pertinent, additional information in this patient's case includes the history of bilateral carpal tunnel syndrome and macroglossia on physical examination. In working up a suspected patient with amyloidosis, in addition to the comprehensive clinical evaluation, it is important to undertake an expedited multisystemic investigative approach. This should include laboratory tests and pertinent imaging studies to assess the extent of organ involvement with amyloidosis (Table 2), screening for a plasma cell disorder, and identifying amyloidosis on a biopsy.

Laboratory tests

A comprehensive battery of tests to assess for organ amyloidosis should include a complete blood count with differential, liver function tests, renal function tests, PT/aPTT, cardiac biomarkers (NT-proBNP or BNP, troponin T or I), and a 24-hour urine protein test.

Advanced imaging

Mr. Smith had a recent transthoracic echocardiogram that showed multiple findings, including increased left ventricular wall thickness and restrictive diastolic filling pattern[13]; these should have triggered a suspicion of infiltrative cardiomyopathy. An echocardiogram with strain imaging to measure global longitudinal strain (GLS) can provide the characteristic appearance of cardiac amyloidosis, that is, abnormal GLS with apical sparing pattern.[13] Other advanced noninvasive imaging studies include scintigraphy with Tc-99m-pyrophosphate (PYP scan), which is widely available in the United States and can be positive in both ATTR (nearly always) and AL cardiomyopathy (sometimes).[14] Cardiac uptake is evaluated visually and scored comparing tracer uptake in the myocardium and ribs (grade 0–3), with a grade 2 or 3 pattern strongly suggestive of ATTR cardiomyopathy, with variable tracer uptake seen in AL cardiomyopathy. Using a threshold for higher visual score (a heart to contralateral ratio ≥1.5) has 97% sensitivity and 100% specificity for ATTR.[15] A cardiac magnetic resonance imaging demonstrating delayed gadolinium enhancement has high sensitivity and specificity for amyloidosis.[16] More advanced imaging studies such as the SAP scintigraphy scan[17] and PET/CT using radionuclides such as 18F-florbetapir[18] and investigational imaging techniques[19] can be undertaken if available as part of clinical care or research protocols. Abdominal imaging studies can provide insight in organomegaly.

Screening for a plasma cell disorder

A serum protein electrophoresis and immunofixation electrophoresis (IFE) are fundamental tests to detect M-proteins in the serum. The serum protein electrophoresis identifies the presence of abnormal protein bands, while the IFE determines the specific immunoglobulin class involved. The free light chain assay allows measurement of serum kappa and lambda free light chains, providing additional information. The urine protein electrophoresis and IFE on a 24-hour urine sample is an additional valuable test to detect and characterize monoclonal proteins in urine. A comprehensive screen for a plasma cell disorder should include all these tests and has 98.1% sensitivity for AL amyloidosis.[20] Finally, a bone marrow aspiration and biopsy examination is needed to confirm the presence of a clonal plasma cell disorder and can provide prognostic information such as the percentage of clonal plasma cells and cytogenetic abnormalities.

Tissue diagnosis

If the PYP scan is abnormal and a plasma cell disorder screen shows no evidence of a monoclonal process, a tissue biopsy is not needed as this constellation has high specificity and sensitivity for ATTR cardiomyopathy.[14] In this setting, the next step includes a genetic test to identify whether there is a pathogenic mutation in the transthyretin gene (ATTRv) or whether the gene is normal (ATTRwt). In any other scenario, it is critical to confirm amyloidosis with a tissue biopsy. Staining for amyloidosis on a bone marrow biopsy combined with a fat aspirate has a high sensitivity at high-volume specialty amyloid centers.[21,22] However, outside of high-volume centers, the yield on a fat aspirate has been, anecdotally, notoriously low and may be secondary to inadequate tissue quantity, inadequate staining, or improper use of polarizing instrument.[23,24] In this setting, amyloidosis is not ruled out, and additional tissue (eg, salivary gland biopsy or biopsy of the target organ) should be expeditiously obtained to confirm or rule out amyloidosis.

Key next steps after diagnosing amyloidosis

Typing amyloid deposits

While the presence of Congo red positive stain, which exhibits apple-green birefringence under polarized light microscopy, confirms the presence of amyloid, additional tests are needed to determine the specific protein or peptide involved in the amyloid deposits. Immunohistochemical staining has good performance when used with custom-made antibodies specific to different amyloid proteins.[25] Other antibody-based methods, such as immunofluorescence, are used especially in nephropathology. Immuno-gold labeling has high accuracy but is not widely available.[26] Proteomics using liquid chromatography–tandem mass spectrometry can directly assay the protein composition of microdissected amyloid deposits and determine the subtype.[27] This technique is expensive, requires experience in evaluating results, and has a lead time of several days to obtain results.[28] However, it has high accuracy and has revolutionized amyloid typing, has led to discovery of new subtypes, and can also shed light on the rare (<1%) but clinically important phenomenon of co-occurrence of two different types of amyloidosis in the same patient.[29]

Identify whether the patient has localized versus systemic amyloidosis

Differentiating between localized and systemic amyloidosis is an important step in management of patients with amyloidosis. While the presence of amyloid in certain organs such as the heart, kidneys, or liver or in the nervous system is always indicative of a systemic process, in some tissue, such as cutaneous, breast, pulmonary, and gastrointestinal, amyloidosis is either localized or systemic. Some other locations, such as the urinary bladder, aerodigestive tract, and orbit, are almost always localized. In a patient diagnosed with AL amyloidosis in one of these organ systems, it is important to take a systems approach with laboratory, imaging, and plasma cell disorder testing to identify whether a patient has localized or systemic amyloidosis. Localized amyloidosis can be surgically managed with debulking or managed conservatively with monitoring as patients may have local or, rarely, systemic progression.[30,31]

Staging of systemic AL amyloidosis

Staging of systemic AL amyloidosis is an important step to determine the extent of systemic AL disease, predicting prognosis, and guiding treatment decisions. The two commonly used staging systems include the 2004 Mayo staging system[32] with the 2015 European modification[33] and the 2012 revised Mayo staging system.[34] In addition to the cardiac biomarkers, renal staging system is also helpful in determining renal prognosis.[35] Table 3 provides the staging systems along with cross-reference among the various biomarkers. Similar staging systems for ATTR cardiomyopathy using cardiac biomarkers also exist.[36,37]

Referral to specialty centers

Given that amyloidosis remains a rare disease, patients diagnosed with amyloidosis can benefit from evaluation at a specialty center at least once. These specialty centers have multidisciplinary physicians and health care providers who collaborate to

Table 3. Staging systems in AL amyloidosis

	Biomarker thresholds	Staging
2004 Mayo stage with 2015 European modification of 2004 staging system[32,33,38]	NT-proBNP ≥332 ng/L (or BNP ≥81) cTnT ≥0.035 ng/ml (or cTnI ≥0.1 or hsTnT ≥50)	Stage I: 0 markers above cutoff Stage II: 1 marker above cutoff Stage IIIa: both markers above cutoff and NT-proBNP <8500 (or BNP >700) Stage IIIb: both markers above cutoff and NT-proBNP ≥8500 (or BNP >700)
2012 Mayo staging system[34,38]	NT-proBNP ≥1800 ng/L (or BNP ≥800) cTNT ≥0.025 ng/ml (or hsTnT ≥40) dFLC ≥180 mg/L	Stage I: 0 markers above cutoff Stage II: 1 marker above cutoff Stage III: 2 markers above cutoff Stage IV: 3 markers above cutoff
2014 Palladini renal staging system[35]	eGFR ≤50 ml/min/1.73m^2 Proteinuria ≥5 g/24h	Stage I: 0 thresholds met Stage II: either threshold met Stage III: both thresholds met

BNP, brain natriuretic peptide; cTnT, cardiac troponin T; cTnI, cardiac troponin I; hs-TnT, high-sensitivity cTnT; dFLC, difference between involved and uninvolved free light chain; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal of propedtide of BNP.

provide comprehensive care, including accurate diagnosis, treatment options including clinical trials and stem cell transplantation, and a comprehensive approach to supportive care. Further patient and caregiver education through organizations such as the Amyloidosis Foundation, Amyloidosis Support Groups, and Amyloidosis Research Consortium are critical to provide additional support to patients and caregivers, establish collaborative care, and provide research opportunities.

Amyloidosis is not identified on workup

Amyloidosis may not be identified despite clinical manifestations, advanced imaging, and organ biopsy. While it may seem reassuring that a diagnosis of amyloidosis has been refuted, if amyloidosis remains suspected, close follow-up and reevaluation of the clinical course and symptoms is necessary. Monitoring for progressive organ dysfunction or the emergence of additional clinical features consistent with amyloidosis is crucial. Repeat investigations, including laboratory tests, imaging studies, and biopsies, may be necessary over time to capture evolving disease manifestations.

Summary

The detection and diagnosis of amyloidosis can appear daunting. A systems approach to suspecting and working up the disease can make the process easier. There are specific clinical findings that should raise the suspicion and prompt a comprehensive systematic diagnostic approach. Earlier diagnosis prior to the development of advanced end-organ damage is crucial for improving the morbidity and mortality of patients with amyloidosis.

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Anita D'Souza: Nothing to disclose.

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MRD-directed therapy in CLL: ready for prime time?

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In recent years, the treatment paradigm for patients with chronic lymphocytic leukemia (CLL) has moved away from chemoimmunotherapy (CIT) toward the use of novel targeted agents. Commercially available drugs, including Bruton's tyrosine kinase inhibitors and the BCL2 inhibitor venetoclax, often used in combination with anti-CD20 monoclonal antibodies, are now the mainstay of therapy both in the frontline and in relapsed settings. As the landscape for CLL management evolves, therapeutic endpoints need to be redefined. Detection of measurable residual disease (MRD) is a sensitive tool to identify disease burden following treatment with several therapeutic regimens in CLL (including CIT, venetoclax-based regimens, and cellular therapies), and it has demonstrated prognostic value. Despite recent advances, the utility of MRD-directed therapy and attempts to eradicate it in routine clinical practice remain debated. There is little comparative data from clinical trials on the best assay to determine undetectable MRD (U-MRD) and whether its monitoring can lead to changes in treatment strategies. Our review discusses the definitions of MRD, assays for its detection, and its impact on long-term survival outcomes for patients with a CLL diagnosis.

LEARNING OBJECTIVES

- Review the definitions, terminology, and methods for detection of measurable residual disease (MRD) in CLL
- Review current data using undetectable measurable residual disease and its potential prognostic role for survival outcomes
- Discuss potential clinical applications of current findings from clinical trial data and areas for further study

Introduction

Treatments for chronic lymphocytic leukemia (CLL) have shifted from chemoimmunotherapy (CIT) to novel targeted therapies with the approval of the first-in-class Bruton's tyrosine kinase inhibitor (BTKi), ibrutinib,[1] the second-generation covalent BTKis acalabrutinib[2] and zanubrutinib,[3] and the BCL2 inhibitor venetoclax.[4-7] Despite improvements in survival outcomes, CLL remains incurable, and patients may ultimately require treatment with multiple lines of therapy.

Measurable residual disease (MRD) has been used in several hematologic malignancies with demonstrated prognostic value. In CLL, MRD has demonstrated prognostic value, prompting the study of several MRD response adapted therapies. In this review, we define MRD, discuss MRD assays, and examine clinical outcomes with novel targeted therapies (Table 1) and novel doublet/triplet combination currently under development (Table 2). Finally, we discuss the role of MRD testing in clinical practice.

CLINICAL CASE

JT is a 73-year-old man with a past medical history of hypertension and hyperlipidemia who was diagnosed with CLL six years ago, when an isolated lymphocytosis was identified on preoperative blood work for an elective knee replacement. At this visit, he endorses worsening fatigue and new early satiety. His blood work demonstrates a white blood cell count of 113,000 cells/μL, a hemoglobin of 9.3 g/dL, and platelet count of 93,000 cells/μL. He now meets International Workshop on CLL (iwCLL) criteria to initiate treatment, and you discuss with him starting a

Table 1. Summary table of U-MRD rates and outcomes of pivotal clinical trials in patients with treatment-naive and relapsed/refractory CLL

Trial	Phase	Treatment setting	Treatment	MRD endpoint	MRD detection method	MRD detection level	% U-MRD PB	% U-MRD BM	PFS/mPFS	OS/mOS
CLL8[23]	3	TN	FC FCR	Secondary	Flow cytometry ASO-PCR	10^{-4}	35% 63%	28% 44%	32.9 mo 56.8 mo	86 mo NR
NIH[24]	2	TN/RR	I	Exploratory	Flow cytometry	10^{-4}	10.2%	8%	**5-year:** **TP53 cohort:** 58.2% **Elderly cohort:** 81.2%	**5-year:** **TP53 cohort:** 75.7% **Elderly cohort:** 83.85%
E1912[21]	3	TN	IR FCR	Exploratory	Flow cytometry	10^{-4}	7.9% 30.3%	NA	**5-year:** 78% 51%	**mOS:** NR NR
ELEVATE-TN[25]	3	TN	A AO CO	Exploratory	Flow cytometry	10^{-4}	7% 49% 61%	0% 61% 10.9%	**48 mo:** 77.9% 87% 25.1%	**mOS:** NR NR NR
CLL14[7]	3	TN	VO CO	Secondary	ASO-PCR	10^{-4}–10^{-6}	39.6 mo: 81% 49.5%	17.1% 56.9%	**5-year:** 62.6% 27%	**5-year:** 87% 87%
FLAIR[27]*	3	TN	FCR IR	Secondary	Flow cytometry	10^{-4}	75% 10%	46% 4%	**mPFS:** 67 mo NR	**mOS:** NR NR
CAPTIVATE: MRD[28]*	2	TN	IV + Placebo IV + I	Secondary	Flow cytometry	10^{-4}	75%	68%	**4-year:** 88% 95%	**4-year:** 100% 98%
CAPTIVATE: Fixed Duration[29,30]	2	TN	IV	Secondary	Flow cytometry	10^{-4}	77%	60%	95% at 24 mo	98% at 24 mo
GLOW[32]	3	TN	IV CO	Secondary	NGS	10^{-4}, 10^{-5}	43.4% 18.6%	40.6% 7.6%	**30 mo:** 86.7% 35.5%	**mOS:** NR NR
MDACC[31]	2	TN	IV	Secondary	Flow cytometry	10^{-4}	NA	75%	**3-year:** 93%	**3-year:** 96%
CLL13[33]	3	TN	FCR/BR VR VO VIO	Primary	Flow cytometry	10^{-4}	52% 57% 86.5% 92.2%	37.1% 43% 72.5% 77.9%	**3 year:** 75.5% 80.8% 87/.7% 90.5%	**3-year:** 95% 96.5% 96.3% 95.3%
MURANO[34]	3	RR	BR VR	Secondary	ASO-PCR Flow cytometry	10^{-4}	13.3% 62.4%	1.5% 27.3%	**mPFS:** 17 mo 53.6 mo	**60 mo:** 62.2% 82.1%
CLARITY[35]*	2	RR	IV	Primary	Flow cytometry	10^{-4}	53%	36%	NR	100%
CLL3X[36]	2	RR	Allogeneic HSCT	Secondary	Flow cytometry ASO-PCR	10^{-4}			**10-year:** 34%	**10-year:** 51%
TRANSCEND-004 CLL[41]	1–2	RR	Lisocabtagene maraleucel	Secondary	NGS	10^{-4}	63%	59%	17.97 mo	43.17 mo

*Indicates a trial that used MRD to guide treatment decisions.

A, acalabrutinib; AO, acalabrutinib-obinutuzumab; ASO-PCR, allele-specific oligonucleotide polymerase chain reaction; BM, bone marrow; BR, bendamustine-rituximab; CO, chlorambucil-obintuzumab; FC, fludarabine-cyclophosphamide; FCR, fludarabine-cyclophosphamide-rituximab; HSCT, hematopoietic stem cell transplant; I, ibrutinib; IV, ibrutinib-venetoclax; IR, ibrutinib-rituximab; mOS, median OS; mPFS, median PFS; MRD, measurable residual disease; NA, not available; NGS, next-generation sequencing NR, not reached; OS, verall survival; PFS, progression-free survival; PB, peripheral blood; RR, relapsed/refractory; TN, treatment-naive; U-MRD, undetectable-MRD; VR, venetoclax-rituximab.

Table 2. Selected ongoing clinical trials for CLL with MRD-guided primary endpoints

Clinicaltrials.gov ID	Study title	Primary outcomes
NCT05478512	Front-line VenObi Combination Followed by Ven or VenZan Combination in Patients With Residual Disease: a MRD Tailored Treatment for Young Patients With High-risk CLL (VIS)	• U-MRD at month 9 • U-MRD at month 21
NCT04941716	Acalabrutinib in Combination With Venetoclax for the Treatment of Refractory or Recurrent Chronic Lymphocytic Leukemia or Small Lymphocytic Lymphoma	• Rate of U-MRD
NCT04501939	Cirmtuzumab Consolidation for Treatment of Patients With Detectable CLL on Venetoclax	• Percentage of subjects with U-MRD after 6 months of cirmtuzumab and venetoclax treatment
NCT04754035	Clinical Study With Ibrutinib and Venetoclax for Patients With Relapsed/Refractory Chronic Lymphocytic Leukemia (IMPROVE)	• U-MRD rate evaluated by multi-color flow cytometry analysis (limit of detection 10^{-4}) within the treatment period
NCT05677919	Pirtobrutinib and Venetoclax Combined With Minimal Residual Disease Detection for Previously Untreated Chronic Lymphocytic Leukemia, MIRACLE Study	• Success of U-MRD (< $1/10^4$) will be measured by ClonoSEQ in both PB and BM; the proportion of successes will be estimated by the number of successes divided by the total number of evaluable patients; exact binomial 95% confidence intervals for the true rate of U-MRD by ClonoSEQ in both PB and BM after cycle 15 will be calculated
NCT05650723	Zanubrutinib and Venetoclax as Initial Therapy for Chronic Lymphocytic Leukemia (CLL) With Response-based Obinutuzumab	• Percentage of total patients that have achieved U-MRD at cycle 16, as assessed via PB and BM aspirate • Percentage of eligible patients that have achieved U-MRD at cycle 23, as assessed via PB and BM aspirate
NCT05317936	Pirtobrutinib (LOXO-305) Consolidation for MRD Eradication in Patients With Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL) Treated With Venetoclax	• Rate of U-MRD in the PB
NCT05168930	Zanubrutinib and Venetoclax in CLL (ZANU-VEN)	• Rate of U-MRD
NCT03580928	Acalabrutinib, Venetoclax, and Obinutuzumab for Initial Therapy of CLL (AVO)	• Rate of BM U-MRD complete response
NCT04908228	Fixed-duration Therapy With Ibrutinib and Obinutuzumab (GA-101) in Treatment-naïve Patients With CLL (FIGHT)	• BM U-MRD (<10^{-4}) at 30 days follow-up
NCT02158091	A Phase 1b/2 Study of IPI-145 Plus FCR in Previously Untreated, Younger Patients With CLL	• Number of patients who had a U-MRD complete response (CR) 2 months after chemotherapy • Number of patients who experienced a dose limiting toxicity during phase 1
NCT03708003	Ibrutinib lead-in Followed by Venetoclax Plus Ibrutinib in Patients With RR CLL	• U-MRD CR/CRi at end of cycle 30
NCT04285567	A Study to Compare the Efficacy and Safety of a Combined Regimen of Venetoclax and Obinutuzumab Versus Fludarabine, Cyclophosphamide, and Rituximab (FCR)/Bendamustine And Rituximab (BR) in FIT Patients With Previously Untreated Chronic Lymphocytic Leukemia (CLL) Without DEL (17P) or TP53 Mutation (CRISTALLO)	• Minimal residual disease (MRD response rate using NGS in the first 140 participants recruited
NCT05336812	Acalabrutinib in Combination With Venetoclax or Obinutuzumab for the Treatment of Treatment-naive Chronic Lymphocytic Leukemia	• Rate of BM U-MRD, defined as tumor cell in 10,000 cells using standard flow-based assay, achieved after completion of therapy
NCT04169737	Acalabrutinib and Venetoclax With or Without Early Obinutuzumab for the Treatment of High Risk, Recurrent, or Refractory Chronic Lymphocytic Leukemia or Small Lymphocytic Lymphoma	• Disease assessment of BM U-MRD (<10^{-4}, MRD4) (TN cohort) • Disease assessment of BM undetectable-MRD4 (RR cohort)
NCT04010968	Evaluation of Risk-Adapted and MRD-Driven Strategy for Untreated Fit Patients With Intermediate Risk Chronic Lymphocytic Leukemia (ERADIC)	• Minimal residual disease (MRD) in BM <0.01% at month 27
NCT05791409	Venetoclax Treatment (26 Cycles) With 6 Cycles or 12 Cycles of Epcoritamab in Patients With Relapsed or Refractory CLL or SLL (AETHER)	• Proportion of U-MRD in BM by NGS and no progression according to iwCLL criteria after 26 cycles (i.e., 12 weeks after cycle 26) for all intent to treat patients • Recommended phase 2 dose for the combination of venetoclax and epcoritamab based on dose limiting toxicity
NCT04523428	REtreatment With VEnetoclax and Acalabrutinib After Venetoclax Limited Duration (REVEAL)	• U-MRD in BM by flow cytometry after 26 cycles (2 acalabrutinib and 24 acalabrutinib-venetoclax)

BM, bone marrow; CRi, incomplete bone marrow recovery; iwCLL, International Workshop on CLL; MRD, measurable residual disease; NGS, next-generation sequencing; Obi, obinutuzumab; PB, peripheral blood; RR, relapsed/refractory; TN, treatment-naive; U-MRD, undetectable MRD; Ven, venetoclax; Zan, zanubrutinib.

continuous BTKi or treating with venetoclax-obintuzumab (VO) for one year. Before his clinical visit, he read about MRD testing, so he asks which treatment will be best and give him the deepest response.

What defines MRD?

MRD is defined by the number of CLL cells detected within a sample. A recent expert panel convened to standardize nomenclature and assessment of MRD in CLL.[8] Undetectable MRD (U-MRD) is the preferred term. The levels of MRD have been defined as MRD4 (10^{-4}, or 1 in 10,000 leukocytes), MRD5 (10^{-5} or 1 in 100,000 leukocytes), and MRD6 (10^{-6} or 1 in 1,000,000 leukocytes). Given the heterogeneity of the available assays, the method utilized, the sensitivity threshold, and the tested compartment need to be reported when discussing MRD results. Identification of the tested compartment in the MRD results is important because there can be discordance between the peripheral blood (PB) and bone marrow (BM). The expert panel, as well as the iwCLL guidelines,[9] define a U-MRD threshold of at least 10^{-4} (MRD4). Though there is no specific guidance on timing for testing and monitoring of MRD, the expert panel consensus recommendation is to consider testing at a minimum of two months after completion of fixed-duration therapy. Currently, there are no consensus guidelines on how to use MRD testing results in routine clinical practice.

How is MRD detected?

There are various methods for MRD detection (Figure 1). Flow cytometry–based assays are the most widely available and frequently used. The European Research Initiative on CLL[10,11] provided consensus guidelines for harmonization of MRD detection by flow cytometry. Current recommendations are to use an antibody panel consisting of CD5, CD19, CD20, CD43, CD79b, and CD81, which can detect MRD4 by four-color flow. Six and 10-color flow cytometry can increase sensitivity, allowing for detection of MRD5.[12,13] Polymerase chain reaction (PCR) to the immunoglobulin heavy chain variable (IGHV) gene can also be performed and can reach a sensitivity of MRD6 but requires patient-specific primers. As such, this was used in clinical trials but is not used frequently in clinical practice. Next-generation sequencing (NGS) testing and tests for rearrangements of the IGH VDJ or DJ, IgK and IgL receptor gene sequences, or translocated BCL1/IgH(J) and BCL2/IgH(J)[14] have become more readily available. NGS-based assays have good concordance with flow cytometric assays, are sensitive up to MRD6, and have been incorporated into prospective clinical trials.[15] These tests require baseline samples to establish clonality before initiating treatment. Emerging tests for MRD include digital droplet PCR (ddPCR)[16] and cell-free DNA (cfDNA),[17] which have both demonstrated excellent sensitivity but are not used routinely.

MRD with continuous treatment with targeted agents

Initial approvals of BTKi monotherapy[18] have dramatically changed the treatment landscape, leading to improvements in progression-free survival (PFS) and overall survival (OS) when compared to CIT.[19-21] Several studies have demonstrated the prognostic value of MRD in patients treated with CIT. For patients treated with fludarabine, cyclophosphamide, and rituximab (FCR), U-MRD responses predict PFS, particularly for patients with MRD6 responses that have IGHV mutations.[22] Given the prognostic value of U-MRD responses in predicting outcomes with CIT,[23] several clinical trials have studied the role of U-MRD in patients treated continuously with BTKis.

Studies of treatment with continuous ibrutinib have demonstrated low rates of complete response (CR) as well as low rates of U-MRD. Despite these findings, we continue to see impressive PFS and OS, including in patients with high-risk genetic features (e.g., del17p/TP53, unmutated IGHV, complex karyotype). In a phase 2 CLL study conducted by the NIH with elderly patients or patients with TP53 aberrations, continuous treatment with ibrutinib monotherapy demonstrated a deepening of responses over time, with increased CR rates from 0% at 6 months to 28.4% at 60 months on treatment[24] with low rates of U-MRD at four years: 10.2% (5/49 patients) in PB and 8% (2/25 patients) in BM. Despite the low rates of U-MRD, five-year PFS was excellent at 58.2% for patients with TP53 aberrations and 81.2% for elderly patients. Similar findings were seen in E1912, the pivotal phase 3 trial comparing ibrutinib-rituximab (IR) (with continuous ibrutinib) to six cycles of FCR in treatment-naive fit patients <65 years old with CLL. At 5.8 years of follow up, median PFS was longer for IR compared to FCR (Hazard Ratio [HR] 0.37) with small but statistically significant improvement in OS for patients with unmutated IGHV (HR 0.35; 95% CI 0.15-0.80; p=0.01) but not for patients with mutated-IGHV (HR 0.72; 95% CI 0.15-3.47; p=0.68).[21] Patients treated with FCR had higher rates of U-MRD4 at 3, 12, 24, and 36 months compared to patients treated with IR (29.1% at 3 months, 30.3% vs 7.9% at 12 months, 23.4% vs 4.2% at 24 months, and 8.6% vs 3.7% at 36 months). An updated four-year follow-up from ELEVATE-TN, a phase 3 trial comparing acalabrutinib, acalabrutinib-obinutuzumab (AO), and chlorambucil-obinutuzumab (CO),[25] also demonstrated an improvement in four-year PFS for patients treated in the acalabrutinib-containing arms,[26] despite low rates of U-MRD. These results demonstrate that outcomes remain excellent with BTKis, even after years on therapy. As such, current recommendations are for continuous treatment until progressive disease or unacceptable toxicity, and therefore routine MRD testing is not recommended.

The FLAIR trial compared treatment with IR to FCR in fit patients with treatment-naive CLL without a del(17p).[27] Ibrutinib was continued for six years, until U-MRD4, toxicity or disease progression. MRD in both PB and BM was assessed at 9 months and 12 months after randomization, and then every 6 months thereafter. Treatment with ibrutinib was discontinued if U-MRD was attained, and treatment duration was based on the time from randomization to U-MRD. With median follow-up of 53 months, median PFS was not reached for patients treated with IR and was 67 months for patients treated with FCR. Rates of U-MRD were lower at 9 months for patients treated with IR (3.9%) compared to patients treated with FCR (55.3%), again demonstrating that U-MRD is not necessarily associated with PFS for patients on continuous BTKi therapy. Updated results of the influence of MRD on PFS and the time to next treatment after ibrutinib discontinuation will provide a potential role for utilizing MRD testing to shorten the duration of therapy with BTKis.

MRD in frontline fixed-duration treatment of CLL with targeted treatments

MRD endpoints have been studied in clinical trials of fixed-duration treatments. CLL14 is a pivotal randomized phase 3 trial

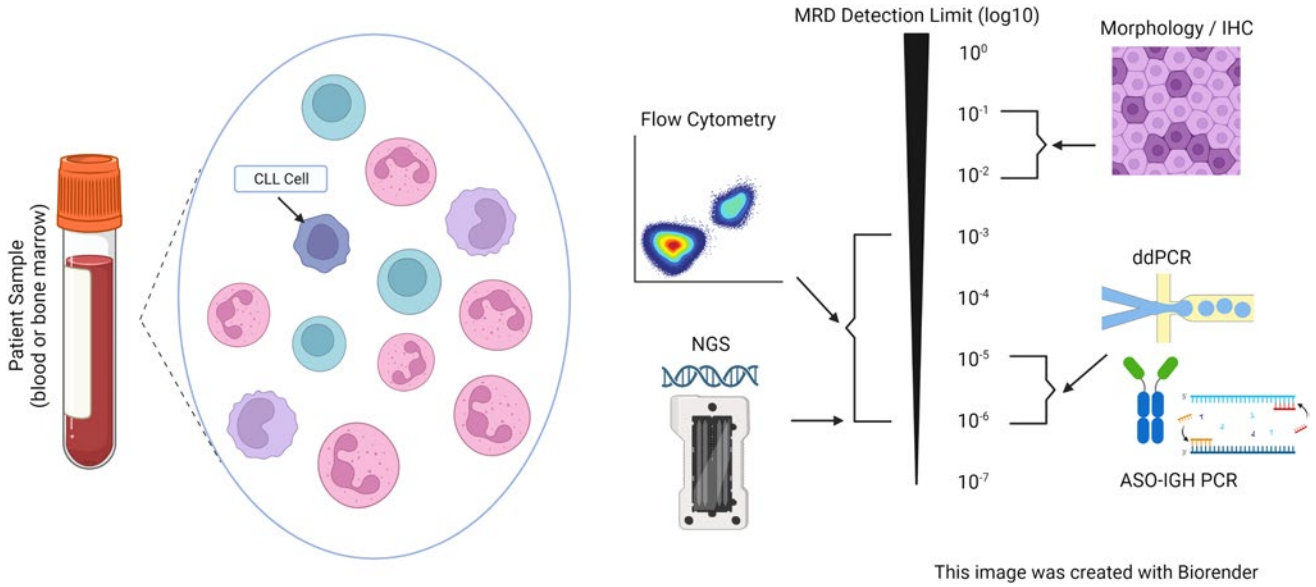

Figure 1. Measurable residual disease (MRD) testing modalities. ASO-IGH PCR, allele-specific oligonucleotide immunoglobulin heavy locus PCR; ddPCR, droplet digital polymerase chain reaction; IHC, immunohistochemistry; NGS, next-generation sequencing.

that led to the approval of VO for frontline treatment of CLL. Patients were randomized 1:1 to receive VO for 12 months vs 6 months of CO. U-MRD4 rates by allele-specific oligonucleotide (ASO) PCR and four-color flow cytometry were higher with VO than CO (76% at end of treatment +3 months vs 35%). The recurrence of measurable disease was also delayed for patients treated with VO compared to CO (21 months vs 6 months), demonstrating not just the importance of reaching U-MRD at the end of treatment (EOT) but also highlighting the differences in duration of U-MRD responses by treatment.

The phase 2 CAPTIVATE trial examined patients with CLL who were ≤65 years old treated with frontline ibrutinib and venetoclax (IV). Patients received 3 months of ibrutinib lead-in treatment followed by IV for 12 months. The study consisted of both a fixed duration (FD) cohort and a randomized MRD cohort. MRD4 was measured by eight-color flow cytometry in both PB and BM and confirmed that U-MRD was defined as two separate U-MRD measurements.[28] U-MRD was achieved by 75% of patients in the PB and 68% in BM at EOT. High rates of U-MRD were seen across high-risk subgroups, including in patients with del17p and unmutated IGHV. In the MRD cohort, patients with confirmed U-MRD were randomized to receive either placebo or ibrutinib. Patients with unconfirmed U-MRD were randomized to receive ibrutinib or IV. Four-year PFS was 95% in patients treated with ibrutinib and 88% in patients treated with placebo.[29] In the unconfirmed MRD cohort, 30-month PFS was 95% for patients treated with ibrutinib and 97% for patients treated IV. In the FD cohort, four-year PFS rates were higher for patients with U-MRD at EOT +3 compared to those with detectable MRD (90% vs 66%), although there were no differences in OS.[30] Similar results were seen in a phase 2 study of 24 months of IV treatment for patients with treatment-naive CLL with high-risk genetic features (i.e., del17p, TP53, del11q, or unmutated IGHV). Complete response rate at EOT (18 cycles of combination) was 96%, with 61% also achieving U-MRD in BM initially. With longer follow-up, 75% of patients achieved U-MRD in BM as best response with associated three-year PFS of 93%.[31]

The phase 3 GLOW trial studied fixed duration IV compared to CO for frontline treatment of CLL in patients ≥70 years of age or unfit for CIT.[32] MRD4 and MRD5 were assessed by NGS. IV achieved higher rates of MRD5 in BM and PB at EOT +3 (40.6% and 43.4% vs 7.6% and 18.1%) and EOT +12 in PB (36.8% vs 6.7%) compared to CO. Patients treated with IV were more likely to sustain their U-MRD compared to CO, which demonstrated shorter duration or U-MRD. There were no significant differences in PFS for patients treated with IV if they were U-MRD4 vs detectable MRD4 in BM (96.3% vs 93.3%), demonstrating excellent PFS irrespective of achieving U-MRD status.

Recently, the CLL13 trial compared venetoclax combinations to CIT. It randomized fit patients 1:1:1:1 to receive CIT (with FCR or bendamustine-rituximab [BR]) for six cycles, 12 cycles of venetoclax-rituximab [VR], 12 cycles of VO, or 12 cycles of venetoclax-obinutuzumab-ibrutinib [VOI] with co-primary endpoints of U-MRD4 at 15 months (EOT +3) and PFS.[33] Rates of U-MRD4 by flow cytometry were higher in PB and BM in the VO and VOI arms compared to CIT (72.5%, 77.9%, 37.1%, respectively). At a median follow-up of 38.8 months, three-year PFS was 76.4% for VO, 82.9% for VOI, and 65.5% for CIT. At 15 months, U-MRD was associated with improvement in PFS in the venetoclax-containing arms compared to CIT, although there were no significant differences between doublet and triplet combinations.

What potentially accounts for the above differences in the association of MRD with PFS? GLOW has a shorter follow-up than CAPTIVATE, and it may take longer to see differences in PFS with BTKi-containing regimens given their long-term PFS benefits with continuous BTKi therapy. Importantly, GLOW enrolled an older population, and several deaths were reported in the study. This suggests that the toxicity of regimens needs to be considered when choosing therapy for older patient populations and that in certain populations, attaining

U-MRD may not be optimal due to the toxicity associated with the regimen.

CLINICAL CASE (continued)

JT decides that he wants to start treatment with acalabrutinib monotherapy. You discuss that in this setting, you would not recommend checking MRD testing, as outcomes do not correlate well with U-MRD status. After five years on acalabrutinib, he develops progressive disease and starts treatment with VR. He asks about MRD testing and if it should be performed now.

MRD for relapsed/refractory disease with targeted agents

Given the improvements in PFS and OS for patients with U-MRD responses to frontline CIT, MRD has been explored as an endpoint in patients treated with targeted agents for relapsed/refractory CLL. MURANO is the practice-changing phase 3 clinical trial comparing VR to BR for patients with relapsed/refractory CLL. MRD was assessed by ASO-PCR and four-color flow cytometry. In a recent update, seven-year PFS was 23% for patients treated with VR compared to 0% for patients treated with BR.[34] Seven-year OS rates were 69.6% for patients treated with VR compared to 51% for patients with BR (HR 0.53). For patients with U-MRD at EOT who have not had progressive disease, median PFS was 52.5 months compared to 18 months for patients who had detectable MRD, demonstrating that patients with U-MRD at the end of VR treatment have prolonged PFS, and U-MRD status can be prognostic in this setting.

The CLARITY study is a phase 2 trial that treated relapsed CLL patients with the combination of IV and looked at the primary endpoint of MRD4 in PB and BM after 12 months of combination therapy.[35] Unlike CLL14 and MURANO, where all patients discontinued treatment at 12 months irrespective of MRD status, patients could stop combination therapy anywhere from 8 to 26 months if U-MRD was confirmed. Patients continued ibrutinib treatment if they were not U-MRD4 at 26 months. At 14 months on therapy, 36% (19/53) of patients achieved U-MRD in BM, and 53% (28/53) of patients achieved U-MRD in PB at 14 months (12 months combination therapy).[35] There was a deepening of U-MRD responses by month 26 (44% [11/25]). Twenty-three patients with U-MRD have stopped all therapy, and 14/18 remain U-MRD4 at 38 months. These data demonstrate that responses to IV can deepen over time; further follow up for PFS while off therapy, during the time to next treatment, can help inform if targeting U-MRD specifically with FD treatments improves outcomes.

MRD with cellular therapy

Allogeneic stem cell transplantation remains part of the treatment paradigm for fit patients with high-risk CLL and has curative potential, although it is used less frequently given the efficacy of our novel targeted therapies. There is limited prospective data on U-MRD responses to transplant. The German CLL3X trial looked at reduced intensity conditioning allogeneic stem cell transplant in poor-risk patients with relapsed/refractory CLL and found a U-MRD rate of 52% of patients at 12 months in those patients who were alive, including in patients with del17p. Additionally, U-MRD at 12 months was prognostic for a reduced relapse risk,[36] with a six-year OS of 58% irrespective of high-risk genomic features.[37]

Chimeric antigen receptor (CAR) T-cells were initially studied in patients with CLL, with early trials demonstrating durable responses.[38,39] Several products are currently under study and have demonstrated the ability to achieve U-MRD, even in high-risk patients. A study of CD19 CAR-T cell therapy in combination with ibrutinib in patients who progressed on ibrutinib therapy demonstrated a four-week overall response rate of 83% with 61% of patients having U-MRD in BM.[40] A phase 1-2 study of lisocabtagene maraleucel in patients with relapsed/refractory CLL demonstrated a CR rate of 18% and overall response rate of 43%.[41] U-MRD rate was 63% in the PB and 59% in BM. Median PFS for patients with U-MRD was 26.2 months compared to 2.8 months in those with detectable MRD. These data demonstrate the prognostic value for U-MRD with cellular therapies, though longer follow-up is needed with CAR T-cells to determine the durability of these responses.

CLINICAL CASE (revisited)

After two years of VR therapy, you check U-MRD using four-color flow cytometry at EOT and detect a clone in 0.02% of cells.

Is MRD ready for prime time?

U-MRD at EOT has demonstrated important prognostic value with several treatments in CLL, including CIT, venetoclax-based combinations, and cellular therapies. As such, it is reasonable to evaluate U-MRD status at the end of these finite treatments if testing is readily available. At present, the value of MRD testing on survival requires further investigation for future combination regimens because it is unclear whether MRD-guided approaches will be standardized. Currently, there is no evidence that an MRD-guided approach should be used outside clinical trials. U-MRD remains an attractive endpoint for clinical trials because median OS has not been reached for many pivotal clinical trials, and surrogate endpoints are needed.

While U-MRD is prognostic, other disease-specific factors continue to influence outcomes. Patients with high-risk genomic features such as del17p and unmutated IGHV have similar rates of U-MRD compared to those with low-risk disease, but they continue to have shorter PFS, demonstrating a potential difference in MRD kinetics based on disease biology.[7,34] As of today, there is no clear evidence as to how to adapt treatment based on MRD. The BOVen study treated treatment-naive patients with CLL with the triplet of zanubrutinib, venetoclax, obinutuzumab, with 89% of patients having a U-MRD response in the PB and BM after a median of 10 cycles of therapy.[42] Longer follow-up is needed, but the level of MRD seems to correlate with the durability of remission, with 94% of patients remaining U-MRD after an additional 15.8 months of follow-up. The CLARITY study was amended to increase the duration of treatment with IV, with nearly all patients discontinuing treatment by the end of two years.[35] The FLAIR study also used an individualized treatment duration, but longer follow-up (especially after ibrutinib discontinuation) is needed to

determine if this is an effective approach.[27] The CLL17 trial of ibrutinib, IV, or VO for frontline treatment of CLL will help determine whether continuous time-limited therapy for frontline treatment is best, although translating this data into outcomes with second-generation BTKis that have become a preferred treatment will be challenging.

Despite recent consensus guidelines, there are still key unanswered questions: What level of MRD detection should be targeted? Which testing method should be used? When and how often should testing be performed, and with what compartment? Finally, is MRD the correct endpoint for all CLL patients? Outcomes with continuous BTKi therapy are excellent, and U-MRD may not be necessary to improve survival in all patients.

Conclusions

MRD remains a powerful tool for response assessment for certain FD treatment regimens, and it has the potential to become an important surrogate for PFS. Despite MRD's prognostic utility, further research and follow-up is needed to determine its role in routine clinical practice, both for prognostication and for guiding treatment decisions in routine clinical practice. Finally, U-MRD may not be a one-size-fits-all endpoint, and individualized treatment decisions will be needed that consider patient factors, treatment, and desired endpoints. Clinical trials are currently underway to begin to answer these questions, and we eagerly await these results.

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Off-label drug use

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Dual-targeted regimens for the frontline treatment of CLL

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The treatment landscape of chronic lymphocytic leukemia (CLL) has evolved considerably over the past decade due to the development of effective novel agents with varying mechanisms of action, including Bruton tyrosine kinase (BTK) and B-cell lymphoma 2 (BCL2) inhibitors. Extrapolating upon the success of anti-CD20–directed chemoimmunotherapy, a dual-targeted approach has been explored in treatment-naive patients with CLL. Anti-CD20 monoclonal antibody combinations with BTK inhibitors as well as BCL2 inhibitors have demonstrated superiority over traditional cytotoxic chemoimmunotherapy regimens such as fludarabine, cyclophosphamide, and rituximab; bendamustine-rituximab; and obinutuzumab-chlorambucil. Impressive clinical benefit is seen in both younger and older patients, those with comorbidities, and, most importantly, those with poor prognostic features. Given this success, combinations of BTK inhibitors and venetoclax have been explored in clinical trials. These dual-targeted regimens provide remarkable efficacy while allowing for an all-oral approach and fixed duration of treatment. Current investigations under way are evaluating the utility of a triplet approach with the addition of obinutuzumab in comparison to a doublet approach.

LEARNING OBJECTIVES

- Learn about the approved dual-targeted regimens for the frontline treatment of chronic lymphocytic leukemia
- Learn about Bruton tyrosine kinase and B-cell lymphoma 2 inhibitor combinations under investigation in frontline chronic lymphocytic leukemia

CLINICAL CASE

A 66-year-old man was diagnosed with chronic lymphocytic leukemia (CLL) after presenting with cholecystitis and was found to have a lymphocytosis of 54×10^3/μL. Peripheral blood flow cytometry demonstrated a monoclonal B-cell population with expression of CD5, $CD20^{dim}$, and CD23 and λ light chain restriction; cyclin D1 and CD10 were negative. As other complete blood count (CBC) parameters were within normal limits and he was asymptomatic, he was monitored closely. After 4 years of watch and wait, his white blood cell count increased to 220×10^3/μL. He also developed worsening fatigue and progressive anemia with a hemoglobin of 9.7 g/dL. Prognostic testing identified del(13q) by fluorescence in situ hybridization and immunoglobulin heavy chain (IGHV) somatic hypermutation at 5.8%. He presents to discuss potential treatment options. You share that a number of targeted therapies have been approved for the treatment of CLL, including Bruton tyrosine kinase (BTK) inhibitors, the B-cell lymphoma 2 (BCL2) inhibitor venetoclax, and anti-CD20 monoclonal antibodies.

BTK inhibitors

Given the additive benefit of rituximab to chemotherapy in CLL, it was quickly extrapolated that the addition of an anti-CD20 monoclonal antibody to a small-molecule inhibitor may improve depth and durability of response. Several phase 3 trials have shown this to be true, demonstrating superiority of the BTK inhibitor plus anti-CD20 monoclonal antibody combination over chemoimmunotherapy (Table 1). The ECOG 1912 study compared ibrutinib-rituximab to fludarabine, cyclophosphamide, and rituximab (FCR) in patients ≤70 years old (n=529).[1] This cooperative group trial noted a significant progression-free survival (PFS) benefit with the dual-targeted regimen; the 5-year PFS rate was 78% with ibrutinib-rituximab and 51% with FCR ($P<.0001$). An improvement in PFS was seen regardless of IGHV mutational status. There was also a modest overall

Table 1. Phase 3 trials comparing novel agents to chemoimmunotherapy in treatment-naive CLL

Reference	Study name	Regimen	Eligibility	n	Survival
Shanafelt et al[1]	ECOG 1912	Ibrutinib + rituximab vs FCR	≤70 years old without del(17p)	(n=529)	5-year PFS: 78% vs 51% (*P*<.0001) 5-year OS: 95% vs 89% (*P*=.018)
Hillmen et al[2]	NCRI FLAIR	Ibrutinib + rituximab vs FCR	≤75 years old without del(17p)	(n=771)	Median PFS not reached vs 67 months (*P*<.001) No difference in OS
Woyach et al[3]	Alliance A041202	Ibrutinib ± rituximab vs BR	≥65 years old	(n=547)	Median PFS not reached with either ibrutinib arm vs 44 months (*P*<.0001) No difference in OS
Moreno et al[4]	ILLUMINATE	Ibrutinib + obinutuzumab vs obinutuzumab + chlorambucil	≥65 years old or younger coexisting conditions	(n=229)	Median PFS not reached vs 22 months (*P*<.0001) No difference in OS
Sharman et al[5]	ELEVATE TN	Acalabrutinib ± obinutuzumab vs obinutuzumab + chlorambucil	≥65 years old or younger with comorbidities	(n=535)	Estimated 60-month PFS: 84% (A+O), 72% (A) vs 21% (O+C) No difference in OS
Tam et al[27]	SEQUOIA	Zanubrutinib vs BR	Without del(17p)	(n=479)	2-year PFS: 86% vs 70% (*p*<.0001) No difference in OS
Al-Sawaf et al[7]	CLL14	Venetoclax + obinutuzumab vs chlorambucil + obinutuzumab	CIRS >6 and/or CrCl 30–69 mL/min	(n=432)	Median PFS not reached vs 36 months (*P*<.0001) No difference in OS

CIRS, cumulative illness rating score; CrCl, creatinine clearance.

survival (OS) benefit with ibrutinib-rituximab: 95% at 5 years compared to 89% (*P*=.018). The NCRI FLAIR study had a similar trial design in patients ≤75 years old (n=771).[2] The PFS was significantly longer with ibrutinib-rituximab compared to FCR (*P*<.001), but at a median follow-up of 53 months, there was no difference in OS.

Alliance A041202 was a cooperative group study comparing ibrutinib with or without rituximab to bendamustine-rituximab (BR) in patients ≥65 years old (n=547).[3] There was a significantly longer PFS for both ibrutinib-containing arms compared to BR. At a median follow-up of 55 months, the median PFS was 44 months with BR but had not been reached with either ibrutinib arm (*P*<.0001). This benefit was seen even in higher-risk prognostic subgroups, including del(17p), del(11q), and unmutated IGHV. Interestingly, there appeared to be no significant difference in PFS between the ibrutinib monotherapy and ibrutinib-rituximab arms (*P*=.96). The ILLUMINATE study compared ibrutinib-obinutuzumab to chlorambucil-obinutuzumab in patients ≥65 years old or younger with coexisting conditions (n=229).[4] As expected, the targeted doublet produced a significantly longer PFS. At a median of 45 months of follow-up, the median PFS had not been reached in comparison to 22 months with the chlorambucil arm (*P*<.0001).

These studies highlight the efficacy and appropriateness of targeted therapy over chemoimmunotherapy, but Alliance A041202 also raises the question as to whether a dual-targeted approach is necessary when using a BTK inhibitor. The apparent lack of benefit with the addition of an anti-CD20 antibody may be attributed to the specific agents. In the ELEVATE TN study, which compared acalabrutinib (± obinutuzumab) to chlorambucil-obinutuzumab in patients ≥65 years old or younger with comorbidities, both acalabrutinib arms performed better than the chlorambucil arm (n=535).[5] However, in a post hoc analysis, the combination of obinutuzumab and acalabrutinib appeared to have a longer PFS than acalabrutinib monotherapy. At a median follow-up of 58 months, the estimated 60-month PFS rates were 84% and 72%, respectively. As the study was not powered to determine a difference between these arms, either can be considered for patients. It should be noted that the addition of obinutuzumab was associated with a greater incidence of cytopenias and grade ≥3 infections in addition to the expected infusion-related reactions.

Venetoclax + anti-CD20 monoclonal antibody

In addition to increased durability of response, it was also theorized that the addition of an anti-CD20 monoclonal antibody to venetoclax could potentially allow for a time-limited course of therapy. The feasibility and efficacy of a fixed-duration regimen was first demonstrated in the MURANO study in relapsed/refractory patients.[6] The median PFS with 2 years of venetoclax-rituximab was 54 months compared to 17 months with BR (*P*<.0001). Furthermore, there was a significant difference in OS: 82% vs 62% at 5 years (*P*<.0001). The CLL14 study subsequently evaluated a shorter course of venetoclax (1 year) with obinutuzumab in comparison to chlorambucil and obinutuzumab in treatment-naive patients (n=432).[7] At a median follow-up of 52 months, the median PFS had not been reached with venetoclax-obinutuzumab but was only 36 months with obinutuzumab-chlorambucil (*P*<.0001). The PFS benefit was seen in high-risk patients, including those with TP53 aberrations and unmutated IGHV. There was no difference in OS or an apparent difference in incidence in Richter's transformation. Both studies highlighted the utility of undetectable minimal residual disease (uMRD) in predicting a longer PFS in patients receiving venetoclax. Furthermore, CLL14 showed that patients who completed therapy with uMRD had a longer OS, 92% at 3 years after completing therapy compared to 73% in those who had detectable minimal residual disease (MRD). Rituximab and obinutuzumab

were evaluated as partners to venetoclax as part of the frontline phase 3 GAIA/CLL13 trial (n=926).[8] While these arms were not compared directly as part of the statistical design, the 15-month uMRD and 3-year PFS rates favored obinutuzumab-venetoclax (87% and 88%) over rituximab-venetoclax (57% and 81%), respectively.

Ibrutinib-venetoclax combinations

The ability to achieve uMRD has increasingly become a focus of clinical research, particularly as a tool in directing course of therapy. While the BTK inhibitors alone are typically incapable of achieving uMRD, pairing with a BCL2 inhibitor can resolve this issue (Table 2). Jain and colleagues[9] were among the first to demonstrate the success of ibrutinib and venetoclax in a phase 2 frontline study. Despite most patients possessing high-risk features, 72% achieved uMRD (defined as 10^{-4}) in the bone marrow. Patients received treatment for 24 cycles, but a trial amendment allowed for those who continued to have detectable MRD to receive an additional 12 cycles of treatment. The 4-year PFS was 95% for the entire cohort (n=120) and an impressive 91% for those with del(17p)/TP53 mutation (n=27).

The phase 2 CAPTIVATE study also evaluated the combination of ibrutinib and venetoclax with duration of therapy determined by an MRD-guided approach in previously untreated patients ≤70 years old. The trial design consisted of 2 cohorts: an MRD-driven cohort and a separate fixed-duration cohort. After 3 cycles of ibrutinib lead-in, patients received ibrutinib and venetoclax for 12 cycles. In the MRD cohort (n=164), the best uMRD response rates were 75% (peripheral blood) and 68% (bone marrow).[10] Patients were subsequently randomized. Those with uMRD were randomized to receive a placebo (n=43) or ibrutinib (n=43), whereas those who did not have confirmed uMRD received ibrutinib (n=31) or ibrutinib plus venetoclax (n=32). For those with uMRD, the estimated 30-month PFS was 95% with placebo and 100% with ibrutinib. For those who did not have confirmed uMRD, the estimated 30-month PFS rate was 95% with ibrutinib and 97% with ibrutinib-venetoclax. In the subsequent fixed-duration cohort, therapy consisted solely of 3 cycles of ibrutinib lead-in, followed by 12 cycles of the combination (n=159).[11] The overall response rate (ORR) was 96% with a complete response (CR) of 55%. uMRD rates in the peripheral blood and bone marrow were 77% and 60%, respectively. At 36 months, PFS was

Table 2. Frontline trials of BTK-BCL2 inhibitor combinations in CLL

Reference	Regimen	Eligibility (n)	Response rate	PFS	MRD
Jain et al[9]	Ibrutinib + venetoclax	del(17p), TP53 mutation, del(11q), unmutated IGHV, or ≥65 years old (n=120; n=27 del(17p)/TP53 mutation)	ORR 100% (CR 88%)	4-year PFS: 95% 4-year PFS for del(17p)/TP53 mutation: 91%	BM-uMRD at 12 cycles: 56% BM-uMRD at 24 cycles: 66%
CAPTIVATE[10–12]	Ibrutinib + venetoclax	≤70 years old MRD cohort (n=164) FD cohort (n=159)	MRD cohort: ORR 97% (CR 46%) FD cohort: ORR 96% (CR 55%)	MRD cohort: Estimated 30-month PFS for all randomized cohorts: ≥95% FD cohort: 3-year PFS: 88%	MRD cohort: BM-uMRD at 12 cycles: 68% FD cohort: BM-uMRD at 12 cycles: 60%
Kater et al[13]	Ibrutinib + venetoclax vs obinutuzumab + chlorambucil	≥65 years old or younger with CIRS >6 or CrCl <70 mL/min, without del(17p) or TP53 mutation (n=211)	ORR 87% (CR 39%) vs 85% (CR 11%)	Estimated 3.5-year PFS: 75% vs 25%	BM-uMRD at 3 months after end of treatment: 52% vs 17%
Eichhorst et al[8]	Ibrutinib + venetoclax + obinutuzumab	CIRS ≤6, normal CrCl, without del(17p) or TP53 mutation (n=230)	ORR 94% (CR 62%)	3-year PFS: 91%	PB-uMRD 15 months: 92%
Rogers et al[28]	Ibrutinib + venetoclax + obinutuzumab	Excluded patients with known BTK cysteine 481 mutation (n=50)	End of therapy ORR 90%	Estimated 48-month PFS: 96%	BM-uMRD at end of treatment: 67%
Ryan et al[19]	Acalabrutinib + venetoclax + obinutuzumab	≥18 years old No stipulation based on comorbidities or prognostic markers (n=56; n=29 TP53 aberrant)	ORR 98% (CR 48%) ORR TP53 aberrant: 100% (CR 52%)	NA	BM-uMRD by cycle 16: 86% BM-uMRD by cycle 16 for TP53 aberrant: 83%
Woyach et al[29]	Acalabrutinib + venetoclax + obinutuzumab	≥18 years old Intermediate- or high-risk CLL (n=9)	ORR 100% (CR/CRi 50%)	Estimated 18-mo PFS: 100%	PB-uMRD at cycle 10: 75%
Tedeschi et al[18]	Zanubrutinib + venetoclax	del(17p) (n=36)	ORR 97% (CR/CRi 14%)	NA	NA
Soumerai et al[20]	Zanubrutinib + venetoclax + obinutuzumab	del(17p) (n=37)	ORR 100% (CR 57%)	Median PFS not reached at 30 months	BM-uMRD by cycle 17: 89%

CRi, complete response with incomplete count recovery; FD, fixed duration.

88%.[12] Given these impressive results with the fixed-duration cohort, it is unclear whether an MRD-guided approach is truly warranted in this setting and more follow-up is necessary.

Given these exciting findings, the GLOW study sought to compare the ibrutinib-venetoclax regimen to obinutuzumab-chlorambucil in patients ≥65 years old or younger with comorbidities who did not have del(17p) or TP53 mutation.[13] The duration of ibrutinib-venetoclax was 12 cycles. While the ORRs were similar between the arms, the ibrutinib-venetoclax combination produced a significantly longer PFS (P<.001), regardless of age or comorbidities. Neutropenia was the most common grade ≥3 adverse event noted, occurring in 35% of patients receiving the biologic doublet and 50% of those receiving chemoimmunotherapy; grade ≥3 infection was seen in 17% and 12% of patients, respectively. Tumor lysis syndrome did not occur in the ibrutinib-venetoclax arm but did occur in 6% of patients receiving obinutuzumab-chlorambucil. There was no difference in OS between the arms.

CLINICAL CASE (continued)

The patient has a history of paroxysmal atrial fibrillation and would like to avoid ibrutinib. He is extremely interested in an oral regimen with a fixed duration, however, and inquires as to whether there are other options. You inform him that the BTK-BCL2 inhibitor combination has not yet been approved by the US Food and Drug Administration for CLL, but there are data to support the use of other BTK inhibitors in combination with venetoclax.

Second-generation BTK inhibitor combinations with venetoclax

Toxicity is the most common reason for discontinuation of ibrutinib in the frontline setting.[14] The second-generation BTK inhibitors, acalabrutinib and zanubrutinib, have shown improved safety profiles when compared directly to ibrutinib in relapsed/refractory CLL.[15,16] Additionally, it has become increasingly evident that ibrutinib is associated with a significant risk of ventricular tachyarrhythmias and sudden death.[17] As such, there is considerable interest in second-generation BTK inhibitor combinations with venetoclax.

Arm D of the SEQUOIA study evaluated the combination of zanubrutinib and venetoclax in treatment-naive patients with del(17p).[18] Efficacy data available from 36 patients indicated an ORR of 97% and CR/CR of 14%. Longer follow-up is needed, but the regimen appears well tolerated. Davids and colleagues[19] expanded on this concept by designing a multitargeted regimen of acalabrutinib, venetoclax, and obinutuzumab for treatment-naive patients. After a staggered initiation of each agent, patients were eligible to discontinue therapy after cycle 15 if in a CR with uMRD or after cycle 24 if with uMRD in a partial response. Of the 56 patients evaluable, the ORR was 98%, and 86% had uMRD in the bone marrow by cycle 16. In a similar frontline study of zanubrutinib, venetoclax, and obinutuzumab, the ORR was 100% (CR 57%) among the 37 evaluable patients. Eighty-nine percent of patients had achieved uMRD and were able to discontinue therapy after a median of 10 cycles.[20]

Whether a triplet regimen is necessary with these highly active targeted agents remains a question. The previously mentioned GAIA/CLL13 trial suggests that there might not be a need based on the results of a third arm consisting of obinutuzumab, venetoclax, and ibrutinib. The 15-month uMRD and 3-year PFS rates were similar to the obinutuzumab-venetoclax arms: 92% and 91% with the triplet and 87% and 88% with the doublet.[8] Several ongoing phase 3 studies are formally comparing triplet to doublet regimens (Table 3). These all-oral doublet regimens are impressive, and they challenge even the newer standard of obinutuzumab-venetoclax. The ongoing MAJIC study of obinutuzumab-venetoclax vs a fixed duration of acalabrutinib and venetoclax will help answer that question. The CLL17 study of obinutuzumab-venetoclax vs a fixed duration of ibrutinib-venetoclax vs ibrutinib monotherapy will also address this matter as well as compare the efficacy of continuous BTK inhibition.

Table 3. Ongoing phase 3 trials of BTK-BCL2 inhibitor combinations in treatment-naive CLL

Study name	ClinicalTrials.gov Identifier	Regimen	Eligibility
CLL16 (German CLL Study Group)	NCT05197192	Acalabrutinib + obinutuzumab + venetoclax vs obinutuzumab + venetoclax	High risk with either del(17p), TP53 mutation, or complex karyotype
A041702 (Alliance)	NCT03737981	Ibrutinib + obinutuzumab + venetoclax vs ibrutinib + obinutuzumab	≥65 years old
EA9161 (ECOG-ACRIN)	NCT03701282	Ibrutinib + obinutuzumab + venetoclax vs ibrutinib + obinutuzumab	<70 years old without del(17p)
MAJIC	NCT05057494	Acalabrutinib + venetoclax vs obinutuzumab + venetoclax	≥18 years old No stipulation prognostic markers
CLL-17 (German CLL Study Group)	NCT04608318	Continuous ibrutinib vs obinutuzumab + venetoclax vs fixed-duration ibrutinib + venetoclax	≥18 years old No stipulation prognostic markers

How I treat frontline CLL in 2023

Chemoimmunotherapy is no longer considered appropriate for most patients with CLL. With the plethora of novel agents now approved, oncologists have several effective options to choose from for frontline treatment. A myriad of factors should be taken into consideration, including prognostic factors, comorbidities, feasibility of administration, financial cost, and patient preference. The first decision point is whether to use a single-agent BTK inhibitor or the obinutuzumab-venetoclax combination. One of the most important details in making this decision is the presence or absence of del(17p)/TP53 aberration.

Although venetoclax was initially approved in relapsed/refractory patients with del(17p), there are limited data with obinutuzumab-venetoclax in frontline patients. It appears that a fixed duration of venetoclax cannot completely overcome the inferior prognosis associated with TP53 aberrancy. In the CLL14 study, the 4-year PFS for patients with TP53 mutation/deletion (n=25) was only 53% compared to 77% in those without (n=184).[7] In contrast, the prolonged use of a BTK inhibitor appears to be more beneficial in this population. In a pooled analysis of 89 patients with TP53 aberrations receiving ibrutinib in the frontline setting, the estimated 4-year PFS was 79%, and the median PFS had not been reached at a median follow-up of 50 months.[21] The ELEVATE-TN and SEQUOIA studies showed similar efficacy with acalabrutinib and zanubrutinib in this high-risk population; the 5-year PFS in both acalabrutinib arms was 71% and the 42-month PFS with zanubrutinib was 79%.[5,22]

Medical comorbidities such as cardiac history, bleeding tendencies, and renal dysfunction are also important in determining appropriate therapy. As previously discussed, ibrutinib is associated with significant cardiac complications. There is a suggestion that this might be true with acalabrutinib, but more data are needed.[23] All of the approved covalently binding BTK inhibitors are associated with increased bruising, which is less attractive for patients in whom there are concerns about bleeding complications. In contrast, venetoclax requires hospitalization and rigorous monitoring for tumor lysis syndrome (TLS) in patients with a decreased creatinine clearance. Both classes of drugs decrease the efficacy of the SARS-CoV-2 vaccines, which is of particular concern in this vulnerable population.[24] A fixed-duration regimen may be attractive as there appears to be some immune reconstitution after drug discontinuation that allows for a serologic response.[25]

Patient preference and feasibility of administration should also be considered. BTK inhibitors are self-administered and require relatively few clinic visits but are prescribed indefinitely and are associated with ongoing risk of adverse events. Obinutuzumab-venetoclax requires a cumbersome initial start, including frequent infusion visits and laboratory monitoring, but allow for a limited duration of therapy as well as a shorter interval for drug and financial toxicity. Furthermore, it appears that patients can be effectively retreated with venetoclax.[26]

The treatment algorithm for CLL will continue to evolve in coming years as we learn more about the BTK-BCL2 inhibitor combinations, including the populations they most benefit, the role for MRD, and how best to sequence these agents. For now, the BTK-BCL2 inhibitor combinations, with or without anti-CD20 monoclonal antibodies, remain investigational.

CLINICAL CASE (continued)

The patient completes the remainder of workup for CLL. Laboratory testing indicates a decreased creatinine clearance of 70 mL/min and mildly elevated lactate dehydrogenase (LDH) of 283. Computed tomography imaging indicates abdominal nodes of up to 6 cm. After thorough discussion with you, the patient elects to receive venetoclax and obinutuzumab. He lives close to your clinic/hospital and is comfortable with laboratory monitoring schedule required for the venetoclax dose ramp-up. Peripheral blood flow cytometry indicates uMRD at the end of the fixed duration of venetoclax.

Conflict-of-interest disclosure

Chaitra Ujjani: consultancy for Abbvie, Astrazeneca, Pharmacyclics, Janssen, Genentech, Beigene, and Eli Lilly; research funding from Abbvie, Astrazeneca, Pharmacyclics, and Eli Lilly.

Off-label drug use

Chaitra Ujjani: This article includes discussion of off-label drug use.

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Richter transformation—is there light at the end of this tunnel?

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Richter transformation (RT) represents an uncommon (2% to 10%) but feared complication of chronic lymphocytic leukemia (CLL). The disease is characterized by rapid disease kinetics, a high-risk genetic mutational profile, chemoimmunotherapy resistance, and consequent poor survival. The typical overall survival (OS) from the pre-Bruton tyrosine kinase (BTK)/B-cell lymphoma 2 (BCL2) inhibitor CLL era is 6–12 months, and recent series of RT complicating progression on a BTK or BCL2 inhibitor in heavily pretreated relapsed CLL patients suggests an OS of only 3–4 months. Despite these sobering survival statistics, novel agents have the potential to impact the natural RT disease course. This article reviews recent therapeutic developments, focusing on inhibitors of BTK, BCL2, the PD1-PDL1 axis, and T-cell–activating/engaging therapies. Herein, I discuss the importance of randomized clinical trials in a disease where small single-arm studies dominate; industry engagement, including the role of registrational studies; and the need to integrate prospectively planned correlative biological studies embedded within future clinical trials to help discover which patient benefits most from each class or combination of novel targets.

LEARNING OBJECTIVES

- To better understand the currently applied management strategy for patients with Richter transformation
- To obtain knowledge of the key novel agents in development in Richter transformation management

CLINICAL CASE

A previously fit 57-year-old man with untreated chronic lymphocytic leukemia (CLL) presented with a large, rapidly growing right-sided cervical neck mass. A biopsy revealed a CD5-positive, CD20-positive diffuse large B-cell lymphoma (DLBCL). The immunohistochemical MIB-1 (a proliferation-related antigen) index was 80%, and immunohistochemical staining revealed a nongerminal center B-cell (non-GCB) phenotype by the Hans algorithm. Fluorescence in situ hybridization and next generation sequencing was performed. No myelocytomatosis (*MYC*) or B-cell lymphoma 2 (*BCL2*) rearrangements were noted by fluorescence in situ hybridization. A *TP53* mutation/17 p deletion was observed in both the DLBCL biopsy and the peripheral blood CLL population. Given the patient's history of untreated CLL, the diagnosis of Richter transformation (RT) was made. Fluorodeoxyglucose–positron emission tomography staging demonstrated nonbulky stage III disease with a maximum standardized uptake value of 35 in the right side of the neck. The patient received 6 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) and achieved a partial metabolic remission at the end of treatment positron emission tomography/computed tomography response assessment. He received an unrelated reduced-intensity allogenic stem cell transplantation in first remission as consolidation and remained in remission for 9 months before relapsing with biopsy-confirmed stage IV non-GCB DLBCL. He was considered fit for clinical trial assessment and enrolled in a noncovalent Bruton tyrosine kinase inhibitor (BTKi) trial but unfortunately progressed after an initial partial response and died 4 months later with palliative care support.

The challenge of Richter transformation

Richter transformation (RT) represents one of the most feared complications for individuals with CLL and occurs in 2% to ~10% of patients. Three percent of a series of 2975 chemoimmunotherapy (CIT) treated CLL patients within German CLL Study Group (GCLLSG) front-line clinical trials developed RT.[1] RT most commonly represents a large cell transformation from CLL to a DLBCL-type histology. Hodgkin-like transformation, T-cell transformation, and Burkitt-like/lymphoblastic transformations are all described but are rare. RT is characterized by rapid disease kinetics,

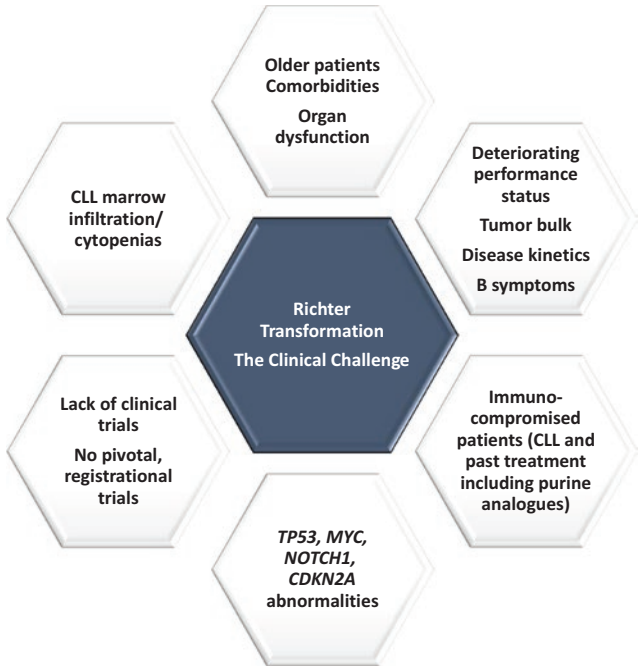

Figure 1. The clinical challenge of RT.

a poor-risk genomic profile (*TP53, MYC, NOTCH1, CDKN2A*,[2] and *DNA* damage response mutations[3]), chemotherapy-resistance and typically poor overall survival (OS) (see Figure 1). Recent comprehensive paired analysis of RT and CLL samples have improved understanding of the biological drivers of RT. RT is characterized by profound genomic instability, associated with chromothripsis/chromoplexy and whole genome duplication. Moreover, multiplexed in vivo CRISPR-Cas9 B-cell editing analysis has demonstrated tonic PI3K signaling and activation of MYC/mTOR/PI3K as a key pathway in RT.[4] For further detail on this topic, see the recent review by Parry et al. in *Blood* 2023.[5]

The median OS observed from nonrandomized studies of CIT from the pretargeted inhibitor CLL era typically ranged from 6 to 12 months.[6] Intensification of treatment beyond standard CHOP (rituximab, doxorubicin, cyclophosphamide, vincristine, prednisolone) plus anti-CD20 monoclonal antibody (mab) therapy[7,8] using infusional EPOCH-R[9] (etoposide, doxorubicin, vincristine, cyclophosphamide, prednisolone, rituximab) or combinations including purine analogues,[10] cytarabine,[11] or platinum-based therapy[12] did not improve survival outcomes but resulted in substantial treatment-related infectious morbidity and mortality. More intensive chemotherapy was clearly not better. Heavily pretreated CLL patients developing RT in the contemporary era following a targeted inhibitor such as a Bruton tyrosine kinase inhibitor (BTKi) have potentially an even worse outlook, with series demonstrating an OS of only 3–4 months.[13,14]

Although there are possible exceptions, such as patients with clonally unrelated DLBCL[15] and *TP53*-intact patients with treatment-naïve CLL,[16,17] these patients are generally in the minority, and routine, widespread testing of the clonal relationship of DLBCL to the underlying CLL by next generation sequencing or Sanger sequencing of the immunoglobulin heavy chain variable region (*IGVH*) gene or by B-cell polymerase chain reaction (PCR) clonality is limited. Most patients either do not respond to front-line CIT or progress early after an initial response, leading some to debate whether R-CHOP genuinely represents a de facto standard-of-care first-line therapy. Consolidation with allogenic stem cell transplantation (SCT) (as described in our clinical case) or autologous SCT represent standard options for patients achieving a first remission.[18] A recent Center for International Blood and Transplant Research (CIBMTR) registry study evaluated outcomes for patients following autologous SCT (*N*=53) or allogenic SCT (allo-SCT) (*N*=118).[19] The allo-SCT cohort was a higher-risk group compared with the autologous SCT cohort because a higher proportion had a 17 p deletion, more patients received prior targeted agents, and more individuals were less often in a complete remission pre-SCT. In the auto-SCT cohort, the 3-year relapse incidence, PFS, and OS were 37%, 48%, and 57%, respectively. In the allo-SCT cohort, the 3-year relapse incidence, PFS, and OS were 30%, 43%, and 52%, respectively. Depth of response prior to allo-SCT but not 17 p deletion status or prior novel agent exposure were associated with improved survival outcomes. Overall, these sizeable series suggest either approach remains valid for suitable patients obtaining a stable first remission. However, the broad applicability of allo- or auto-SCT is limited by the lack of durable disease control in first response and therefore an inherent selection in bias in published transplant series, and the patient's ability to withstand the well-documented toxicity risks with transplantation. Patient age, fitness, and comorbidity burden, and the history of CLL including prior CLL-directed treatment and its complications (eg, immunosuppression, infection, bronchiectasis), all impact these decisions.

Tight eligibility criteria for clinical trials,[20] a relative lack of available RT-specific trials, a lack of histopathological diagnostic RT reporting concordance,[21] RT kinetics, and a lack of RT cell lines and animal models have all impacted our ability to make progress in this disease. The sobering reality is that a high unmet medical need continues to exist for novel, efficacious, and well-tolerated treatment.

Light at the end of the tunnel?

So, is there light at the end of this long and rather bleak tunnel? Despite the challenges described, broader therapeutic advances in hemato-oncology are starting to impact the RT space. This includes BTK inhibitors (reversible [covalent] and nonreversible [non-covalent]), PD1-PDL1 inhibition, BCL2 inhibition, bispecific antibodies, chimeric antigen receptor (CAR) T-cell therapy, and combination strategies. Key selected recently published data and ongoing clinical trials are presented in Tables 1 and 2, respectively.

BTK inhibitors

Covalent BTK inhibitors have been transformational in CLL, mantle cell lymphoma (MCL), and Waldenström macroglobulinemia and have demonstrated some efficacy as monotherapy in RT. The second-generation BTKi acalabrutinib was shown to be active in 25 RT patients (including relapsed RT), with an overall response rate (ORR) of 40% (complete response [CR] 8%) and a median duration of response (mDOR) of 6.2 months.[22] Small case series (*N*=4) have shown activity with ibrutinib (2 PR, 1 CR, 1 clinical response).[23] Two small recently published series[24] suggest activity with the second-generation BTKi zanubrutinib as monotherapy (ORR 61.5%, CR 15.4%) and combination with the PD1 inhibitor tislelizumab (ORR 42.9%, CR 14.3%). A relatively large phase 2 GCLLSG (NCT04271956) group cooperative CLL-RT1 trial

Table 1. Clinical trials: novel agents in development in Richter transformation (RT)

Reference	Treatment	Number	ORR	Survival
Eyre et al., Lancet Haem 2021	Acalabrutinib	*N*=25	ORR 38% CR 14%	mPFS 3.2 m mDOR 5.7 m
Tsang et al., Blood 2015	Ibrutinib	*N*=4	2 PR, 1 CR, 1 clinical benefit	Median duration on treatment 6.1 m
Wierda et al., ASH 2022	Pirtobrutinib	*N*=75	ORR 52% CR 13%	mPFS 3.7 m
Tam et al., HemaSphere 2023	Zanubrutinib	*N*=13	ORR 61.5% CR 15.4%	mPFS 17.3 m
Tam et al., HemaSphere 2023	Zanubrutinib-tislelizumab	*N*=7	ORR 42.9% CR 14.3%	mPFS 2.9 m
Jain et al., Blood Adv. 2022	Ibrutinib-nivolumab	*N*=24	ORR 42% CR 34%	mOS 13 m
Ding et al., Blood 2017	Pembrolizumab	*N*=9	ORR 44% CR 11%	mOS 10.7 m
Armand et al., BJH 2020	Pembrolizumab	*N*=23	ORR 13% CR 4%	mOS 3.8 m
Davids et al., JCO 2017	Venetoclax	*N*=7	ORR 43% No CRs	NK
Davids et al., Blood 2022	Venetoclax-EPOCH-R	*N*=12 evaluable *N*=20 total	ORR 75% CR 67%	mPFS is 10 m mOS is 16.3 m
Davids et al., ICML 2023	R-CHOP-venetoclax	*N*=25 evaluable *N*=27 total	ORR 68% CR 48%	mPFS 7.2 m mOS 19.5 m
Mato et al., ASH 2020	Novel BTKi, DTRMWXHS-12 (DTRM-12), everolimus and pomalidomide	*N*=11	ORR 36%	NK
Kater et al., ASH 2022	Epcoritamab	*N*=10	ORR 60% CR 50%	NK
Carlo-Stella et al., ICML 2023	Glofitamab	*N*=11	ORR 63.6% CR 45.5%	NK

CHOP-R, doxorubicin, vincristine, cyclophosphamide, prednisolone, rituximab; CR, complete response; EPOCH-R, etoposide, doxorubicin, vincristine, cyclophosphamide, prednisolone, rituximab; m, month; mDOR, median duration of response; mOS, median overall survival; mPFS, median progression-free survival; NK, not known; ORR, overall response rate; PR, partial response.

(*N*=52) of zanubrutinib in combination with the tislelizumab[25] has fully enrolled and the results are eagerly awaited. The National Cancer Research Institute UK-wide first-line STELLAR trial is currently enrolling to test whether the addition of acalabrutinib to R-CHOP provides a progression-free survival (PFS) improvement compared with R-CHOP alone.[26] This is the first and currently the only randomized clinical trial globally in RT.

Pirtobrutinib is a first-in-class, noncovalent, reversible BTKi. Pirtobrutinib inhibits both wildtype and C481-mutant BTK with equal low nanomolar potency and has a favorable oral pharmacology that enables continuous BTK inhibition throughout the dosing interval regardless of intrinsic rate of BTK turnover.[27] Drug plasma exposures exceeds BTK IC_{90} throughout the 24-hour dosing interval. These favorable pharmacokinetic properties may enable enhanced therapeutic activity in more highly proliferative tumors that remain dependent on B-cell receptor signaling, such as MCL and RT. The phase 1/2 BRUIN trial has recruited 82 RT patients with efficacy data available for 75 patients to date and included 68 patients who had received prior RT treatment (median prior lines of RT treatment was 2 [0–8]).[28] The ORR was 52% and CR rate 10%, with an ORR of 47% in patients who received a prior covalent BTKi and an ORR of 50% with RT who had received prior RT-directed therapy. The mDOR was 5.6 months, median PFS 3.7 months and median OS 13.1 months. The toxicity prolife for pirtobrutinib across all B-cell histologies (*N*=773) and the RT cohort (*N*=82) was favorable, with only 2.6% discontinuing due to treatment-related adverse events and only 4.5% requiring dose reductions, lending itself well to future combination strategies. The combination of time-limited pirtobrutinib-venetoclax-obinutuzumab is currently being studied in RT (NCT05536349).

PD1-PDL1 axis inhibitors

The PD1-PDL1 axis is known to be upregulated in the RT microenvironment, although the data regarding efficacy of PD1-PD1L axis inhibition are mixed. A small series (*N*=9) provides proof of principle of the activity of PD1 inhibitors in RT. Pembrolizumab monotherapy delivered at 200mg every 3 weeks has demonstrated an ORR of 44%, a CR rate of 11%, and a median PFS of 10.7 months.[29] A trend of increased expression in PD1 was observed in the tumor microenvironment in RT patients who had confirmed responses. PD1 inhibitors are also well tolerated, and combination strategies have also been tested. The nivolumab-ibrutinib combination provided an ORR of 42%, with potentially deeper responses than with a PD1 inhibitor or BTKi alone (CR rate 34%).[30] Less encouraging was a small nontrial cohort (*n*=10) from The Ohio State who had

Table 2. Summary of key ongoing trials in Richter transformation (RT)

Trial name and identifier	Planned enrollment	Trial design	Novel treatment
Bispecific antibody			
EPCORE CLL-1, NCT04623541	102 (RT and CLL)	Single-arm phase 2	Anti-CD20/CD3 bispecific antibody in recruiting patients with CLL or RT
Doublet/triplet combination			
GCLLSG CLL-RT1, NCT04271956	52	Single-arm phase 2	Tislelizumab, a PD1 inhibitor, with zanubrutinib, a 2nd BTK inhibitor in R/R or 1L RT
Israeli CLL Study Group, GIVeRS, NCT04939363	15	Single-arm phase 2	Obinutuzumab, ibrutinib, and venetoclax for 1L or R/R RT
Acalabrutinib, Venetoclax and Durvalumab for the Treatment of RT, NCT05388006	33	Single-arm phase 2	Time-limited acalabrutinib, venetoclax, and durvalumab for patients with 1L RT
Atezolizumab (PD-L1 mAb) in Combination With Obinutuzumab and Venetoclax for Patients With Chronic Lymphocytic Leukemia and Richter Transformation, NCT02846623	65 (RT and CLL)	Single-arm phase 2	Time-limited atezolizumab, venetoclax, and obinutuzumab for patients with 1L RT
Pirtobrutinib, Venetoclax, and Obinutuzumab, NCT05536349	60 (RT and CLL)	Single-arm phase 2	Time-limited pirtobrutinib, venetoclax, and obinutuzumab for patients with 1L CLL or RT
BTK inhibition			
BRUIN, NCT03740529	82	Single-arm phase 1/2	Pirtobrutinib monotherapy in 1L and R/R RT
Chemoimmunotherapy plus targeted inhibitor			
NCRI STELLAR trial, NCT03899337	60	Randomized phase 2	R-CHOP versus R-CHOP-acalabrutinib in 1L RT
Venetoclax Plus Dose-Adjusted R-EPOCH or R-CHOP for RT, NCT03054896	66	Single-arm phase 2	Venetoclax plus dose-adjusted R-EPOCH (*N*=26) or R-CHOP (*N*=40) for 1L RT
CAR-T–based combinations			
Lisocabtagene Maraleucel, Nivolumab and Ibrutinib for the Treatment of RT, NCT05672173	20	Single-arm phase 2	Lisocabtagene maraleucel, nivolumab, and ibrutinib

BTK, Bruton tyrosine kinase; CAR-T, chimeric antigen receptor T cell; CHOP-R, doxorubicin, vincristine, cyclophosphamide, prednisolone, rituximab; CLL, chronic lymphocytic leukemia; EPOCH-R, etoposide, doxorubicin, vincristine, cyclophosphamide, prednisolone, rituximab; GCLLSG, German chronic lymphocytic leukemia study group; NCRI, National Cancer Research Institute; R/R, relapsed/refractory; RT, Richter transformation.

only a 10% ORR with PD1 inhibitor combinations/monotherapy and a trial cohort of 23 patients in which the ORR was only 13%.[31,32] Ongoing trials are testing triplet combinations including a PD1-PD-L1 axis inhibitor in RT, namely acalabrutinib, venetoclax, and durvalumab (PD-L1 mab) (NCT05388006) and obinutuzumab, venetoclax, and atezolizumab (PD-L1 mab) (NCT02846623).

B cell lymphoma 2 inhibitors

Promising early data of the BCL2 inhibitor venetoclax in RT patients has led to its exploration in combination with both targeted inhibitors and CIT. Initially, responses were seen in 3 of 7 patients receiving monotherapy in a B-cell malignancy basket phase 1 trial.[33] The combination of venetoclax with standard CIT has been explored in a first-line single-arm phase 2 trial[34] with the hypothesis that the BCL2 inhibitor may sensitize the RT tumor to CIT. Venetoclax was delivered with an accelerated daily ramp-up to the target dose of 400mg after cycle 1 and continued across the following 5 cycles of CIT. The CR rate for 26 patients receiving venetoclax plus dose-adjusted EPOCH-R was 50%, with 11 achieving bone marrow minimal residual disease for the CLL disease component. The ORR was 62%, median PFS 10.1 months, and median OS 19.6 months. Hematological toxicity was notable in this study, with grade ≥3 neutropenia in 65%, febrile neutropenia in 38%, and a single fatal episode of sepsis observed with venetoclax-EPOCH-R. Daily venetoclax ramp-up was safe with no tumor lysis syndrome events reported. The encouraging deep and durable responses have led to an extension of this study with a total of 67 patients enrolled (NCT03054896), and the final results are awaited. The CIT backbone was deintensified from dose-adjusted EPOCH-R to R-CHOP because of excess toxicity (cytopenias, infection). Forty patients received R-CHOP-venetoclax, an approach that enabled outpatient delivery (personal communication), with initial results of the first 27 patients presented at ICML 2023 (ORR 68%, CR 48%).[35] A real-world analysis[36] from the Mayo Clinic and MD Anderson suggests that R-CHOP-venetoclax may improve PFS compared with standard CIT approaches or the BTKi-BCL2i-Obinutuzumab triplet, although a prospective randomized first-line trial is required to formally answer this question. BCL2 targeting has also formed part of a range of combination strategies in ongoing, enrolling clinical trials as already discussed (NCT05388006, NCT05536349, NCT02846623, NCT04939363).

Anti-CD20-CD3 bispecific antibodies

Early data with the anti-CD20-CD3 bispecific antibody epcoritamab have been recently presented.[37] Epcoritamab and glofitamab bind to CD3 on T cells and CD20 on B cells to induce T-cell–mediated killing of CD20-positive malignant B cells. Bispecific antibody development in RT/CLL has been slow compared with DLBCL and follicular lymphoma (FL) in part because of the rarity of the phenomenon (RT) but also because of (a) the risk of severe cytokine release syndrome considering the circulating

peripheral blood CLL component and (b) T cell dysfunction in CLL patients. Epcoritamab is delivered subcutaneously to progression or intolerance whereas glofitamab is delivered intravenously for a fixed duration. The initial results from the RT cohort in the ongoing phase 1b/2 EPCORE CLL-1 trial observed an ORR of 60% and CR of 50% with epcoritamab in 10 RT patients.[37] This study (NCT04623541) continues to accrue patients (the aim is for 102 RT or CLL patients), and we await a mature larger data set with great interest. Recently, responses were reported in 63.6% (CR 45.5%) in 11 RT patients receiving glofitamab[38] providing further early evidence of anti-CD20-CD3 bispecific antibody activity in RT.

Chimeric antigen receptor (CAR) T-cell therapy

Finally, anti-CD19–directed CAR-T therapy has changed the treatment paradigm of relapsed, refractory large B-cell lymphoma,[39] MCL,[40] and FL[41] over recent years. Although data specifically in RT remain limited, the treatment approach is highly promising. Two recently published real-world series from the US[42] and Israel[43] have demonstrated ORRs of 89% with Axi-cel (*N*=9, CR *N*=5) and 71% (*N*=8 including 1 "accelerated CLL" and 1 prolymphocytic transformation, CR *N*=5), respectively. Global availability of anti-CD19 CAR-T-cell therapy for RT remains highly variable. At present, CAR-T therapy is considered a standard treatment option in the third-line setting in the United Kingdom for RT patients who have received ≥2 prior DLBCL treatments including R-CHOP.[44] An important ongoing clinical trial is assessing the anti-CD19 CAR-T lisocabtagene maraleucel in combination with nivolumab and ibrutinib. Both nivolumab and ibrutinib have the potential to enhance the activity of CAR-T-cell therapy via independent mechanisms (PD1-PDL1 axis and upregulation of T-cell activity via ITK inhibition,[45] respectively), and both also provide direct anti-RT tumor activity (NCT05672173).

Future targets?

Most current studies involve combinatorial approaches targeting BTK, BCL2, or PD1 or integrating T-cell engaging therapy. Examples of future targets beyond these approaches may include targeting the feto-embryonic antigen ROR1, the MAPK pathway,[3] MYC/mTOR-PI3K signaling the cell cycle regulator CDK9, and the nuclear pore complex (XPO1).[5]

Future directions

The recent explosion in targeted therapeutics across hemato-oncology has started to impact RT management. Promising targets include inhibition of BTK, BCL2, the PD1-PDL1 axis, and T-cell–activating/engaging therapies. Many of these therapies are particularly well tolerated and lend themselves well to combinatory studies. Despite this promise, no agents have been licensed or reimbursed specifically for RT in the United States and Europe. Genuine progress in RT suffers from the relative rarity of the disease, the small commercial impact of any potential future drug approval, and the consequent lack of investment into registrational trials from major industry partners. This must change if we are to see the impact of novel agents in this catastrophic disease—the light at the end of the tunnel. Although the portfolio of agents studied over recent years is increasingly impressive, the relative lack of clinically meaningful, correlative biological sub-studies is also noteworthy. The role of precision medicine will become increasingly important in a disease where multiple agents have potential efficacy but typically modest response rates. Carefully designed, planned correlative biological embedded studies should be strongly encouraged within future clinical trials to help discover which patient benefits from which class or combination of novel targets.

Conflict-of-interest disclosure

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A rational approach to functional high-risk myeloma

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Multiple myeloma is a clinically and biologically highly heterogeneous disease, as the overall survival can vary from more than a decade in patients with standard risk disease treated with intensive chemotherapy to 2–3 years in patients with high-risk features. The current staging systems, which rely on baseline biological risk factors to stratify patients into groups with differing risks of progression or death, are sometimes suboptimal tools for identifying high-risk patients. This is particularly evident when considering the so-called functional high-risk patients—patients who do not necessarily display baseline high-risk features but typically show a suboptimal response to induction therapy or relapse early after treatment initiation: the survival of these patients is particularly poor even in the context of newer therapies. The prompt identification, as well as a consistent definition, of this subset of patients, as well as their management, currently represents an unmet medical need. In this review we explore the main characteristics of functional high-risk patients, the available known risk factors and scoring systems, and the possible management.

LEARNING OBJECTIVES

- Identify the patients with functional high-risk multiple myeloma
- Outline a possible therapeutic strategy for patients with functional high-risk multiple myeloma
- Define possible risk factors of suboptimal response and early relapse

CLINICAL CASE

A 58-year-old man with newly diagnosed (ND), International Staging System (ISS) stage I, Revised ISS (R-ISS) stage II IgG-κ multiple myeloma (MM) was referred to our center. The patient was symptomatic for bone lesions (L3 vertebral fracture) and presented a paraskeletal plasmacytoma involving the right and left pedicles on magnetic resonance imaging. The bone marrow biopsy showed 30% plasma cell infiltration, and fluorescent in situ hybridization (FISH) analysis on bone marrow aspirate was negative for del(17p), t(4;14), t(14;16), and chromosome 1 abnormalities. The patient had no comorbidities and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 1, related to the bone disease.

The patient started treatment with 4 cycles of daratumumab, bortezomib, thalidomide, and dexamethasone (DVTd), achieving a partial response (PR) after the first cycle, with no significant decrease in the monoclonal (M) component during the subsequent cycles. After the induction phase, the patient underwent stem cell mobilization and collection and high-dose melphalan and autologous stem cell transplantation (HDM-ASCT), without a further decrease in the M-component. Two months after ASCT, a sudden increase of the M-component was observed along with the onset of hypercalcemia. The FISH analysis carried out on bone marrow plasma cells at relapse showed the acquisition of del(17p). A second-line treatment with carfilzomib, lenalidomide, and dexamethasone (KRd) was started. The patient achieved a very good partial response (VGPR), which is currently ongoing 24 months after treatment initiation.

How do we define high risk in MM?

The prognosis of MM has greatly improved in the last 2 decades as a result of the introduction of new agents, their combinations into multidrug regimens, and the use of HDM-ASCT. However, the biological and clinical diversity of MM reflects its heterogeneous clinical courses and prognosis; therefore, the overall survival (OS) of a NDMM patient ranges from 2 to 3 years in the presence of high-risk features to more than 10 years in standard-risk disease.[1]

Table 1. Risk factors and stratification models in patients with multiple myeloma

ISS[59]	R-ISS[17]	R2-ISS[18]	Other risk factors
Stage I: serum β2M < 3.5 μg/L and serum albumin ≥3.5 g/dL **Stage II:** not ISS stage I or III **Stage III:** serum β2M ≥ 5.5 μg/L	**Stage I:** ISS stage I, t(4;14), and/or t(14;16) and/or del(17p) negativity by FISH and normal serum LDH **Stage II:** not ISS stage I or III **Stage III:** ISS stage III and either elevated serum LDH or t(4;14) and/or t(14;16) and/or del(17p) positivity by FISH	Additive score: ISS II: 1 point ISS III: 1.5 points Del(17p): 1 point Elevated serum LDH: 1 point t(4;14): 1 point 1q+: 0.5 point Groups: **Low risk**: 0 **Low intermediate:** 0.5–1 **Intermediate-high**: 1.5–2.5 **High**: 3–5	Genetic lesions: deletion and mutations of *TP53*[5]; deletion chromosome 1p detected by FISH[4] Extramedullary disease[60] CTCs detected in the peripheral blood by flow cytometry[14,15] Plasma cell leukemia and plasma cell leukemia–like disease[16,61] GEP: high-risk signatures[9–12]

Several biological and clinical risk factors correlate with an aggressive disease, and risk models have been developed to predict the risk of relapse or death. High serum values of β_2-microglobulin (B2M), a marker of tumor burden and renal insufficiency; high lactate dehydrogenase (LDH) serum values linked to plasma cell proliferation; and low albumin values, reflecting systemic inflammation, are validated risk factors that correlate with disease aggressiveness.[2]

Recurrent chromosomal abnormalities detected by FISH, including t(4;14), t(14;16), and del(17p), are detected in up to 15% to 20% of MM patients at diagnosis, and their presence is associated with shorter progression-free survival (PFS) and OS.[2] Copy number alterations involving the long arm of chromosome 1 (1q), detected in up to 30% of patients at diagnosis, portend a worse survival.[3] Del(1p32) is another adverse feature.[4] The number of high-risk chromosomal abnormalities, or the co-occurrence of mutations such as *TP53* inactivation,[5] are additional prognostic factors, as patients with so-called double-hit or ultra-high-risk myeloma (two or more high-risk genetic lesions) consistently showed worse survival outcomes compared to those with 1 or no high-risk genetic alteration.[6-8] In addition to cytogenetics, different gene expression profile (GEP) signatures have been demonstrated to be independent prognostic factors for both PFS and OS, thus providing an additional method to identify high risk.[9-12] The spread of myeloma cells outside the bone marrow is another unfavorable prognostic factor. The presence of extramedullary plasmacytomas is an established risk factor for both PFS and OS.[13] Several groups have demonstrated that circulating tumor cells (CTCs),[14,15] even when the criteria for plasma-cell leukemia are not fulfilled, correlate with shorter survival. Furthermore, MM with plasma-cell leukemia–like status, identified by transcriptome profile, exhibits an aggressive disease course.[16]

The current risk-stratification model recommended by the International Myeloma Working Group, the R-ISS,[17] stratifies patients into 3 risk groups with a different OS (stage I: not reached [NR]; stage II: 83 months; and stage III: 43 months); although the majority of patients (62%) fall into the intermediate-risk category. To account for this issue, while also including chromosome 1q alterations, the European Myeloma Network has recently proposed a second revision of the R-ISS (R2-ISS) that stratifies patients into 4 risk categories, with a more homogeneous repartition (Table 1).[18]

What is functional high risk?

Despite the improvement in baseline risk-stratification, a significant proportion of patients not classified as high-risk at diagnosis will progress within 12 to 18 months from treatment initiation despite an optimal initial therapy: these are considered *functional high-risk* (FHR) patients.[19,20] Studies focusing on early relapse and associated risk features are heterogeneous. They include transplant-eligible and non-eligible patients, treated up front with immunomodulatory agents (IMiDs) and proteasome inhibitors (PIs) in most cases, while data in patients treated up front with anti-CD38 monoclonal antibodies (MoAbs) are so far lacking. Early relapse is commonly defined as occurring within 12 to 18 months from initial treatment,[21,22] 24 months in a few previous reports.[23,24] Patients experiencing early relapse will display a short OS, ranging from 18 to 32 to 44 months (Table 2).

Currently approved regimens incorporating up-front anti-CD38 MoAbs have significantly reduced the risk of early relapse at 12 to 24 months to approximately less than 10% in transplant-eligible and 20% in non–transplant-eligible patients compared to older regimens.[25-27] Given these positive results, the design of specific clinical trials for these high-risk populations has become more challenging. The case presentation described a patient with FHR MM: despite the lack of a baseline high-risk feature, the disease relapsed early (12 months since initial diagnosis), thus indicating an aggressive clinical course.

How can we identify early FHR?

Several groups have made the effort to define risk factors for an early relapse and to incorporate them into a scoring system (Tables 2 and 3).[28-32] Markers of high tumor burden and organ damage (anemia, thrombocytopenia, high plasma cell infiltration, hypercalcemia, renal insufficiency, high LDH),[21,22,24] advanced myeloma stage (Durie and Salmon stage III,[23] ISS stage III,[21-23,33] R-ISS stage III[21,34]), and high-risk cytogenetic features are frequently observed in patients experiencing early relapse.[22,33,35] Nevertheless, a proportion of "standard-risk" patients relapse early. As an example, ISS-I was reported in 22% of early-relapse patients and standard-risk cytogenetic in 12% to 28%.[22,33] Studies are heterogeneous in terms of baseline features analyzed, and only the most recent reported a more comprehensive evaluation including R-ISS, extended cytogenetic evaluation (1q and 1p abnormalities), and mutational status (p53, *IGLL5* mutation, interleukin 6/JaK/STAT3 pathway).[35,36] Indeed, as both GEP and the presence of CTCs have been shown to complement and

Table 2. Studies evaluating functional high risk

Reference	Study population, N	ER or FHR definition	ER, N (%)	First-line treatment, N (%)	Median OS (early vs late relapse)	Factors influencing ER		
						Baseline factors	Treatment related	Impact of response
Jimenez-Zepeda et al 2015[40]	Princess Margaret Cancer Center, N=184	ER: progressive disease within 12 months from transplant	27 (14)	In overall population: • PI based: 119 (64.7) • IMiD based: 65 (35.3) • ASCT: 184 (100)	20 mo vs 93 mo (*P*=.001)		**Favoring** • Thalidomide induction regimens (*P*=.04)	Patients with ER showed a lower ≥ VGPR rate that those with non-ER
Kumar et al 2018[23]	Center for International Blood and Marrow Transplant Research database, N=3256	ER: progressive disease within 24 months from transplant	1239 (38)	In overall population: • Bort based: 748 (22) • Len based: 342 (10) • Len-Bort based: 545 (17) • ASCT: 3256 (100)	44.7 mo vs 113.7 mo (*P*<.001)	**Favoring:** • DS/ISS III (*P* = .02) **Protective:** • Chemo sensitivity (*P* = .007)	**Protective:** • Transplant after 2008 (*P* = .02) • Post-ASCT maintenance with novel agent (*P*=.02)	
Spencer et al 2019[21]	Australian and New Zealand Myeloma and Related Diseases Registry, N=1320	FHR: ER + SOR ER: progressive disease within 12 months of commencing 1st line of therapy SOR: best response to 1st line minimal response or stable disease	FHR: 270 (20.4) ER: 118 (8.9) SOE: 152 (11.5)	N/A	ER, 20.2 mo vs 60.7 mo (*P*<.001) SOR, 57.8 mo vs 59.3 mo	**ER, favoring:** • Higher ISS (*P*<.001); • Higher R-ISS (*P*<.001); • Inferior ECOG (*P* =.007); • Hypercalcemia (*P*= .002); • Renal insufficiency (*P*<.001); • Anemia (*P*<.001) **SOR, favoring**: • Age >70y (*P*= .01)	**SOR, favoring:** • Bort based (*P* = .001)	SOR (MR or SD) in early vs late relapse: 25% vs 11% (*P*<.001)
Kastritis et al 2020[24]	Department of Clinical Therapeutics, Athens (Greece), N=297	ER: progressive disease within 12 months from transplant	43 (14.5)	In overall population: • Bort based: 139 (47) • Len based: 248 (6) • Bort + IMiD: 26 (9) ASCT: 297 (100)	mOS 18 mo (early relapse) vs 5-years OS 71% (late relapse)	**Favoring:** • LDH ≥ ULN (*P* =.018) • Hypercalcemia (*P*= .034)	**Protective:** • Consolidation therapy (*P*<.001) • Maintenance (*P*<.048)	Response rates and depth of response to induction therapy were not significantly different among those with early vs later relapse
Corre et al 2020[33]	Retrospective study, N=2627 patients	ER: progressive disease within 18 months from initial therapy or within 12 months from transplant	496 (18.9)	In overall population: • PI based: 1129 (43) • PI + IMiD: 1485 (57) ASCT: 2627 (100)	HR 4.40 (*P*<.0001)	**Favoring:** • ISS II/III (*P*<.001) • High-risk cytogenetics, including del(17p) or t(4;14) or gain 1q or del(1q32) (*P*<.001) **Protective:** • Trisomy 5 (*P* = .0024)		• Poor response to treatment (<VGPR) (*P*<.001)

Table 2 Studies evaluating functional high risk (Continued)

Reference	Study population, N	ER or FHR definition	ER, N (%)	First-line treatment, N (%)	Median OS (early vs late relapse)	Factors influencing ER		
						Baseline factors	Treatment related	Impact of response
D'Agostino et al 2020[35]	CoMMpass data set, N=926	ER: progressive disease within 18 months from diagnosis	191 (20.6)	In overall population • Bort based: 83 (9) • Len based: 63 (7) • Bort + Len based: 319 (34) • Carf based: 215 (23) ASCT: 440 (53)	32.8 mo vs 54 mo (ISS III) vs 65 mo (cytogenetics high risk)	**Favoring:** • *TP53* mutation (*P*<.001) • High LDH (*P*=.006) • L-chain translocation (*P*=.033) • *IGLL5* mutation (*P* = .007)	**Favoring:** • Refractoriness to PIs (*P*<.001) or IMiDs + PIs (*P*<.001) **Protective:** • Carfilzomib-based induction (*P*=.01)	• Lower ORR (*P*<.001; • Poor response to treatment (<VGPR) (*P*<.001)
Bygrave et al 2021[22]	NCRI Myeloma XI, N=1349	ER: progressive disease within 12 months from transplant	174 (12.9)	CTd vs CRd as induction treatment If ≤ VGPR prior ASCT: VTd ASCT = 1349 (100)	26 mo vs 91 mo (*P*<.001)	**Favoring:** • Anemia (*P*<.0001) • Low platelet count (*P* = .0001) • Heavy plasma cell infiltration (*P*<.0001) • Advanced ISS stage (*P* = .0029) • High-risk genetic (*P*<.0001)	**Protective:** Len-based maintenance (*P* = .0005)	
Soekojo et al 2022[36]	CoMMpass data set, N=512	FHR: primary refractory to induction therapy plus progressive disease within early relapse within 12 months of starting induction therapy without high-risk cytogenetics (ER)	FHR: 61 (11)	In FHR population: • PI based: 43 (36) • IMiD based: 18 (15) • PI + IMiD: 50 (42.7) ASCT = N/A	27.6 mo (FHR) vs 44.7 mo (GHR) vs NR (SR) (*P*<.001)	**Favoring:** mutations affecting the IL-6/Jak/STAT3 pathway, associated with aberrant mitosis and DNA damage response		

Bort, bortezomib; Carf, carfilzomib; CTd, cyclophosphamide-thalidomide-dexamethasone; ER, early relapse; GHR, genomic high risk; IL-6, interleukin 6; Len, lenalidomide; mOS, median overall survival; MR, minimal response; N/A, not available; NCRI, National Cancer Research Institute; ORR, overall response rate; SOR, suboptimal response; SR, standard risk; STAT3, signal transducer and activator of transcription 3; ULN, upper limit of normal; VTd, bortezomib-thalidomide-dexamethasone.

Table 3. Studies evaluating scoring systems to identify the risk of early relapse

Score	Variables	Risk groups (sum)	Clinical outcomes
CIBMTR scoring system[28]	• High-risk cytogenetics[a]: +4 points • Pre-ASCT BMPCs ≥10%: +4 points • Albumin at diagnosis ≤3,5 g/dL: +2 points • Standard-risk cytogenetic: +1 point • No cytogenetic abnormality, BMPCs <10% at ASCT, and albumin ≥3.5 g/dL at diagnosis: +0 point	• Low risk (0–3) • Intermediate risk (4–8) • High risk (9–10)	3-year PFS: 58% vs 49% vs 31% (*P*<.001) 3-year OS: 88% vs 81% vs 64% (*P*<.001)
S-ERMM(18) score[29]	• LDH > ULN: +5 points • Presence of t(4;14): +5 points • Presence of del(17p): +3 points • Abnormal albumin: +3 points • BMPCs >60%: +3 points • FLC λ: +2 points	• Low risk (≤5) • Intermediate risk (6–10) • High risk (≥11)	Median OS: NR vs 59.5 mo vs 31.5 mo (*P*<.001) Median PFS2: 62.3 mo vs 40 vs 19.8 mo (*P*<.001)
DS-ERMM score[29]	• S-ERMM score (0–21 points) • Achievement of at least VGPR: −4 points	• Low risk (≤0) • Intermediate risk (1–5) • High risk (≥6)	Median OS: NR vs NR vs 57.3 mo (*P*<.001) Median PFS2: NR vs 53.8 mo vs 40.2 mo (*P*<.001)
EBMT scoring system[30]	• Disease status at ASCT: 0–3 points CR/VGPR: +0 point PR/SD/MR: +1 point Rel/prog: +3 points • ISS: ISS I: +0 point ISS II: +1 point ISS III: +2 points • Age (years): −1 to −3 points ≤55: −1 point; 55–75: −2 points ≥75: −3 points	Score −2 Score −1 Score 0 Score 1 Score 2	12-mo PFS2, score −2 vs score 2: 91% vs 65%
EBMT scoring system[31]	• Disease status at auto-HSCT: 0–4 points CR/VGPR: +0 point PR: +1 point PR/SD/MR: +2 points Rel/prog: +3 points- ISS: 0–2 points ISS I: +0 point ISS II: +1 point ISS III: +2 points • Karnofsky performance status: +1 point	• Risk score 0 (0) • Risk score 1 (1) • Risk score 2 (2) • Risk score 3 (3) • Risk score 4 (≥4)	12-mo PFS, risk score 0 vs risk score 4: 91.7% vs 57.1%

[a]t(4;14),t(14;16),t(14;20), del(13q/monosomy 13 on karyotype), del(17p),1q gain,1p del.

BMPCs, bone marrow plasma cells; CIBMTR, Center for Blood and Marrow Transplant Research; CR, complete response; DS-ERMM, dynamic simplified early relapse in multiple myeloma; EBMT, European Society for Blood and Marrow Transplantation; FLC, free light chain; MMRF, Multiple Myeloma Research Foundation; MR, minimal response; NR, not reached; PFS2, progression-free survival-2; Rel/prog, relapse/progression; SD, stable disease; S-ERMM18, simplified early relapse in multiple myeloma (18 months); ULN, upper limit of normal.

refine the prognostic information provided by commonly evaluated risk factors, the lack of access to such tools in the community setting limits our ability to properly identify high-risk patients at diagnosis.[14,15,37,38] Their integrations in clinical practice could allow a more precise identification of patients at high risk of early relapse, although some patients with FHR will likely be identified only due to disease evolution. However, whether an early relapse is due to a treatment-induced clonal selection that leads to the early emergence of a highly resistant MM clone or simply to an inadequate risk evaluation at baseline remains to be determined.

Many reports consistently highlight the potential impact on survival of response to therapy as a dynamic factor, particularly when considering minimal residual disease (MRD) negativity.[39] Unfortunately, most of the studies focusing on the risk of early relapse included data on patients treated in the last 10 years with IMiDs and/or PI-based regimens and lack MRD data. In these studies the achievement of a suboptimal response (eg, less than VGPR) was more frequent in patients with early relapse.[22,33,35,40] Similarly, a large metanalysis on 2190 patients showed that the incorporation of the response achieved (at least VGPR vs not) into the baseline risk score changed the risk status in 56% of patients, with the rate of patients at risk of an early relapse increasing from 7% to 20%.[29]

In today's clinical practice, the achievement of at least a VGPR could be an acceptable early dynamic prognostic factor, being a standard biochemical response evaluation achievable in a significant proportion of patients with most of the current therapies and supported by data from numerous reports. MRD status, which is a better predictor of outcome than VGPR, may replace the current response system and become a dynamic predictor of early relapse in the near future. In this regard both the incorporation of imaging techniques (eg, positron emission tography/computed tomography), demonstrated to be complementary to bone

marrow MRD testing and possibly of particular importance in high-risk patients, where extramedullary disease is more common,[41,42] and sustained MRD negativity may play a key role in modulating the risk of early relapse,[6,43,44] thus impacting treatment strategies for standard-risk—and, more importantly, for high-risk— disease.

How can we manage FHR patients?

Patients with FHR currently represent an unmet medical need. In general, for patients with high-risk disease, up-front multiagent chemotherapy, single or tandem transplant, and single- or double-agent maintenance, when tolerated, are generally recommended.[45,46] The treatment-free interval should be limited, as the disease may respond to therapy but rapidly relapse, especially if treatment is interrupted or de-escalated.[44] Data from the MASTER trial showed that treatment interruption in very high-risk patients, even when MRD negativity is achieved, leads to a higher risk of MRD resurgence and suboptimal PFS.[47] In addition, post hoc analysis of the FORTE study showed that doublet maintenance (carfilzomib-lenalidomide) compared with single-agent lenalidomide reduced the risk of MRD resurgence, but this is true only during doublet therapy, as after stopping carfilzomib the risk is equal to a patient receiving lenalidomide alone, and this is particularly evident in patients with high-risk disease.[44]

As FHR is currently defined by the pattern of relapse, specific considerations must be made. First, disease progression during treatment or soon after stopping therapy means the disease is refractory to that treatment; studies reported a high proportion of refractory patients in the early relapsed group.[35] The patient discussed in our clinical case relapsed 2 months after HDM and less than 6 months after DVTd, meaning he can be considered refractory to HDM and to have a suboptimal duration of remission after DVTd, which would advise against retreatment with the same agents.[48,49] A study analyzing the pattern of clonal evolution suggests that depth of response to treatment is the main determinant of the evolutionary pattern: patients relapsing early under treatment or with a suboptimal response mostly present a linear clonal evolution pattern, whereas patients achieving deep treatment response (complete response [CR] or MRD-negative status) are more likely to follow a branching evolutionary pattern.[50] These data provide the rationale to investigate intensification strategies in patients with a suboptimal response to up-front therapy or to consider a class agent switch as salvage treatment with different targets and mechanisms of action.

The best combination to be administered in each patient is based on several factors, including refractoriness to prior regimens, expected tolerability, and drug availability.

Considerations can be made based on a post hoc analysis of randomized clinical trials that have established the current standards of care in the relapse setting (Table 4). Many of these trials analyzed the outcomes of patients with early vs late relapse. First, most of the 3-drug regimens currently recommended as salvage therapies also proved to be effective in patients with an early relapse, consistently improving CR and MRD-negativity rates and prolonging PFS. In the POLLUX study, the median PFS observed in patients with an early relapse increased from 12 months with lenalidomide and dexamethasone (Rd) to 37 months with daratumumab (DRd)[51]; in the ASPIRE study, the addition of carfilzomib to Rd prolonged the median PFS from 11 to 21 months in patients who progressed within 12 months from the start of the previous treatment.[52] These regimens can both be considered valuable options in lenalidomide-naive patients who are also not refractory to either DRd or carfilzomib (KRd). Results in favor of a triplet regimen were also reported in the early relapse population treated with daratumumab, carfilzomib, and dexamethasone (DKd; hazard ratio [HR], 0.6, median PFS NR) in the CANDOR study and isatuximab, carfilzomib, and dexamethasone (IsaKd; HR, 0.6, median PFS 25 months) in the IKEMA study as compared to carfilzomib-dexamethasone (Kd) alone (median PFS of 23 months and 17 months, respectively).[53,54] Based on these results, for patients relapsing early after a 3-drug regimen up front who are not daratumumab refractory, a triplet salvage combination based on an anti-CD38 MoAb in combination with either lenalidomide (DRd) or carfilzomib (DKd, IsaKd), if lenalidomide refractory, are the options of choice. Patients with an early relapse who are refractory to daratumumab have limited treatment options. In general, at first and second relapse a 3-drug combination of a proteasome inhibitor (bortezomib or carfilzomib) with pomalidomide (pomalidomide-bortezomib-dexamethasone [PVd], carfilzomib-pomalidomide-dexamethasone [KPd]), or alkylating agents (carfilzomib-cyclophosphamide-dexamethasone [KCd]/bortezomib-cyclophosphamide-dexamethasone [VCd]) are viable treatment options, although efficacy data about these combinations in the early relapse are currently lacking. Similarly, pomalidomide-based regimens in combination with elotuzumab, a MoAb targeting *SLAMF7*, can also be considered as a third line.

Despite the efficacy demonstrated by these regimens in a patient with an early relapse, the survival outcomes observed in this population are still significantly inferior to those reported in patients with a late relapse. Furthermore, as many patients experiencing an early relapse today will also be refractory to daratumumab and/or lenalidomide, since both drugs have become a mainstay of the induction and maintenance strategies, their treatment at the time of relapse poses important challenges. In this light, new salvage agents such as chimeric antigen receptor (CAR) T cells and bispecific antibodies, with different targets and mechanisms of action, represent an appealing option (Table 5). In cohort 2a of the KarMMa-2 study,[55] idecabtagene vicleucel (ide-cel), a B-cell maturation antigen (BCMA)–directed CAR T-cell therapy currently approved for patients with at least 4 prior lines of therapy in the United States and 3 in Europe, is being investigated as a salvage treatment in patients who underwent ASCT and had an early relapse (89% of patients progressed within 12 months from ASCT). Ide-cel resulted in an overall response rate of 84%, with 46% of patients achieving at least a CR, an almost double rate compared to that (24%) reported with the first-line therapy in this patient population.[55] While the median PFS reported in the overall cohort of patients was only 11.4 months, a longer duration of response (24 months) was observed in patients achieving a CR/stringent(s)CR,[55] thus highlighting on one hand the challenges in the treatment of this functional high-risk population and on the other the importance of the depth of response. In a similar phase 2 study (CARTITUDE-2, cohort B) conducted in patients relapsing within 12 months since initial treatment or ASCT, ciltacabtagene autoleucel (cilta-cel), another approved anti-BCMA CAR T cell, induced at least a CR in 89% of treated patients, 75% of whom were also MRD-negative (next-generation sequencing, 10^{-5}): these results translated into

Table 4. Efficacy of approved regimens in patients with early vs late relapse

Clinical trial	Study design	Definition of FHR	Patients, n	Clinical outcomes
POLLUX[51]	DRd vs Rd	Early relapse: progression within 18 months from the start of first-line treatment Late relapse: progression after 18 months from the start of first-line treatment	**Early relapse, 99** DRd arm, 47 Rd arm, 52 **Late relapse, 196** DRd arm, 102 Rd arm, 94	**DRd vs Rd** **PFS, median** Early relapse: 37 vs 12 mo (HR, 0.41; *P* = .0002) Late relapse: 69 vs 28 months (HR, 0.53; *P* = .0007) **CR rates** Early relapse: 53% vs 12% Late relapse: 62 vs 38% **MRD rates (10^{-5})** Early relapse: 30% vs 4% Late relapse: 34 vs 14%
ASPIRE[52]	KRd vs Rd	Early relapse: progression within 12 months from the start of the prior treatment line Late relapse: progression after 12 months from the start of the prior treatment line	**Early relapse, 217** KRd arm, 113 Rd arm, 104 **Late relapse, 520** KRd arm, 263 Rd arm, 267	**KRd vs Rd** **PFS, median** Early relapse: 21 vs 11 mo (HR, 0.7; *P* = .0026) Late relapse: 30 vs 18 mo (HR, 0.68; *P* = .0005)
CASTOR[51]	DVd vs Vd	Early relapse: progression within 18 months from the start of first-line treatment Late relapse: progression after 18 months from the start of first-line treatment	**Early relapse, 49** DVd arm, 30 Vd arm, 19 **Late relapse, 186** DVd arm, 92 Vd arm, 94	**DVd vs Vd** **PFS, median** Early relapse: 15 vs 9 mo (HR, 0.51, *P* = .048) Late relapse: 28 vs 8 mo (HR, 0.2; *P*>.0001) **CR rates** Early relapse: 21% vs 17% Late relapse: 51% vs 14% **MRD rates (10^{-5})** Early relapse: 13% vs 0% Late relapse: 23% vs 13%
ENDEAVOR[52]	Kd vs Vd	Early relapse: progression within 12 months from the start of the prior treatment line Late relapse: progression after 12 months from the start of the prior treatment line	**Early relapse, 239** Kd arm, 123 Vd arm, 116 **Late relapse, 675** Kd arm, 335 Vd arm, 340	**Kd vs Vd** **PFS, median** Early relapse: 14 vs 6 mo (HR, 0.6; *P* = .0017) Late relapse: 22 vs 10 mo (HR, 0.5; *P*<.0001)
CANDOR[53]	DKd vs Kd	Early relapse: progression within 18 months from the start of first-line treatment Late relapse: progression after 18 months from the start of first-line treatment	**Early relapse, 92** DKd arm, 59 Kd arm, 33 **Late relapse, 118** DKd arm, 82 Kd arm, 36	**DKd vs Kd** **PFS, median** Early relapse: NR vs 13 months (HR, 0.6) Late relapse: NR vs NR (HR, 0.7) **CR rates** Early relapse: 29% vs 3% Late relapse: 39% vs 17%
IKEMA[54]	IsaKd vs Kd	Early relapse: progression within 18 months (1 prior line of therapy), 12 months (2 or more prior treatments), or 12 months from ASCT Late relapse: progression after 18 months (1 prior line of therapy), 12 months (2 or more prior treatments), or 12 months from ASCT	**Early relapse, 107** IsaKd arm, 61 Kd arm, 46 **Late relapse, 176** IsaKd arm, 104 Kd arm, 72	**Isakd vs Kd** **PFS, median** Early relapse: 25 vs 17 mo (HR, 0.6) Late relapse: 43 vs 22 mo (HR, 0.5) **MRD rates (10^{-5})** Early relapse: 25% vs 15% Late relapse: 39% vs 17%

an 18-month PFS of 83%, thus already superseding the duration of the first remission for most patients.[56]

Given the promising results of T-cell redirecting therapies also in patients with early relapse and aggressive disease, efforts should be made to grant access to bispecific antibodies and CAR T cells for this high-risk population; however, the current label for both bispecific antibodies and CAR T cells, after the third or fourth line of therapy rather than based on drug class refractoriness, is a clear limitation. Of even more interest is to build up on the correlation between the depth of response at first line and the risk of early relapse, thus looking at an early change of treatment approach in patients with suboptimal responses to first-line therapy. This led to the investigation of a treatment intensification strategy with ide-cel in NDMM patients achieving less than a VGPR after ASCT.[57] Preliminary results in the 31 treated patients demonstrated a promising efficacy: 74% of patients achieved at least a CR, and the MRD negativity (next-generation flow, 10^{-5}) in the overall population was 42%.[57] Altogether, these results, though preliminary, suggest that CAR T cells, either used as salvage therapies after early relapse or as a treatment intensification in the presence of a suboptimal response after transplant, could be promising strategies. Ongoing phase 3 trials are currently investigating intensification in patients with a suboptimal response.

Table 5. Prospective clinical studies with CAR T cells in patients with an early relapse

Clinical trial	Study design	Definition of FHR	Patients	Clinical outcomes
KarMMA-2, cohort 2a[55]	Ide-cel	ER: progressive disease within 18 months from first-line treatment including induction, ASCT, and lenalidomide maintenance	n = 37	ORR, 84% CR rate 46% PFS, median 11.4 mo 2-y OS, 85% DOR, median • Overall population, 16 mo • Patients in CR, 24 mo
KarMMA-2, cohort 2c[57]	Ide-cel	Inadequate response (less than VGPR) after up-front ASCT	n = 31	ORR, 87% CR rate, 74% MRD rates (10^{-5}), 42%
CARTITUDE 2, cohort b[56]	Cilta-cel	ER: progressive disease after initial therapy including PIs and IMiDs within 12 months since ASCT or start of first-line treatment	n = 19	ORR, 100% CR or better rates, 90% MRD rates (10^{-5}), 74% 18-mo PFS, 83% 18-mo OS, 83%

CR, complete response; DOR, duration of response; ER, early relapse; ORR, overall response rate.

Finally, optimal timing to start therapy and the role of continuous treatment should be considered. Prospective and retrospective studies in relapse showed a potential benefit in patients who received therapy at biochemical rather than at clinical relapse.[58] It is true that in patients with high-risk disease there is often a short interval between biochemical and clinical relapse, but if one may argue that we lack sufficient evidence for changing the treatment approach for suboptimal response, it could be reasonable to change therapy in early relapse at first signs of confirmed serological relapse. Continuous treatment proved to be effective up front and at relapse. This can suggest the potential importance of prolonged therapy even following newer anti-BCMA agents in the context of early relapse and to help prolong the duration of response.

Conclusions

FHR patients represent an unmet medical need even in the context of highly effective up-front and salvage multidrug regimens. Current challenges in managing FHR patients consist of a correct identification of patients at higher risk of early relapse through baseline and dynamic risk factors as well as the development of strategies that aim to prevent early relapse in high-risk patients together with effective salvage treatments. In this light, the use of the most effective regimen up front (quadruplets rather than triplets), incorporating response to treatment in dynamic risk stratification models, early treatment intensification in patients with a suboptimal response and class-drug/switch at relapse, as well as the early use of new immunotherapeutic approaches (CAR T cells and bispecific antibodies) and early treatment in case of MRD-resurgence or biochemical relapse are promising strategies to be validated in clinical studies.

Conflict-of-interest disclosure

Francesca Gay: honoraria: Janssen, Celgene/Bristol Myers Squibb, Takeda, Amgen, Sanofi, GSK, Roche, Abbvie; advisory board: Janssen, Celgene/Bristol Myers Squibb, Takeda, Amgen, Sanofi, GSK, Roche, Abbvie, Pfizer, Oncopeptides.

Giuseppe Bertuglia: no competing financial interests to declare.

Roberto Mina: honoraria: Janssen, Celgene/Bristol Myers Squibb, Takeda, Amgen; advisory board: Janssen, Celgene/Bristol Myers Squibb, Takeda, Amgen; consultancy: Janssen, Takeda, Sanofi.

Off-label drug use

Francesca Gay: nothing to disclose.
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Considerations for next therapy after anti-CD38 monoclonal antibodies used as first line

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In the current treatment paradigm, the use of anti-CD38 monoclonal antibodies (mAbs) in frontline has notably increased, for both transplant-ineligible and transplant-eligible patients with newly diagnosed multiple myeloma (NDMM) patients. As a result, patients with multiple myeloma (MM) are frequently exposed to or develop resistance to anti-CD38 mAb therapy during the initial stages of treatment. Here, we review second-line (first relapse) and some third-line (second relapse) therapies for patients with MM with disease progression after exposure to anti-CD38 mAb-based therapy. We discuss therapies including B-cell maturation antigen (BCMA)–targeted and non-BCMA-targeted therapeutic options in the setting of prior anti-CD38 mAb exposure/refractoriness.

LEARNING OBJECTIVES

- Discuss non–B-cell maturation antigen (BCMA)–directed multiple myeloma (MM) treatment options in first and second relapse after anti-CD38 monoclonal antibody (mAb) exposure
- Review the use of BCMA-directed MM therapies in first and second relapse after anti-CD38 mAb exposure
- Review management of MM in second relapse after anti-CD38 mAb exposure

CLINICAL CASE

A 63-year-old African American man was diagnosed with multiple myeloma (MM) 3 years ago. At the time of diagnosis, he had immunoglobulin G (IgG) κ, stage II disease per the Revised International Staging System. Additionally, bone marrow pathology revealed standard-risk features, including t(11;14) and monosomy 13. Positron emission tomography/computed tomography showed no fluorodeoxyglucose (FDG) avid osseous lesions. He was treated with daratumumab (Dara), lenalidomide (Len), bortezomib (Bortez), and dexamethasone (Dex) (DRVd) for 4 cycles. He then underwent stem cell harvest, followed by another 4 cycles of DRVd consolidation. Given his standard-risk disease and achievement of stringent complete remission after the first 4 cycles of induction therapy, he opted to forego autologous stem cell transplantation (ASCT). He received maintenance therapy with Dara-Len for 2 years and then Len single-agent maintenance onward. The following year, he developed relapsed disease with a new paraspinal L5 plasmacytoma and symptomatic bilateral rib lytic lesions. M spike was 1.8 g/dL, repeat bone marrow biopsy showed 40% plasma cell involvement, and fluorescence in situ hybridization analysis detected an additional finding of 3 copies of duplication 1q.

Use of anti-CD38 mAbs in frontline treatment of NDMM

The integration of anti-CD38 monoclonal antibodies (mAbs) in the frontline treatment of newly diagnosed multiple myeloma (NDMM) has become a standard-of-care (SoC) approach based on various clinical trials. The pivotal MAIA trial, which encompassed a cohort of patients with transplant-ineligible (TI) NDMM treated with Dara in the frontline setting laid the foundation for this approach. Data published in 2022 showed a 44% reduction in risk of death or progressive disease and sustained measurable residual disease (MRD) negativity (using next-generation sequencing at 10^{-5}),[1] with nearly 15% of patients with TI NDMM maintaining MRD negativity at or beyond 6 months and ~11% at or beyond 12 months, which translated to favorable progression-free survival (PFS) outcomes.[1] The tolerability and safety of Dara-Len-Dex in the MAIA regimen proved to be reassuringly comparable to those of Len-Bortez-Dex,[2,3] leading to a surge in the utilization

of anti-CD38 mAb therapy as a primary treatment option for patients with TI NDMM.

In the United States, there is an increasing trend in the utilization of quadruplet mAb-based therapy as frontline treatment for patients with transplant-eligible (TE) NDMM based on the phase 2 GRIFFIN trial. Results published in 2022 showed an impressive MRD-negative rate of 64% in the Dara cohort compared to 30% in the non-Dara arm ($P < .0001$), with 44% of patients sustaining MRD negativity at or beyond 12 months,[4] as well as superior PFS and MRD negativity and deepening of complete remission rates over time in the Dara-containing arm. No additional safety concerns and no notable differences in ability to proceed with ASCT were observed in the Dara-quadruplet group compared to the triplet group.[4] The PERSEUS phase 3 clinical trial evaluation of this quadruplet regimen in patients with NDMM with PFS as the primary end point is ongoing.[5]

In Europe, the phase 3 randomized CASSIOPEIA trial assessed Dara in combination with Bortez, thalidomide, and dex (D-VTd) vs VTd induction in patients with TE NDMM. The first randomization was 1:1 to quadruplet versus triplet therapy, and the second randomization allowed patients with partial or better response to receive maintenance with Dara vs observation only. The D-VTd group had clinically superior complete remission (CR) of 39% vs 26% in the VTd group, with MRD-negative rates of 64% and 44% ($P < .0001$), respectively.[6] At a median follow-up of 35.4 months after the second randomization, median PFS for the Dara cohort was not reached, whereas it was 46.7 months for the VTd cohort.[6]

Another notable European quadruplet phase 3 trial (ALCYONE) evaluated Dara, Bortez, melphalan, and prednisone (D-VMP) vs VMP in TI NDMM, focusing on PFS. In the final analysis at a 40.1-month median follow-up, both PFS and overall survival (OS) were significantly better in the D-VMP cohort, with hazard ratios of 0.60 ($P = .0003$; 95% confidence interval [CI], 0.46-0.80) and 0.42 ($P < .0001$; 95% CI, 0.34-0.51), respectively.[7] Additionally, the overall response rate (ORR), CR or better, and MRD negativity rates in the D-VMP-treated patients were all significantly superior to those in the VMP arm. The infectious adverse events (AEs) were notably more increased in the Dara cohort.[7]

Finally, the GMMG-HD7 trial, a phase 3 trial, successfully achieved its primary end point in just 18 weeks, with the isatuximab (Isa)–RVd quadruplet regime attaining >50% MRD negativity in the quadruplet group pre-ASCT. The post-ASCT results from this trial will possibly be practice informing, given the planned double-randomization study design before and after ASCT.[8]

What would best be used for second-line therapy for this patient?

There are several factors to consider when choosing the next best regimen for a patient with MM with relapsed disease. These can be categorized as follows: (1) prior regimen received, (2) disease risk stratification, and (3) patient conditions (Table 1). In this clinical case, the patient experienced disease progression in less than 4 years with prior Dara exposure and prolonged Len therapy with progression on Len. It is important to factor in his prior

Table 1. Factors to consider when selecting the next line of therapy in patients with relapsed MM

Prior MM directed therapies (induction/first line)	MM disease risk stratification	Patient conditions
Triplet vs quadruplet therapy • Triplet ◦ DRd vs RVd • Quadruplet ◦ D-RVd* ◦ Clinical trial	High risk vs standard risk • Cytogenetics ◦ Hypoploidy ◦ Hyperploidy • Fluorescence in situ hybridization ◦ Del 17p, t(4;14), t(14; 16), t(14;20), gain 1q, loss 1p • Next-generation sequencing • Genomic expression profiling	Renal disease • Cast nephropathy • Nonmalignant etiology • Severity • Medication toxicity
Alkylator† • Bendamustine • Cyclophosphamide Anthracycline • Doxorubicin • Doxil	Time to relapse • Early (<12 mo vs <24 mo) • Late (>48 mo) • Slow (asymptomatic/biochemical) • Rapid (symptomatic)	Neuropathy • MM related • Nonmalignant etiology
Proteasome inhibitor • Bortezomib • Carfilzomib • Ixazomib	Extramedullary disease • Plasmacytomas ◦ Visceral ◦ Skin • Central nervous system disease	Bone lesions (symptomatic vs asymptomatic)
ASCT • Transplant ineligible • Transplant eligible	Peripheral blood plasma cells • <5% • >5% • >20%	Steroid (tolerant vs intolerant)
Institutional/resources • Advanced cancer care center • Community medical center		Non-MM-related health conditions • Cardiovascular • Renal disease

*Based on the GRIFFIN trial, a phase 2 trial; the phase 3 (PERSEUS) trial needed to support FDA approval of the quadruplet regimen is ongoing.

†Some patient cases require induction triplet inclusive of traditional chemotherapeutic agent, for various reasons, such as renal insufficiency.

exposure to Dara and prolonged Len exposure when determining the best next course of therapy.

In cases where the patient does not exhibit refractoriness to Dara, the APOLLO and CANDOR trials provide appropriate second-line options. The APOLLO trial evaluated 304 patients randomized to receive Dara-pomalidomide-Dex (DPd; n = 151) vs Pd (n = 153) post–first relapse (1 to 3 prior lines of therapy). Most patients (79.6%) were Len refractory, with almost half (48%) being proteasome inhibitor (PI) refractory and 42.4% being both PI and Len refractory. After a median follow-up of 39.6 months, DPd had a median OS of 34.4 months (95% CI, 23.7-40.3) vs 23.7 months for Pd (95% CI, 19.6-29.4). Almost 10% of the DPd cohort was exposed to prior anti-CD38 mAb therapy.[9] The CANDOR trial compared Dara-carfilzomib-Dex (DKd) vs Kd after 1 to 3 prior lines of therapy and primarily assessed PFS. Final analysis showed that at a median follow-up of 50 months, the DKd group demonstrated a median PFS of 28.4 months, whereas the Kd group had a PFS of 15.2 months, with MRD negativity rates of 28% in the DKd group and 9% in the Kd group.[10] The use of carfilzomib instead of Bortez in the CANDOR trial is supported by several trials, including the pivotal phase 3 ENDEAVOR trial, which compared carfilzomib-Dex (Kd) to Bortez-Dex (Vd) in patients with relapsed or refractory MM (RRMM). The trial showed a median PFS of 18.7 months with Kd vs 9.4 months with Vd (hazard ratio = 0.53; 95% CI, 0.44-0.65; $P < .0001$). Updated interim analysis also showed improved OS for the carfilzomib group compared to the Bortez cohort.[11]

Isatuximab (Isa) is another MAb that targets CD38 plasma cell surface antigen but differs from Dara in epitope binding.[12] This difference may translate to distinct treatment outcomes when either agent is used. An in vitro study suggested that Isa exhibits a higher potency than Dara in inhibiting CD38 enzymatic activity[13]; despite this, a phase 2 study evaluating the use of Isa in patients with Dara-refractory MM (within 6 months of Dara exposure) yielded disappointing results.[14] In the same study, minimal responses were observed in patients with ≥6 months from the last Dara dose treated with Isa. This distinction highlights the importance of considering the timing of prior Dara exposure when treatment strategies involving another anti-CD38 mAb are planned.

Notably, isatuximab is approved by the Food and Drug Administration (FDA) in the United States for use in combination with carfilzomib and Dex (IsaKd) in patients with RRMM after 1 to 3 prior lines of therapy, based on the IKEMA study.[15] The final analysis, after a median follow-up of 44 months, showed a median PFS of 35.7 months with IsaKd (95% CI, 25.8-44.0) vs 19.2 months for Kd (95% CI, 15.8-25.0), translating to a 42% risk reduction of death or MM disease progression for the Isa-containing regimen.[16]

For the patient in this clinical case, retaining Bortez as a component of his second-line therapy is a reasonable consideration. This decision is grounded in the facts that his prior exposure to Bortez was limited to just 8 cycles and his last encounter with this drug was over 12 months ago.

The BOSTON regimen, which combines selinexor (Seli) with Bortez and Dex (SVd), is a viable non–B-cell maturation antigen (BCMA) second-line treatment option for this patient. Seli is an oral selective inhibitor of nuclear export medication that binds and inhibits XP01, resulting in myeloma cell destruction. Seli has shown efficacy in Len-resistant and mAb-exposed patients with RRMM. The BOSTON trial had a median PFS for SVd of ~14 months compared to ~9.5 months for Vd (P=.0075).[17,18] Another key trial (STORM) that investigated Seli in patients with RRMM showed favorable efficacy and tolerability in combination with pomalidomide and Dex (SPd).[19-21] Experience with Seli use has shown the importance of anticipatory management of potential gastrointestinal and electrolyte adverse effects, often well mitigated with acceptable safety and tolerance, especially in the combination regimens with doses of Seli below 80 mg weekly. For many patients, especially in the community oncology setting, the BOSTON regimen may be an ideal option to salvage patients in second relapse and/or bridge patients to clinical trial opportunities or next therapeutic care such as immune cellular therapy or ASCT for those eligible. The triplet regimens from the OPTIMISMM (PVd) and CASTOR (DVd) trials are potential Bortez-based second-line therapies.

Two other potential second-line non-BCMA therapeutic options to consider are carfilzomib, pomalidomide, and Dex (KPd), and carfilzomib, cyclophosphamide (Cy), and Dex (KCyd), as supported by findings from the EMN011/HOVON114 and MCRN-003/MYX.1 phase 2 trials.[22] The caveat is that those trials were smaller phase 2 institutional studies with no phase 3 data support, although the trials' results are compelling.

It is worth noting that the TOURMALINE-MM1[23] and ELOQUENT[24] studies, which evaluated ixazomib (Ixa) with Len-Dex (IxaRd) and elotuzumab (Elo) with Len-Dex (EloPd), respectively, in patients after 1-3 prior lines of therapy, would not be suitable next-line options in this case. This is because the patients enrolled in these studies were not exposed to Dara.

The case described here, use of ASCT in second-line treatment is possible, given the patient completed stem cell harvest and deferred frontline transplantation. Both the phase 3 IFM 2009 and DETERMINATION trials, which evaluated ASCT in frontline vs later in relapse, indicate that ASCT remains important for select MM patients.[25,26] The IFM 2009 trial included 700 patients randomly assigned to ASCT early after 3 cycles of RVd with consolidation of 2 RVd cycles, compared to RVd for 8 cycles. Len maintenance was for 1 year. The primary end point was PFS, and at a median follow-up of 93 months, the median PFS was 47.3 months vs 35 months for the ASCT group vs the RVd-alone group, respectively (hazard ratio = 0.70; 95% CI, 0.95-0.83). The MRD negativity rates were superior in the ASCT group (29.8%) vs RVd alone (20%; P = .01). Of the patients who relapsed, 68.4% proceeded to second-line therapy (ASCT, n =217; RVd alone, n = 262); 22.6% received a second ASCT and 76.7% of the RVd-alone group received ASCT post–first relapse. The median PFS showed no statistical difference between the groups, and the median OS was 62.2% and 60.2% in the ASCT and RVd-alone groups, respectively.[12,25]

The DETERMINATION trial evaluated patients with NDMM who received RVd induction with ASCT up front (n = 365) vs no frontline ASCT (n = 357), with both groups receiving Len maintenance until disease relapse. PFS was the primary end point of the trial. The median follow-up was 76.0 months, with a median PFS of 67.5 months vs 46.2 months, respectively. The OS at 5 years was 80.7% and 79.2%, respectively.[26]

These studies suggested that whether ASCT is performed up front or delayed, the overall survival benefit does not seem to be significantly different. It is important to note that both DETERMINATION and IFM 2009 were preplanned for PFS as the

primary end point. Additionally, only ~35% of the patients in the DETERMINATION trial received ASCT at the time of first relapse, which may have impacted the OS outcome.[25,26] Consequently, the timing of ASCT frontline vs post first relapse remains controversial, with the data suggesting that ASCT is a reasonable option for second-line treatment if not performed previously.

CLINICAL CASE (continued)

The patient is fully cognizant of the reoccurring nature of his myeloma disease and is concerned about treatment options in the event of a second relapse.

What would best be used for third-line therapy for this patient?

Depending on the choice of second-line therapy, third-line treatment options may include options as discussed above (Table 2). The choice of third-line therapy may also depend on the nature of the relapse, whether it is rapidly progressive or indolent. Treatment options in this scenario can encompass combination chemotherapy such as Dex, Cy, etoposide, and cisplatin (DCEP) or Bortez, Dex, cisplatin, doxorubicin, Cy, and etoposide (VD-PACE), as well as BCMA-targeted therapies such as chimeric antigen receptor T-cell (CAR-T) therapies. Some examples of sequencing therapies from NDMM through second RRMM are listed below:

1. DRVd (TE) → ASCT → DR → first relapse → PVd, SVd, or clinical trial → second relapse → clinical trial, BCMA-targeted therapy (CAR-T or bispecific [eg, teclistamab]), or non-BCMA-targeted therapy (eg, talquetamab)
2. DRd (TI) → R → first relapse → DPd, DVd, KRd, or clinical trial → second relapse → clinical trial, SVd, PVd, BCMA-targeted therapy (CAR-T or bispecific [eg, teclistamab]), or non-BCMA-targeted therapy (eg, talquetamab)
3. RVd (TI or TE) → R → first relapse → ASCT (TE), PVd, DPd, SVd, DVd, or clinical trial → second relapse → clinical trial, BCMA-targeted therapy (CAR-T or bispecific [eg, teclistamab]), or non-BCMA-targeted therapy (eg, talquetamab).

CLINICAL CASE (continued)

The patient is interested in newer therapies and asks about BCMA-targeted treatment options in first and second relapse. He is aware of the market withdrawal of belantamab mafodotin (belamaf) in 2022 and has many questions about this treatment.

What is belamaf and how does it work? Why was it withdrawn? What other BCMA-directed second-line therapeutic options exist for patients with MM after anti-CD38 resistance?

Belamaf is an antibody-drug conjugate that targets BCMA found on the surface of the malignant plasma cells in MM. Belamaf is

Table 2. Current US FDA-approved triplet regimens for management of RRMM in the second line

Regimen (study)	Survival outcomes, PFS (mo) and OS (mo)	Median lines of therapy (range)	Included anti-CD38 mAb–exposed/ refractory patients	Included Len-refractory patients
DPd (APOLLO)	Median OS 34.4 (95% CI, 23.7–40.3) DPd vs 23.7 (95% CI, 19.6–29.4) Pd	2 (1–5)	No	Yes
KRd (ASPIRE)	Median PFS 26.1 (95% CI, 23.3–30.5) KRd vs 16.6 (95% CI, 15–20.6; P<.001) Rd Median OS 48.3 (95% CI, 42.4–52.8) KRd vs 40.4 (95% CI, 33.6–44.4; P=.0045) Rd	2 (1–3)	No	No
SVd (BOSTON)	Median PFS 13.9 SVd vs 9.46 Vd (P=.0075)	2 (1–3)	Yes	Yes
DKd (CANDOR)	Median PFS 28.4 DKd vs 15.2 Kd	2 (1–5)	No	Yes
DVd (CASTOR)	Median PFS 16.7 DVd vs 7.1 Vd (P<.0001)	2 (1–9)	No	Yes
EloRd (ELOQUENT-2)	Median PFS 19.4 EloRd vs 14.9 Rd (P=.014) Median OS 43.7 EloRd vs 39.6 Rd (P=0.025)	2 (1–4)	No	No
IsaKd (IKEMA)	Median PFS 35.7 (95% CI, 25.8–44.0) IsaKd vs 19.2 (95% CI, 15.8–25.0) Kd	2 (1–4)	No	Yes
PVd (OPTIMISMM)	Median PFS 11.2 (95% CI, 9.66–13.73) PVd vs 7.1 (95% CI, 5.88–8.48; P<.0001) Vd	2 (1–2)	No	Yes
DRd (POLLUX)	Median PFS 44.5 (95% CI, 34.1-NE) DRd vs 17.5 (95% CI, 13.9–20.8; P<.0001) Rd 65% vs 57% OS	1 (1–11)	No	Yes
IxaRd (TOURMALINE-MM1)	Median PFS 19.4 IxaRd vs 14.9 Rd (P=0.0014) Median OS 43.7 IxaRd vs 39.6 Rd (P=0.025)	Range: 1–3	No	No

Doublet FDA-approved options are not included in this table. KCyD (MYX.1/MCRN-00) and KPd (EMN011/H0114) are not included although they are strongly supported by smaller phase 2 studies.

NE, not evaluable.

conjugated to monomethyl auristatin-F (MMAF), a microtubule inhibitor that binds to tubulin and impedes microtubule assembly, resulting in cell cycle arrest and apoptosis.[27] An additional effect of belamaf is the activation of MM cell death through the antibody-dependent cellular cytotoxicity and antibody-dependent cellular phagocytosis.[28,29] Belamaf gained conditional accelerated FDA approval in August 2020 based on the phase 2 DREAMM-2 trial.[30] The safety and efficacy data were favorable, except for keratopathy, an unusual adverse effect seen only in antibody drug conjugate MM therapy.[30,31] The FDA approval for belamaf was later withdrawn in November 2022, when the phase 3 DREAMM-3 trial failed to meet the conditional requirement, falling short of its primary PFS end point with a hazard ratio of 1.03 (95% CI, 0.72-1.47).[32] Of note, belamaf remains approved for use in Europe.[33]

The ALGONQUIN, DREAMM-12, and DREAMM-15 trials are currently evaluating the management of keratopathy, focusing on both efficacy and mitigation of toxicity. Preliminary findings indicate that when belamaf was combined with pomalidomide and Dex at different doses, the ORR increased from 82% to 95%.[25,26] The belamaf dose of 1.92 mg/kg every 4 weeks in the combination with pomalidomide and Dex has had significant efficacy with reduced keratopathy adverse effects. Further results from the ALGONQUIN, DREAMM-12, and DREAMM-15 trials are forthcoming.

Other BCMA-targeted therapies have shown significant clinical benefit in patients with MM with triple refractory disease (refractory to PIs, immunomodulatory drugs [IMiDs], and anti-CD38 mAbs). The current US FDA-approved BCMA-targeted therapies are the CAR-T therapies idecabtagene vicleucel (ide-cel)[34] and ciltacabtagene autoleucel (cilta-cel),[35] plus the bispecific antibody teclistamab.[36] These newer therapies are currently approved for use in the late refractory and relapsed setting, beyond fourth-line MM therapy. Due to their promising results in treating advanced RRMM, ongoing clinical trials are now assessing the efficacy of these BCMA therapies in the first and second relapse as well (Table 3).

The CARTITUDE-4 trial, a phase 3 randomized study, has shown impressive early results of chimeric antigen receptor T-cell therapy in patients with RRMM who had received 1 to 3 prior lines of therapy, including PIs and IMiDs, and with disease unresponsive to Len. The trial compared cilta-cel to the SoC regimens pomalidomide, Bortez, and Dex (PVd) and Dara, pomalidomide, and Dex (DPd). The primary end point of the study was evaluation of PFS in the intent-to-treat population. At a median follow-up of 16 months, the median PFS was not reached in the cilta-cel–treated patients, while it was 11.8 months in the SoC cohort. This translates to an impressive 74% reduction in the risk of disease progression or death in the cilta-cel cohort (hazard ratio, 0.26; 95% CI, 0.18-0.38; $P < .0001$). ORR was 85% vs 67%

Table 3. Clinical trials assessing the efficacy of BCMA-targeted therapies in earlier lines of therapy

Trial	No. of prior lines of therapy	Median PFS, mo (median follow up)	ORR, n (%); CR, n(%); MRD, n(%)	Hazard ratio (95% CI)
Cilta-cel trials				
CARTITUDE-2[39]	1–3	N/A	18 (88.9); 18 (27.8); 9 (100)	N/A
CARTITUDE-4[37]	1–3	Not reached (15.9 mo)	176 (85); 152 (73); 126 (61)	0.26 (0.18–0.38) (P<.0001)
CARTITUDE-5[40]	0 (NDMM, TI)	N/A (recruiting)	N/A	N/A
CARTITUDE-6 [NCT05257083]	0 (NDMM, TE)	N/A (recruiting)	N/A	N/A
Ide-cel trials				
KarMMa-2[41]	1–3	N/A (recruiting)	37 (83.8); 37 (45.9); 13 (85)	N/A
KarMMa-3[38]	2–4	13.3 (18.6)	181 (71); 98 (39); 50 (20)	0.49 (0.38–0.65) (P<.001)
KarMMa-4[42]	0 (NDMM, high-risk)	N/A	N/A	N/A
KarMMa-7[43]	1–3	N/A	N/A	N/A
Elranatamab trials				
MagnetisMM-1[44]	2–15		55 (64); 35 (31); N/A	
MagnetisMM-3[45]			123 (61); 123 (35); 29 (89.7)	
MagnetisMM- 4[46]	≥3	N/A	N/A	N/A
MagnetisMM-5[47]	>1	N/A	N/A	N/A
Teclistamab trial				
MajesTEC-3[48]	1–3	N/A (recruiting)	N/A	N/A
Talquetamab trials				
TRIMM-2[49]	>3	N/A	50 (78); 50 (16); N/A	N/A
RedirecTT-1[50]	On/after lines of therapy	20.9 (13.4)	93 (86.6); 93 (22); N/A	N/A

N/A, not applicable.

($P < .0001$), and MRD negativity was 61% vs 16% ($P < .0001$) in favor of cilta-cel. These favorable results may lead to use of cilta-cel as second-line therapy after first relapse for patients with RRMM.[37]

Similarly, the international KARMMA-3 trial evaluated ide-cel vs SoC (5 regimens) in patients with RRMM after receiving 2 to 4 prior lines of therapy. The primary end point was PFS, which at a median follow-up of 18.6 months was 13.3 months for ide-cel vs 4.4 months in the SoC, translating to a risk reduction of disease progression or death of 61% for ide-cel (95% CI, 0.38-0.65; $P < .001$). The ORR was 71% vs 42% ($P < .001$), and complete remission rate was 39% vs 5%, favoring ide-cel. There were no unexpected adverse toxicities, and cytokine release syndrome (CRS) and neurotoxicity (NT) rates were similar to other ide-cel trial rates, with the majority being grade 1 (88% CRS; 15% NT) and few grade 3 or above (5% CRS; 3% NT).[38]

Bispecific antibodies are also being assessed in earlier lines of therapy in RRMM; some examples include the MajesTEC trial series evaluating teclistamab in combination with Len and Dara. Other BCMA-targeted bispecifics also being evaluated in combination with Len and Dara include elranatamab in the MagnetisMM trials (Table 3).

Discussion

The treatment landscape for patients with RRMM is rapidly changing, with use of anti-CD38 mAb–based therapy now routinely administered as SoC for both TE and TI patients with NDMM. This change has impacted the next line of therapy offered post–first relapse. In relapsed disease, it is important to consider the prior therapeutic regimen received; the depth and duration of prior treatment response; disease cytogenetic status (high-risk vs standard risk); use of frontline ASCT; refractoriness of disease to PIs, IMiDs, and/or anti-CD38 mAbs; and patient tolerability and fitness when choosing the next therapeutic regimen after anti-CD38 mAb exposure in the front line. Moving anti-CD38 mAbs to NDMM has resulted in newer therapies with novel mechanisms of action, such as BCMA-targeted therapies being moved earlier in the treatment paradigm. These include cellular therapies, such as ide-cel and cilta-cel CAR-T therapies and bispecific antibodies (teclistamab) that have been approved by the FDA within the last 2 years; current clinical trials are addressing evaluation of CAR-T therapies and bispecific antibodies in the early post–first relapse setting, potentially moving us closer to a cure for the disease. The toxicity profile of these therapies, namely, CRS, neurotoxicity, and cytopenias, along with its impact on the T-cell immune milieu of treated patients with MM, remains to be a concern, requiring close monitoring and specialized training.

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Off-label drug use

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Options at the time of relapse after anti-BCMA therapy

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B-cell maturation antigen (BCMA)–directed therapies, including antibody-drug conjugates, bispecific antibodies (BsAbs), and chimeric antigen receptor T cells (CARTs), have shown remarkable efficacy in patients with late-line myeloma with prior exposure to immunomodulatory agents, proteasome inhibitors, and anti-CD38 antibodies. However, optimal sequencing of these agents remains to be determined, and management of these patients once they relapse has become a new unmet need. Fortunately, there are multiple options with demonstrated activity after anti-BCMA therapy, including a different BCMA-directed therapy, non-BCMA-directed CARTs and BsAbs, novel non–T-cell–engaging drugs, and standard triplet/quadruplet regimens or salvage stem cell transplant. Factors to consider when choosing a next therapy after anti-BCMA therapy include patient characteristics and preferences, prior therapies and toxicities, disease biology, timing from last anti-BCMA therapy, and, in the future, BCMA expression and immune profiling. While current data are limited to retrospective studies and small prospective cohorts, the serial use of T-cell–engaging therapies looks particularly promising, especially as BCMA-directed therapies move up earlier in the myeloma treatment course and additional CARTs and BsAbs against alternative targets (eg, G protein–coupled receptor, family C, group 5, member D and Fc receptor-homolog 5) become available. Going forward, ongoing prospective studies, large real-world data sets, and better tools to interrogate antigen expression and immune cell fitness hopefully will provide further insight into how to best individualize therapy for this difficult-to-treat population.

LEARNING OBJECTIVES

- Recognize currently available B-cell maturation antigen–targeted therapies for relapsed/refractory myeloma
- Identify the potential treatment options available after progression on these therapies
- Understand the expected safety and efficacy profiles of these treatment options

CLINICAL CASE

A 60-year-old man is diagnosed in 2015 with IgA κ multiple myeloma, International Staging System (ISS) stage 2 with deletion 13q and gain 1q by fluorescence in situ hybridization. He achieves a very good partial response (VGPR) after bortezomib, lenalidomide, and dexamethasone induction, followed by autologous stem cell transplant and lenalidomide maintenance. His disease progresses in 2019, and he subsequently progresses on daratumumab, pomalidomide, and dexamethasone; cyclophosphamide, bortezomib, and dexamethasone; and carfilzomib, pomalidomide, and dexamethasone. Most recently, he has received idecabtagene vicleucel (ide-cel), achieving complete response, but relapses 10 months later with new bone lesions and anemia. He is now 68 with normal renal function and Eastern Cooperative Oncology Group (ECOG) performance status of 1.

B-cell maturation antigen–directed therapies in late-line, relapsed/refractory myeloma

B-cell maturation antigen (BCMA), a cell surface receptor expressed on plasma cells, is now well established as a target for myeloma therapy.[1] Several BCMA-targeted therapies have activity in relapsed/refractory myeloma, including antibody-drug conjugates (ADCs), bispecific antibodies or T-cell engagers (BsAbs), and chimeric antigen receptor T cells (CARTs). As of mid-2023, 4 of these had received regulatory approval—belantamab mafodotin (belamaf, an ADC), ide-cel (CART), ciltacabtagene autoleucel (cilta-cel, CART), and teclistamab (BsAb)—all for patients with myeloma who had at least 4 prior lines of therapy (3 prior lines in Europe), including a proteasome inhibitor, an immunomodulatory agent (IMID), and an anti-CD38 antibody (ie, "triple-class exposed"). Registration efforts are under way for several additional agents as well (Table 1). Of note, belantamab mafodotin was withdrawn

Table 1. Approved and selected investigational BCMA-targeted therapies for use in late-line MM*

Agent (reference)	Construct	Trial (NCT#, status)	Phase	Design	n	% ORR (% ≥CR); median DOR in months (95% CI); median PFS in months (95% CI) at reported median follow-up	Selected safety event % (G3 + 4%, if any)
Belantamab mafodotin (belamaf)[3†]	Antibody-drug conjugate	DREAMM-2 (NCT03525678, active, not recruiting)	2	Open-label, 2-arm, randomized to receive 2.5 mg/kg or 3.4 mg/kg RP2D	196	2.5 mg/kg: 31% (3% ≥ CR), 13.7 (9.9-NE); 2.9 (2.1–3.7) at 13 months 3.4 mg/kg: 34% (3% ≥ CR); NR; 4.9 (2.3–6.2)	2.5 mg/kg: keratopathy 70% (27%), thrombocytopenia 35% (20%), anemia 24% (20%) 3.4 mg/kg: keratopathy 75% (21%), thrombocytopenia 54% (30%), anemia 37% (25%)
Idecabtagene vicleucel (ide-cel)[5]	Autologous CART	KarMMa-1 (NCT03361748, active, not recruiting)	1/2	Open-label, single-arm, dose escalation and dose expansion	128	ORR 73% (33% ≥ CR); 10.7 (9.0–11.3); 8.8 (5.6–11.6)	CRS 84% (5%), neurotoxicity 18% (3%), neutropenia 91% (89%), anemia 91% (60%), thrombocytopenia 63% (52%), hypogammaglobulinemia (21%)
Ciltacabtagene autoleucel (cilta-cel)[7]	Autologous CART	CARTITUDE-1 (NCT03548207, completed)	1/2	Open-label, single-arm, dose escalation and dose expansion	97	97% (sCR 82.5%); 33.9 (25.5-NE); 34.9 (25.2-NE)	CRS 95% (4%), neurotoxicity 21% (9%), neutropenia 91% (89%), anemia 93% (95%), thrombocytopenia 81% (68%)
Teclistamab[6‡]	BsAb (humanized IgG4)	MajesTec-1 (NCT04557098, recruiting)	1/2	Open-label, nonrandomized, IV or SC teclistamab in RRMM, dose expansion and dose escalation	165	63% (43% ≥ CR); 24 (24-NE); 12.5 (8.8–17.2) at 22 months	CRS 72.1% (0.6%), neurotoxicity 14.5% (0.6%), neutropenia 70.9% (64.2%), anemia 52.1% (37%), pneumonia 18.2% (12.7%), COVID-19 17.6% (12.7%), hypogammaglobulinemia 74.5% (0%)
Elranatamab[38‡]	BsAb (humanized IgG2a)	Magnetissm-3 (NCT04649359, active, not recruiting)	2	Open-label, multicenter, nonrandomized, single-agent SC	123	61% (28% ≥ CR); NE (12-NE); NE (10.4-NE) at 10.4 months	CRS 57.7% (0%), neurotoxicity 3.4% (0%), peripheral neuropathy 17.1% (0.8%), infections 66.7% (35%)
Linvoseltamab (REGN5458)[39‡]	BsAb (Veloci-Bi antibody)	LINKER-MM1 (NCT03761108, active, not recruiting)	1/2	Open-label, multicenter, nonrandomized, single-agent IV	87	64% (24% ≥ CR); NE; NE at 3.2 months	CRS 37% (1%), ICANS 5.6% (1.2%), anemia 28% (24%), neutropenia 20% (17%), thrombocytopenia 15% (10%), infections 54% (29%)
Alnuctamab (CC-93269)[40‡]	BsAb (2+1 humanized IgG1)	(NCT03486067, recruiting)	1	Open-label, multicenter, nonrandomized, single-agent IV or SC	70 (IV), 68 (SC)	IV: 39%; 33.6 (10.6-NE); 3.1 (1.9–5.5) at 8 months SC: 53% (16%,7%); NE; NR at 4.1 months	CRS 53% (0%), peripheral neuropathy 6% (0%), ICANS 3% (0%), anemia 38% (25%), neutropenia 37% (32%), infections 34% (9%)
ABBV-383B[41‡]	BsAb (2+1 humanized IgG4)	(NCT05286229, active, not recruiting)	1b	Open-label, multicenter, nonrandomized, single-agent IV	55 (40mg), 61 (60mg)	40mg: 58% (13% ≥ CR); NE (4.3); 13.7 (3.1-NE) at 3.5 months 60mg: 61% (34% ≥ CR); NE (10.4); 11.2 (4.8-NE) at 12.7 months	CRS 60% (1%), ICANS 4.9% (1.6%), anemia 37% (16%), neutropenia 34% (26%), thrombocytopenia 29% (11%), infections NR (22%)

sCR, stringent complete response; NE, not estimable/reached; NR, not reported; RRMM, relapsed and refractory multiple myeloma.

*Nonexhaustive list of selected trials (search May 18, 2023)—for a comprehensive list, please visit clinicaltrials.gov.

†Withdrawn from the US market in late 2022 due to a negative phase 3 trial (DREAMM-3).

‡Updated data as presented during ASH 2022 or ASCO 2023 meetings.

from the US market in late 2022 due to a negative phase 3 trial (DREAMM-3)[2] but remains available commercially outside the United States, and in the United States via an expanded access program, with other phase 3 trials ongoing.

A summary of the data supporting registration of these therapies is in Table 1; more detailed discussion of these trials is beyond the scope of this review. In general, overall response rates (ORRs) in triple class-exposed, BCMA therapy-naive patients are roughly 30% for belamaf, 60% for teclistamab or other BCMA-targeted BsAbs, 73% for ide-cel, and 97% for cilta-cel. Responses can be quite durable, with median duration of response (DOR) in these trials reported as 11.0, 24.0, 10.0, and 33.9 months for belamaf, teclistamab, ide-cel, and cilta-cel, respectively.[3-7] Unfortunately, however, there does not appear to be a plateau on progression-free survival (PFS) curves with these agents, and most patients ultimately relapse. Thus, additional therapeutic options following a BCMA-targeted therapy remain necessary.

Serial use of BCMA-targeted therapies

With so many BCMA-targeted therapies available, one obvious question is whether these can be used sequentially. We first reported in 2019 on 2 patients who responded serially to BCMA-targeted therapies (ADC→CART or CART→ADC),[8] and safety and efficacy of sequential BCMA-directed therapies have since been confirmed in several retrospective and prospective studies (Table 2). As a caveat, most of these are small case series or clinical trial cohorts, and in general, they demonstrate that while responses can be recaptured by switching to a different BCMA-targeted agent, ORR and DOR appear lower compared with using the same agent in a BCMA therapy-naive population.

BCMA CART following prior BCMA CART: Although experience is limited, retreating with the same BCMA CART product has had disappointing outcomes (Table 2),[5,9,10] possibly due to CAR-specific immune responses. However, subsequent treatment with a different BCMA CART product has demonstrated more promise, with ORR of 75% to 100% in small numbers of patients.[11-13]

BCMA CART following prior BCMA ADC or BsAb: In cohort C of the CARTITUDE-2 phase 2 study, cilta-cel was infused in 20 relapsed/refractory patients (median 8 prior lines) with prior exposure to a BCMA-targeted therapy (13 ADC [belamaf] and 7 BsAb [various]). The ORR was 60% (30% complete response [CR]) and was similar between the ADC-exposed and BsAb-exposed groups. Median DOR was 11.5 and 8.2 months, and median PFS 9.5 and 5.3 months, respectively, for these 2 groups.[14] In a real-world analysis of outcomes following ide-cel infusion, 44 patients had prior belamaf (n=37) or a BCMA-targeted BsAb (n=7). Median prior lines of therapy was 9, with 62% penta-drug-refractory. ORRs were 68% for ADC exposed and 86% for BsAb exposed, with CR rates of 22% and 43%, respectively. However, median PFS was only 3.2 and 2.8 months, respectively, compared with a median 9.0 months for the BCMA treatment-naive population (n=144).[12] The toxicities of BCMA CARTs appear similar when given after a prior BCMA-directed therapy, although high-grade thrombocytopenia and infections may be more common.[12,14] Overall, infusion of BCMA CART after prior a BCMA-targeted ADC or BsAb leads to responses in most patients, but the depth and duration of these responses appear inferior to that seen in BCMA treatment-naive patients.

BCMA BsAb following prior BCMA CART, ADC, or BsAb: In cohort C of the MajesTEC-1 study, patients with prior exposure to a BCMA-targeted ADC (n=25), CART (n=11), or both (n=4) received teclistamab at a dose of 1.5 mg/kg weekly until progression. ORR was 53% and similar in both groups, with 28% CRs and median DOR not reached. Three of 4 patients with both prior ADC and CART responded. PFS and overall survival (OS) were not reported. Rates of cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and infections appeared similar to that seen in BCMA treatment-naive patients.[15] In a pooled analysis of patients (n=86, median 7 prior lines) receiving elranatamab following a prior BCMA-directed ADC or CART, ORR was 45%, with CR in 17%. Reponses were more frequent in CART-exposed patients compared with ADC-exposed patients (53% vs 41%). Median DOR was not reached, with a median PFS of 4.8 months and 60% alive at 10 months.[16] These studies demonstrate the feasibility of using a BCMA-targeted BsAb after a prior BCMA-targeted ADC or CART (or both). No data are yet available for treating serially with different BCMA-targeted BsAbs. While it is suboptimal to compare across studies, current data suggest that the BCMA-targeted BsAbs may have less of a drop-off in response depth and duration between BCMA therapy-exposed and BCMA therapy-naive populations compared with that seen with BCMA CARTs (Table 2). This suggests that using a BCMA CART first followed by a BCMA BsAb later may be the better sequence compared with the other way around, although prospective trials and/or large real-world data sets are required to confirm this hypothesis.

BCMA ADC following prior BCMA CART or BsAb: There are limited data on the use of belamaf following prior BCMA-directed therapy. Gazeau et al[17] described a patient progressing after a second infusion of ide-cel who achieved a VGPR after starting belamaf, with ongoing response at 5 months. Retrospective single-institution studies have reported responses in 0% to 29% of patients receiving belamaf after prior BCMA CART (Table 2).[13,18,19]

Non-BCMA-targeted, T-cell–engaging therapies

Talquetamab is a BsAb targeting G protein–coupled receptor, family C, group 5, member D (GPRC5D), a receptor expressed highly on myeloma cells, with lower levels of expression on normal plasma cells and keratinized tissues (eg, skin, nailbeds, tongue papillae). In an updated analysis of the MonumenTAL-1 study, ORRs at the recommended subcutaneous (SC) phase 2 doses of 405 µg weekly or 800 µg every other week were 74% and 72%, respectively, including roughly one-third with CR. Median DOR was 9.5 months and not reached, respectively.[20] In a cohort of patients who received talquetamab after prior BCMA T-cell–engaging therapy, ORR was 65% (75% for prior CART [n=36] and 44% for prior BsAb [n=18]), with 35% CR and a median DOR of 11.9 months.[20] Forimtamig is another GPRC5D-directed BsAb, with 2 GPRC5D-binding domains. In a preliminary report of a phase 1 study in patients with relapsed/refractory MM, ORR was 71% and 64% for intravenous (IV) (n=49) and SC (n=55) dosing, respectively, and was 52% (11/21) in patients previously exposed to a BCMA-targeted therapy.[21] Common toxicities of GPRC5D-targeted BsAbs include CRS, skin and nail changes, dry mouth, and dysgeusia.

GPRC5D-targeted CART products have also shown efficacy in patients with relapsed/refractory MM, including those with prior BCMA-directed therapies (Table 3). In a phase 1 study, 18 patients received an infusion of MCARH109 at escalating doses. ORR was 71% (35% CR), with a median DOR of 7.8 months, and was 70% in

Table 2. Activity of BCMA-targeting agents following prior anti-BCMA exposure*

Agent	Population/design	n			% ORR (≥CR); median DOR in months (95% CI); median PFS in months (95% CI) at reported median follow-up (if available)			
		Prior BCMA CART	Prior BCMA BsAb	Prior BCMA ADC	Prior BCMA CART	Prior BCMA BsAb	Prior BCMA ADC	Any prior anti-BCMA
Belamaf[13,18,19]	Commercial belamaf after prior anti-BCMA CART; 3 retrospective single-in stitution analysis—pooled results	22	None	None	18% (NR); NR; NR	—	—	—
Ide-cel[5]	KarMMa-1; prospective phase 2 trial. Analysis of patients retreated with ide-cel upon progression.	28	—	—	21% (0% ≥ CR); NR; 1.0 (1.0–2.1)	—	—	—
Ide-cel [12†]	Commercial ide-cel recipients; retrospective multi-institution analysis	5	7	36	100% (60% ≥ CR) NR; NE	85.7% (42.9% ≥ CR); NR; 2.83	67.6% (21.6% ≥ CR); NR; 3.19	74% (29% ≥ CR); NR; 3.2
Cilta-cel[14]	Cilta-cel recipients (cohort C CARTITUDE-2); prospective phase 2 trial	None	7	13	—	57.1% (14.3% ≥ CR); 8.2 (4.4-NE); 5.3 (0.6-NE) at 10.9 months	61.5% (38.5% ≥ CR); 11.5 (7.9-NE); 9.5 (0.99-NE) at 11.8 months	60% (30% ≥ CR) 11.5 (7.9-NE); 9.1 (1.5-NE) at 11.3 months
CT103A[11]	Recipients of CT103A BCMA CART (n=103 total); subgroup analysis, prospective phase 1 trial	12	None	None	75% (41.7% ≥ CR); 6.3 (2.9-NE); NR at 12.2 months	—	—	—
Teclistamab[15†]	Teclistamab recipients (MajesTec-1 cohort C), prospective phase 1/2 trial	15	None	29	53.3% (26.7% ≥ CR); NR; NR at 12.5 months	—	55.2% (24.1% ≥ CR); NR; NR at 12.5 months	52.5% (27.5% ≥ CR); NE (10.5-NE); NR at 12.5 months
Elranatamab[16†]	Elranatamab recipients (4 MagnetisMM prospective studies); pooled sub-group analysis	36	None	59	52.8% (19.6% ≥ CR); NE (9.8-NE); 10.0 (1.9-NE) at 11.3 months	—	42.4% (18.7% ≥ CR); 13.9 (6.8-NE); 3.9 (1.9–6.6) at 11.3 months	46% (18.4% ≥ CR); 17.1 (9.8-NE); 5.5 (2.2–10.0) at 11.3 months

*Nonexhaustive list of selected studies.

†Updated data as presented during ASH 2022, ASCO 2022, or ASCO 2023 meetings.

Table 3. Safety and efficacy of selected late line T-cell–directed therapies not targeting BCMA*

Name	Target	Construct	Trial (NCT#, status)	Design	n		% ORR (sCR, CR); median DOR in months (95% CI); median PFS in months (95% CI) at reported median follow-up		Select safety event % (G3+4%, if any)
					All	BCMA-exposed	All	BCMA-exposed	
MCARH109[22]	GPRC5D	Autologous CART	(NCT04555551, active, not recruiting)	Phase 1, open-label, single-center (MSKCC), dose escalation	17	10	71% (35% ≥ CR); 7.8 (5.7-NE); NR at 10.1 months	70% (40% ≥ CR); NR; NR at 10.1 months	CRS 88% (6%), ICANS 6% (6%), nail changes 65%, rash 18%, infections 18% (12%), cerebellar toxicity (12%)
BMS-986393 (CC 95266)[25]	GPRC5D	Autologous CART	(NCT04674813, recruiting)	Phase 1, open-label, multienter, dose escalation	21	7	86%; NE; NE at 4 months	66.7%; NE; NE at 4 months	CRS 65%, ICANS 12%, neutropenia 41% (NR), thrombocytopenia 35% (NR), AEs of skin 18%, nails 12%, dysgeusia/dysphagia 12%
OriCAR-017[23]	GPRC5D	Autologous CART (bi-epitope nanobody based)	POLARIS (NCT05016778, active, not recruiting)	Phase 1, open-label, single-center (First Affiliated Hospital of Zhejiang University)	10	5	100% (60% ≥ CR); NE; NE at 7.8 months	100% (40% ≥ CR); NE; NE at 7.8 months	CRS 100%, ICANS 0%, anemia 80% (70%), neutropenia 100% (100%), nail disorders 30%
Anti-GPRC5D CAR T[24]	GPRC5D	Autologous CART	(Chinese Clinical Trial Register: ChiCTR2100048888)	Phase 2, open-label, single-center (Hospital of Xuzhou Medical University)	33	9	91% (63% ≥ CR); NE; NE at 5.2 months	100% (40% ≥ CR); NE; NE at 5.2 months	CRS 76%, ICANS 6% (3%), anemia 100% (52%), neutropenia 100% (100%), thrombocytopenia 100% (45%), nail changes 27%
Talquetamab[20] †	GPRC5D	BsAb (humanized IgG4 Fc)	MonumenTal-1 (NCT03399799, recruiting; NCT04634552)	Phase 1/2, multienter, open-label, dose escalation and dose expansion	143 (0.4mg/kg SC weekly), 145 (0.8mg/kg SC q2 wk)	51 (36 CART, 18 BsAb)	0.4 mg/kg: 74.1% (33.6% ≥ CR) 9.5 (6.7–13.3); 35% at 12 months 0.8 mg/kg: 71.7% (38.7% ≥ CR); NE (13.0-NE); 54% at 12 months	64.7% (35.3 ≥ CR %); 11.9 (4.8-NE); 38% at 12 months (ORR 75% in patients with prior CART and 44% with prior BsAb)	0.4mg/kg: CRS 79% (2.1%), dysgeusia 63%, anemia 44.8% (31.5%), skin-related AEs 63%, nail disorders 51.7%, infections 57.3% (16.8%) 0.8mg/kg: CRS 80% (0.7%), dysgeusia 46.2%, anemia 39.3% (24.8%), skin-related AEs 67.6% (0.7%), nail disorders 43.4%, infections 50.3% (11.7%)
Forimtamig (RG6234)[21†]	GPRC5D	BsAb (2:1 humanized antibody)	(NCT04557150, recruiting)	Phase 1, multienter, dose escalation and dose expansion	51 (IV), 57 (SC)	11 (IV), 12 (SC) (21 evaluable)	IV: 71.4% (34.7% ≥ CR); 10.8 (0–17.6); NR at 11.6 months SC: 63.6% (25.5% ≥ CR); 12.5 (1.2–12.5); NR at 8 months	IV: 50%; NR; NR SC: 54.5%; NR; NR	IV: CRS IV 82.4% (2.0%), ICANS 9.8% (2.0%), skin toxicity 78.4% (11.8%), hair and nail toxicity 23.5%, infections 56.9% (19.6%) SC: CRS 78.9% (1.8%), ICANS 12.3% (3.6%), skin 86% (22.8%), hair and nail toxicity 28.1%, infections 37.0% (24.1%)
Cevostamab[26†]	FCRH5	BsAb (Humanized IgG1 Fc)	GO39775 (NCT03275103, recruiting)	Phase 1, multienter, fixed-duration of 17 cycles; 90 and 160mg dose expansion cohorts	161 (86 90mg and 44 160mg target dose level evaluable patients)†	54 (27 CART, 13 BsAb, 27 ADC)—some patients had multiple BCMA therapies	90mg: 36.1% (9.6% ≥ CR); 11.5 (6.0–18.4; NR at 14.3 months 160mg: 56.7% (8.4% ≥ CR); NR; NR at 6.5 months	All: 36.4% CART: 44.4% BsAbs: 33.3% ADCs: 50.0%	CRS 80.7% (1.2%), ICANS 14.3% (0.6%), infections 42.5% (18.8%), neurological/psychiatric 40.6% (3.8%), anemia 31.9% (21.9%)

AE, adverse event; RP2D, recommended phase 2 dose.

*Nonexhaustive list of select ongoing trials (search May 18, 2023)—for a comprehensive list, please visit clinicaltrials.gov.

†Updated data presented during ASH 2021, ASH 2022, or ASCO 2023 meetings.

the 10 patients with prior BCMA-directed therapies (8 with prior CART). Typical CART-related (eg, CRS, ICANS, cytopenias) and GPRC5D-related (eg, skin and nail changes, dysgeusia) toxicities were seen, although 2 patients developed grade 3 cerebellar toxicity at the highest dose level (450×10e6 CART cells).[22] Several additional GPRC5D-targeted CART products have reported preliminary data, with similar efficacy in both BCMA treatment-naive and treatment-exposed patients, and no further cerebellar toxicity was reported.[23-25] Of note, loss of GPRC5D expression has been described in several patients progressing after GPRC5D CARTs or BsAbs, suggesting this may emerge as a mechanism of resistance to this approach.[22]

Another emerging target for myeloma therapy is Fc receptor-homolog 5 (FcRH5), a cell surface receptor highly expressed on myeloma cells, as well as on normal plasma and a subset of B cells. Cevostamab, a T-cell–engaging BsAb targeting FcRH5, is being evaluated in an ongoing phase 1 study exploring IV dosing every 3 weeks for a fixed duration (51 weeks). Preliminary analysis of 2 expansion cohorts showed ORRs of 37% (22/60) and 55% (24/44) at target doses of 90mg and 160mg, respectively, with an estimated median DOR of 11.5 months and not reported, respectively. CRS and ICANS were seen in 80% and 13% of patients, respectively. At target doses ≥90 mg, ORRs in patients with prior exposure to BCMA-directed CARTs, BsAbs, and ADCs were 44% (4/9), 33% (3/9), and 50% (7/14), respectively, demonstrating activity of cevostamab post-BCMA therapy.[26] A phase 1/2 study of cevostamab specifically in patients with prior BCMA-directed therapy is ongoing (CAMMA-2, NCT05535244). Overall, T-cell–engaging therapies against GPRC5D and FcRH5 are showing promising efficacy, and these should be considered as next lines of therapy following a BCMA-directed agent as they become available. In fact, in a single-institution, retrospective analysis of various treatments given to patients progressing after BCMA CART therapy, the best OS was seen in patients who received a different T-cell–engaging therapy (ie, BsAb or CART, most targeting GPRC5D) following prior BCMA CART, with a median OS not reached at 21 months.[18] This study, however, is limited by small size and potential selection bias, and prospective studies are needed.

Non–T-cell–engaging therapies

Data are limited regarding use of standard doublet/triplet/quadruplet regimens incorporating IMIDs, proteasome inhibitors, alkylators, and/or monoclonal antibodies post-BCMA therapy. However, these patients are typically triple class-exposed/refractory, and we know from older studies (eg, MAMMOTH) that expected ORRs in this population with these regimens are roughly 30% to 40%, with median PFS 3 to 4 months.[27] Similar efficacy numbers were reported in 2 retrospective single-institution experiences with these approaches for relapse following BCMA CART therapy.[13,18] The use of cytotoxic chemotherapy (eg, dexamethasone, cyclophosphamide, etoposide, and cisplatinum)±stem cell support, or salvage autologous stem cell transplant (SCT), was associated with responses in roughly 45% to 55% of patients in these studies and remains an option for patients with additional stem cells cryopreserved, especially in the setting of rapidly progressive disease or cytopenias.

Several additional novel agents have reported activity following BCMA-directed therapy (Table 4). A selinexor-based triplet or quadruplet combination induced responses in 7 of 11 patients (64%) with prior BCMA-directed therapy (8 prior ADC or mAb, 2 CART, 1 BsAb) in the STOMP trial, with 5 having responses lasting >6 months.[28] Iberdomide, a novel, oral cereblon E3 ligase modulator (CELMoD), was studied in combination with dexamethasone in 38 patients with prior BCMA-directed therapies. ORR was 37% and was similar regardless of type of prior anti-BCMA therapy, with a median DOR of 7.5 months and a median PFS of 2.4 months.[29] Mezigdomide, another potent oral CELMoD, also had significant activity in combination with dexamethasone in 30 BCMA treatment-exposed patients (22 ADC, 3 CART, 8 BsAb), with an ORR of 50%, a median DOR of 6.9 months, and a PFS of 5.4 months.[30] Finally, in a phase 1/2 study of modakafusp alfa, an immunocytokine consisting of an anti-CD38 antibody fused to 2 attenuated interferon alpha molecules, the ORR for the 1.5 mg/kg IV every 4-week dose was 43% and was 27% for the 15 patients with a prior BCMA-targeted therapy, with DOR and PFS not yet reported.[31] As these latter agents continue to move forward in development, they may provide additional non–T-cell–engaging, noncytotoxic options for patients following anti-BCMA exposure.

Factors to consider when choosing treatment after prior anti-BCMA therapy

BCMA expression: BCMA expression on myeloma cells is dynamic and can decrease after BCMA-targeted CART cells, but in most cases, BCMA is still present at time of relapse.[5,9,15] However, rare cases of biallelic genomic loss of BCMA (typically due to 16p deletion causing loss of the *TNFRSF17* (*BCMA*) gene locus, in combination with a BCMA mutation) have been described,[32,33] and the frequency of mutations or complete antigen loss may increase with the BsAb therapies, which provide more prolonged selective pressure due to their long-term administration. BCMA extracellular domain mutations have been identified that confer resistance to multiple BCMA-targeting BsAbs.[34] Unfortunately, while several research tools exist to assess for the presence of BCMA, including serum soluble BCMA assays and immunohistochemistry and flow cytometry assays for myeloma cell BCMA expression, none of these are widely available yet in clinical practice. Hence, currently assessment for BCMA is not required prior to pursuing a second or third BCMA-targeted therapy. However, it is likely that assessing for BCMA protein expression combined with sequencing for BCMA mutations will become a useful tool to help guide therapeutic choice after prior anti-BCMA therapy.

Timing since last anti-BCMA therapy: In cohort C of the CARTITUDE-2 study, where cilta-cel was given after prior BCMA-directed ADC or BsAb, responding patients had a shorter median duration of prior anti-BCMA therapy (29.5 vs 63.5 days) and a longer median time from prior anti-BCMA therapy to CART infusion (235 vs 117.5 days) than nonresponders.[14] A near-identical finding was observed with the use of ide-cel after prior BCMA-directed therapy.[12] While these findings need to be confirmed in larger studies, they suggest that the optimal patient to consider for another anti-BCMA therapy may be one whose prior anti-BCMA exposure was relatively short and occurred remotely (eg, >6 months earlier). For a patient progressing after more recent BCMA-targeted therapy, switching to an alternative target first and then coming back to a different BCMA-directed modality later may potentially be more effective. Of note, response to prior anti-BCMA therapy was not predictive of response or PFS following subsequent cilta-cel or ide-cel therapy.[12,14]

Table 4. Select non–T-cell–engaging therapies with evidence of efficacy following relapse after BCMA-directed therapies

Agent	Population/design	n			% ORR (sCR, CR); median DOR in months (95% CI); median PFS in months (95% CI) at reported median follow-up	
		Prior BCMA CART	Prior BCMA BsAb	Prior BCMA ADC (or mAB)	BCMA-exposed	All patients
Selinexor (+ various)[28]	BCMA-exposed subgroup in the STOMP trial (NCT02343042) —multicenter, open-label, phase 1b/2 study of selinexor in combination with backbone agents	2	1	8	63.6% (0% ≥ CR); NE (10.6-NE); NE (6-NE) at 14.3 months, with various regimens including XPd, XVd, XKd, XPVd, and XPEd	Various
Iberdomide (+ dex)[29,42]	BCMA-exposed cohort in CC-220-MM-001 trial (NCT02773030), multicenter, open-label, phase 1b/2 study	17	9	13	37% (5.3% ≥ CR); 7.5 (3.2-NE); 2.4 (2.1–4.2) at 8.1 months	At RP2D (dose expansion cohort, n=107): 26% (1% ≥ CR); 4 (2.4–10.5); 3.0 (2.8–3.7)
Mezigdomide (+ dex)[30]	BCMA-exposed subgroup in CC-92480-MM-001 (NCT03374085), multicenter, open-label phase 1b/2 study	3	8	22	50% (3.3% ≥ CR); 6.9 (4-NE), 5.4 (2.1–9.4) at 5.8 months	At RP2D (n=101): 39.6% (5% ≥ CR); 8.3 (5.4-NE); 4.6 (3.2–6.3) at 5.8 months
Modakafusp (TAK-573)[31]*	BCMA-exposed subgroup in multicenter, open-label phase 1/2 study (NCT03215030)	15 at RP2D (8 prior CART, prior ADC, and BsAb not specified)			27% (7% ≥ CR); NR; NR at 5.3 months	At RP2D (n=30): 43% (10% ≥ CR); 12.5 (1–21); 5.7 (1.2–14)

Dex, dexamethasone; XKd, selinexor, carfilzomib, and dexamethasone; XPd, selinexor, pomalidomide, and dexamethasone; XPd-40, selinexor 40 mg, bortezomib, and dexamethasone; XPd-60, selinexor 60 mg, bortezomib, and dexamethasone; XPEd, selinexor, pomalidomide, elotuzumab, and dexamethasone; XPVd, selinexor, pomalidomide, bortezomib, and dexamethasone; XVd, selinexor, bortezomib, and dexamethasone.

*Updated data presented during ASH 2022 meeting.

Patient characteristics/disease biology: When choosing a therapy for relapsed/refractory myeloma, patient- and disease-specific features should always be taken into consideration, and this applies after anti-BCMA therapy as well. Comorbidities, performance status, renal function, presence of cytopenias, prior therapies and toxicities, distance from treatment center, and/or willingness to be hospitalized are examples of patient-specific factors that may impact choice of a T-cell–directed therapy (eg, CART or BsAb) vs reexploring a standard triplet or quadruplet regimen that could be given in the community. Disease-specific features may include cytogenetics, extramedullary disease (EMD), and/or rapid progression. Thus, for a t(11;14) patient, one might consider a venetoclax-based combination,[35] and for patients with EMD and/or rapid disease progression, cytotoxic chemotherapy may be required to regain disease control and serve as a bridge to salvage SCT or a clinical trial.

Immune fitness: In additional to antigen loss, several potential immune-mediated mechanisms of resistance to BCMA-targeted BsAbs and/or CARTs have been identified, including a baseline decrease in T-cell receptor diversity, induction of T-cell exhaustion, and emergence of suppressive cell populations (eg, regulatory T cells, myeloid-derived suppressor cells).[36,37] As with BCMA expression/mutation testing, we currently lack easy tools to assess this in clinical practice, but in the future, our workup may include assessment of T-cell fitness to help guide whether another T-cell–directed therapy vs non–T-cell–directed therapy has the highest likelihood of response after anti-BCMA treatment.

CLINICAL CASE (continued)

The patient was offered a clinical trial of cevostamab but declined as he wished to avoid hospitalization and receive treatment closer to home. He started isatuximab, carfilzomib, and dexamethasone, with a partial response lasting 5 months, before his myeloma progressed. He has since started teclistamab, with ongoing CR at 6 months.

Conclusions

Management of relapse after a BCMA-directed therapy has become a new unmet need in myeloma. Fortunately, patients can respond to additional BCMA- and non-BCMA-targeted T-cell–engaging therapies, as well as both older and newer myeloma therapies not directly dependent on T-cell engagement. Determining the optimal sequence of these therapies remains challenging, although based on the limited available data, we favor sequential T-cell–engaging strategies targeting different antigens, if possible. As usual with relapsed/refractory myeloma, however,

treatment needs to be individualized for each patient, and ultimately ongoing trials, real-world data sets, and better biomarkers of response/resistance will help guide our decision-making.

Conflict-of-interest disclosure

Beatrice Razzo: no competing financial interests to declare.
Alfred L. Garfall has served as a consultant for BMS, Janssen, Novartis, GSK, and Legend; has received research funding form Novartis, Janssen, Tmunity, and CRISPR therapeutics; and has had intellectual property licensed by his institution to Novartis.
Adam D. Cohen has served as a consultant/advisory board member for GSK, Genentech/Roche, BMS/Celgene, Janssen, Abbvie, Pfizer, and Ichnos; has received research funding from Novartis, Janssen, GSK, Genentech/Roche, and BMS/Celgene; and has had intellectual property licensed from his institution to Novartis.

Off-label drug use

Beatrice Razzo: No off-label recommendations to disclose.
Alfred L. Garfall: No off-label recommendations to disclose.
Adam D. Cohen: No off-label recommendations to disclose.

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Accelerated-phase CML: de novo and transformed

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Despite the dramatic improvements in outcomes for the majority of chronic myeloid leukemia (CML) patients over the past 2 decades, a similar improvement has not been observed in the more advanced stages of the disease. Blast phase CML (BP-CML), although infrequent, remains poorly understood and inadequately treated. Consequently, the key initial goal of therapy in a newly diagnosed patient with chronic phase CML continues to be prevention of disease progression. Advances in genomic investigation in CML, specifically related to BP-CML, clearly demonstrate we have only scratched the surface in our understanding of the disease biology, a prerequisite to devising more targeted and effective therapeutic approaches to prevention and treatment. Importantly, the introduction of the concept of "CML-like" acute lymphoblastic leukemia (ALL) has the potential to simplify the differentiation between BCR::ABL1-positive ALL from de novo lymphoid BP-CML, optimizing monitoring and therapeutics. The development of novel treatment strategies such as the MATCHPOINT approach for BP-CML, utilizing combination chemotherapy with fludarabine, cytarabine, and idarubicin in addition to dose-modified ponatinib, may also be an important step in improving treatment outcomes. However, identifying patients who are high risk of transformation remains a challenge, and the recent 2022 updates to the international guidelines may add further confusion to this area. Further work is required to clarify the identification and treatment strategy for the patients who require a more aggressive approach than standard chronic phase CML management.

LEARNING OBJECTIVES

- Understand the implications of the revised definitions of CML phases with regards to identifying patients of highest potential for transformation
- Understand the recent developments in disease biology and therapeutics in blast phase chronic myeloid leukemia

CLINICAL CASE 1

A 26-year-old man was diagnosed with chronic myeloid leukemia (CML) in the chronic phase (CP) following presentation with a marked leukocytosis (white blood cell count, 360×10^9/L), moderate anemia, and normal platelet count. He was classified as high risk by the ELTS score and was commenced on 100 mg/d dasatinib. While he demonstrated an early hematologic response to dasatinib and rapid fall in *BCR::ABL1* values to below 10%, by 2 months, there was emergence of circulating lymphoblasts, and bone marrow biopsy specimen confirmed progression to lymphoid blast phase (BP).

Introduction

While the introduction of tyrosine kinase inhibitors (TKIs) revolutionized the landscape of therapeutic options in chronic myeloid leukemia (CML), enabling most patients to reach optimal molecular targets and outcomes, there remains a subset of patients who either present in or progress to more advanced stages of the disease. Even upfront therapy with potent second-generation TKIs (2G-TKIs) does not completely negate the risk of progression to either accelerated phase (AP) or blast phase (BP) CML as demonstrated in long-term follow-up data from the key frontline TKI studies (Table 1), although that risk has been markedly reduced compared to frontline imatinib-treated patients. Reversion to chronic phase CML (CP-CML) remains critical,

Table 1. Incidence of progression to accelerated and/or blast phase in the major frontline TKI studies in chronic phase CML

Clinical trial (follow-up in years)	Frontline TKI (dose)			
	Imatinib	Nilotinib	Dasatinib	Bosutinib
IRIS[42] (10 years)	7% (400 mg)			
TOPS[43] (42 months)	4.5% (400 mg)/2.5% (800 mg)			
CML-IV[44] (10 years)	6% (400 mg)/5% (800 mg)			
TIDEL-II[45] (40 months)	3.5% (600 mg)			
ENESTnd[46] (10 years)	8.5% (400 mg)	4% (300 mg BD)/2% (400 mg BD)		
ENESTfirst (24 months)		0.6% (300 mg BD)		
DASISION[47] (5 years)	7% (400 mg)		5% (100 mg)	
BFORE[48] (5 years)	3% (400 mg)			2% (400 mg)

BD, twice daily.

but long-term cure with TKI alone has rarely been achieved,[1] necessitating early intervention with an allogeneic stem cell transplant where possible for those diagnosed with BP-CML who can achieve a second CP-CML.

The difficulties in identifying and managing patients with disease that does not exemplify the classic CP-CML that most clinicians are familiar with will be discussed in this chapter. The challenges associated with the recent updates in international guidelines will also be explored. Understanding the disease biology of progression and/or BP-CML is imperative and differentiating de novo BP-CML from Philadelphia chromosome–positive (Ph^+) acute leukemia is increasingly vital to ensure appropriate interpretation of results and optimal therapeutic decisions. Furthermore, the pathway to progression is not well understood with the pathognomonic *BCR::ABL1* fusion alone likely insufficient to drive progression to BP-CML with studies examining genomic profiles in BP-CML uncovering additional genetic abnormalities in almost all cases.[2] Exploring the impact of next-generation sequencing (NGS) in this context is highly relevant. Finally, appreciating the emerging developments in BP-CML therapeutics and the integration of these findings into the current treatment spectrum is necessary. Clinical cases will be used to illustrate key points raised in this chapter.

Reviewing the definitions

Defining the stages of CML has become more complicated with recent updates to the various classification systems, with the World Health Organization (WHO) abolishing AP-CML altogether (Table 2).[3] Patient staging may be altered, which may in turn impact therapeutic decisions, depending on which guideline is being applied. The reduced incidence of progression to AP in addition to most de novo AP patients having similar responses to patients with CP-CML with TKI therapy formed the basis of the justification for this major alteration to the WHO position,[3] with the overall consensus being that the triphasic natural course of CML has become less relevant in the TKI era. However, the removal of AP as a category implies that there is no intermediary phase where patients may be at higher risk of transformation, which as many clinicians will appreciate is a fallacy.

Surprisingly, the cytogenetic profile is not taken into account at any point with the updated WHO guidelines.[3] Almost all of the other guidelines incorporate additional cytogenetic abnormalities (ACAs) as a key definition of AP-CML with minor differences such as inclusion of 3q26.2 rearrangements and complex cytogenetics in the previous iterations of the WHO.[4-6] The original ACAs are defined as trisomy 8, additional Ph translocation, isochromosome 17q, and trisomy 19. Additional high-risk cytogenetic lesions, including trisomy 21, 3q26.2, monosomy 7/7q-, 11q23, and a complex karyotype, together with the original ACAs were identified as conferring an inferior overall survival (OS) and a higher propensity to be present at BP-CML.[7] In fact, when these events were observed in conjunction with lower blast counts (defined as 1%-15%), it also heralded disease progression and inferior OS.[7] Cytogenetic interrogation of the SPIRIT2 cohort, which compared upfront dasatinib to imatinib in newly diagnosed patients with CP-CML, revealed that the presence of ACAs did not correlate with either the Sokal or ELTS but was independently predictive of progression-free survival (PFS).[8] While the PFS was dominated by non-CML deaths without evidence of progression and so perhaps masks the true impact of ACAs (original and modified), the freedom from progression analysis clearly demonstrated that the presence of any one of these lesions conferred an inferior freedom from progression compared to the absence of ACAs (76% vs 98%, $P<.001$) detected at diagnosis.[8] Data from a retrospective analysis of patients with CML treated at the MD Anderson Cancer Center also confirmed an inferior OS and molecular responses, especially in association with selected ACAs, including i(17), monosomy 7/7q-, and 3q26.2 rearrangements.[9] Specific evaluation for the 3q26.2 abnormalities that contain the *EVI1* locus, which when observed in acute myeloid leukemia characterizes a highly aggressive course with poor prognosis, similarly highlights a subset of patients with CML who have a very poor OS.[10] Emergence of 3q26.2 abnormalities in either CP or AP-CML had a high rate of transformation to BP, with the median time to progression approximating 3 months, while also drawing attention to a group of patients with a substandard response to TKI therapy.[10] A smaller French study evaluating 42 patients with AP-CML also confirmed the presence of ACAs predicted for a higher rate of failure and inferior PFS, especially if the hematologic features of AP-CML were evident.[11]

Perhaps instead of abolishing AP-CML altogether, it may have been prudent to simply tighten the definition surrounding AP-CML as some of these higher-risk patients are not recognized within the current WHO guidelines.[3] The International Consensus

Table 2. Classification systems used in chronic myeloid leukemia, including recent updated guideline recommendations

	European LeukemiaNet[6,49]	WHO 2016[5]	ICC 2022[4]	WHO 2022[3]
Accelerated phase	PB or BM blasts 15%-29%	PB or BM blasts 10%-19%	BM or PB blasts 10%-19%	
	PB blasts + promyelocytes ≥30%			
	PB basophils ≥20%	PB basophils ≥20%	Peripheral blood basophils ≥20%	
	Platelets ≤100×10⁹/L (unrelated to therapy)	Platelets ≤100×10⁹/L (unrelated to therapy) or >1000×10⁹/L (unresponsive to therapy)		
		Splenomegaly (unresponsive to therapy)		
	Cytogenetic evolution on treatment	ACA in Ph⁺ cells at diagnosis, including major route, complex karyotype, or 3q26.2 abnormalities, at diagnosis Cytogenetic evolution on treatment	ACA in Ph⁺ cells	
	Consider: ACAs in Ph⁺ cells Resistance to 2 TKIs Detection of a *BCR::ABL1* kinase domain mutation	*Provisional:* Failure to achieve CHR to first TKI Any indication of resistance to 2 sequential TKIs Occurrence of >2 mutations on *BCR::ABL1* during TKI		
Blast phase	PB or BM blasts ≥30%	PB or BM blasts ≥20%	BM or PB blasts ≥20%	BM or PB blasts ≥20%
	Extramedullary blast proliferation	Extramedullary blast proliferation	Myeloid sarcoma	Myeloid sarcoma
			Presence of morphologically apparent lymphoblasts (>5%) warrants consideration of lymphoid BP-CML	Presence of increased lymphoblasts in PB or BM

ACA, additional clonal cytogenetic abnormalities; BM, bone marrow; CHR, complete hematologic remission; PB, peripheral blood.

Classification (ICC), also updated in 2022, has simplified the definition of AP-CML to only take into account 3 variables—blasts, basophil count, and the presence of ACAs in Ph⁺ cells.[4] The variables that were considered "softer" definers of AP such as platelet count and splenomegaly response are not even considered by the ICC, and it may be reasonable to now omit these in the context of stronger evidence addressing the other parameters. Irrespective of the definition used, AP-CML can be treated as high-risk CP-CML with TKI monotherapy. However, we do recommend close scrutiny of response in patients with AP-CML since TKI monotherapy may be inadequate in select patients, such as those with 3q26.2 rearrangements.[10] Allogeneic stem cell transplant (Table 3) or enrollment in clinical trials investigating agents that can target EVI1, such as BET or PARP inhibitors, should be considered.

While the classification of AP-CML is hotly debated between the 2 recent updates to the ICC and the WHO, BP-CML remains relatively unchanged, although the definition now encompasses lymphoblasts in the peripheral blood/bone marrow as a BP-defining criteria in both guidelines. The ICC goes a step further, including a ≥5% cutoff for circulating lymphoblasts (Table 2).[3,4] The data supporting this change are limited and largely restricted to retrospective case series and are somewhat conflicting, with some reports not able to demonstrate a link between progression to lymphoid BP-CML,[12] whereas others indicate a high propensity for early progression.[13-16] This may be linked to increasing reliance on sensitive flow cytometry and improved discrimination between lymphoblasts and hematogones but also defining a blast threshold below which progression to lymphoid BP is less likely. Our suggestion would be to perform flow cytometry at diagnosis to ensure patients with excess lymphoblasts are identified to enable appropriate treatment to be promptly initiated. However, this may not be a cost-effective screening tool for most institutions globally as an internal audit (unpublished data) has demonstrated that diagnostic flow cytometry only altered the treating approach in <1% of newly diagnosed CML.

CLINICAL CASE 2

A 58-year-old man presents to a peripheral center with 7% lymphoblasts in the peripheral blood with associated leukocytosis with neutrophilia. BCR::ABL1 positivity was confirmed, but bone marrow biopsy a few days later demonstrated CP-CML with no excess of blasts. The peripheral blood blast population also spontaneously cleared. Cytogenetics revealed a deletion

Table 3. Recommendations regarding which patients should be considered for allogeneic stem cell transplantation

High-risk features indicating the need to initiate a donor search for transplant-eligible patients
The presence of specific cytogenetic abnormalities at diagnosis or acquisition while on therapy, including • Isochromosome 17q • 3q26.2 • Monosomy 7/7q- • Complex karyotype
Failure to achieve any cytogenetic or molecular response to 2G-TKI after a minimum of 3 months of therapy
Recurrent grade IV cytopenias despite TKI dose interruptions, dose modifications, and cytokine support, especially within the first 3 months of therapy, leading to EMR failure or ELN-defined treatment failure
Recurrent grade 4 toxicity preventing consistent TKI dose intensity, resulting in EMR failure or ELN-defined treatment failure on 2 or more lines of TKI therapy
Compound kinase domain mutations involving T315I
Lymphoblasts >5% at diagnosis

ELN, European LeukemiaNet; EMR, early molecular response.

of 13q, encompassing *RB1*, in addition to the standard Ph$^+$ chromosome. NGS confirmed the presence of a low-level *RUNX1* nonsense mutation in addition to the *BCR::ABL1* translocation. He was treated as CP-CML and commenced 100 mg/d dasatinib. While he demonstrated a complete hematologic response and an initial significant decline in BCR::ABL1 within the first few months, there was rapid progression to lymphoid BP at 6 months with emergence of an F317L mutation. Lymphoblasts carried the same phenotype as observed at presentation. Cytogenetic analysis revealed clonal evolution, including formation of dicentric chromosomes 7 and 12, partial loss of 7p, and an isochromosome derivative 9. He was referred for an allogeneic stem cell transplant workup and commenced on combination chemotherapy with hyperCVAD in addition to 30 mg/d ponatinib, entering a second CP. He underwent a reduced intensity conditioning allogeneic transplant but relapsed within 5 months, necessitating treatment with blinatumomab. Unfortunately, despite achieving a morphologic remission with blinatumomab, he succumbed to septic shock 2 months following treatment completion.

Discussion points

1. Consider the need for flow cytometry at diagnosis

We recommend flow cytometry to be performed at diagnosis to enable accurate enumeration of the blast percentage but also confirmation of the phenotype of identified blasts. The presence of lymphoblasts should prompt concern that this patient is of high risk of progression lymphoid BP-CML, necessitating more frequent monitoring, including repeat bone marrow biopsies as well as a donor search for consideration of an allogeneic stem cell transplant, especially in light of the recent WHO and ICC updates. Persistence of lymphoblasts should be treated as for lymphoid BP.

2. What is the optimal central nervous system prophylaxis in this scenario?

While this was not specifically addressed in the case vignette, the issue of central nervous system (CNS) prophylaxis is highly relevant. The CNS and testes remain a sanctuary site from conventional chemotherapy, and CNS relapses, while rare, do occur. Whichever chemotherapy protocol is used, CNS-penetrating drugs (such as higher-dose cytarabine and methotrexate) need to be included in the regimen. CNS sampling and imaging are also key to exclude current involvement, and regular intrathecal chemotherapy should also be considered. In the event of CNS disease, regular intrathecal chemotherapy administration is recommended. TKI selection is also vital in this setting as not all agents can cross the blood-brain barrier. Imatinib is not preferred for this reason, but both dasatinib and ponatinib can penetrate the blood-brain barrier, resulting in therapeutic levels in the cerebrospinal fluid in murine models.[17,18] Furthermore, in the setting of pediatric Ph$^+$ acute lymphoblastic leukemia (ALL), the incidence of CNS relapse following intensive chemotherapy was less in the dasatinib arm compared with the imatinib cohort.[19] Therefore, to maximize CNS prophylaxis, either dasatinib or ponatinib would be the preferred TKI in conjunction with CNS-penetrating chemotherapy.

3. Is myeloablative (or reduced intensity) conditioning preferred for an allogeneic stem cell transplant in BP-CML?

Reduced intensity conditioning is becoming an acceptable option for older patients who are unable to tolerate the intensity of myeloablative conditioning (MAC) with similar OS between the 2 regimens.[20] This is largely due to the improved relapse-free survival with myeloablative conditioning balancing out with the lower nonrelapse mortality but higher relapse rate associated with reduced intensity conditioning.[20,21] These data are mostly in the setting of CP-CML, with patients with BP-CML being specifically avoided in these studies. Alternative donors were also generally excluded from these studies. Therefore, in the setting of BP-CML, we recommend myeloablative conditioning, if possible, due to the lower risk of relapse. In this scenario, due to age and comorbidities, reduced intensity conditioning was selected.

Differentiating lymphoid blast phase CML from Ph$^+$ ALL

The possibility that some patients diagnosed with Ph$^+$ ALL actually had de novo lymphoid BP and vice versa has been an ongoing issue that, until recently, could only be the subject of conjecture. In the era of minimal residual disease (MRD) monitoring, some advancement in this area has been possible. Parallel MRD monitoring with immunoglobulin/T-cell receptor (Ig/TCR) gene rearrangements and with *IKZF1* deletion

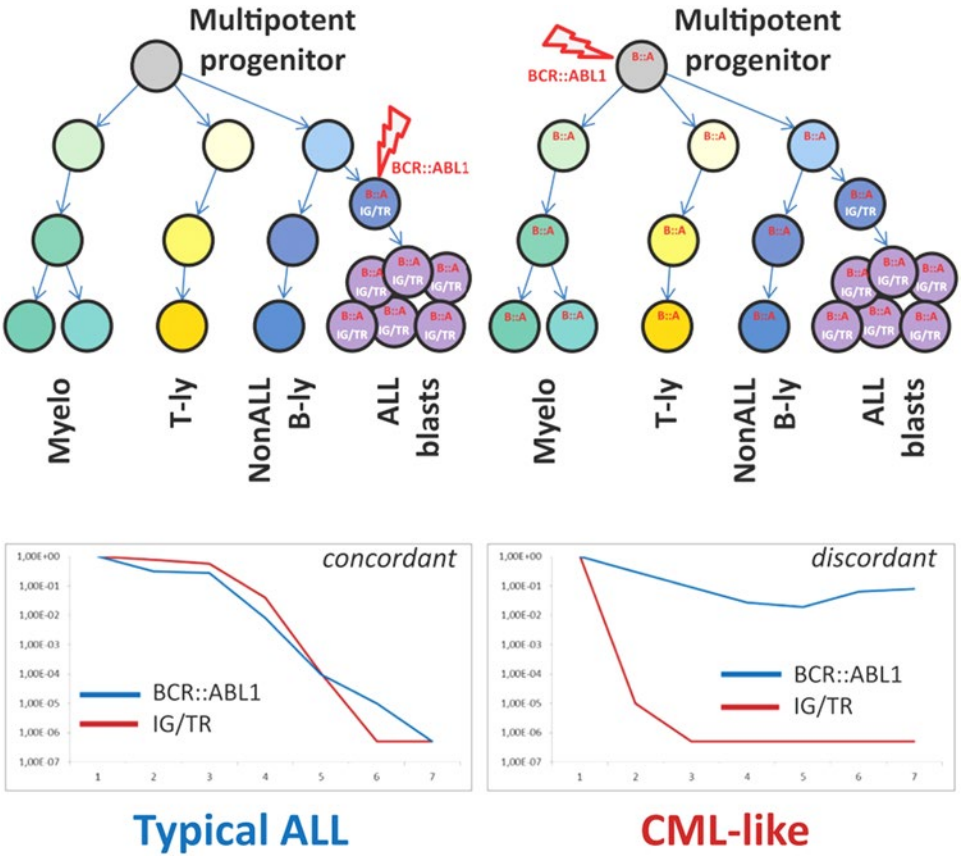


Figure 1. Schematic illustration of key differences between "typical ALL" and "CML-like" disease. Adapted from Zuna et al. with permission.[23]

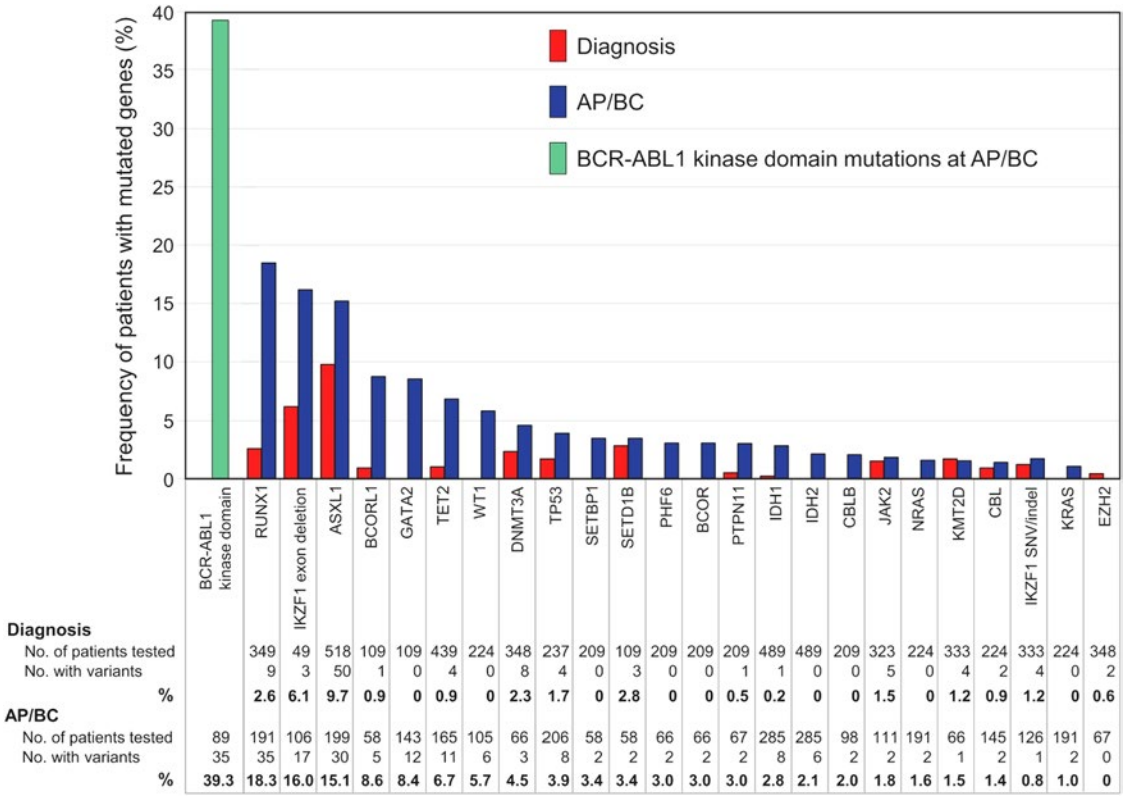
quantification in a population of pediatric Ph⁺ ALL had excellent concordance.[22] However, in a proportion of patients, DNA-based monitoring of the unique *BCR::ABL1* genomic breakpoint revealed consistently higher levels of *BCR::ABL1* fusion compared to Ig/TCR and/or *IKZF1* deletion MRD quantification.[22] Subsequent cell sorting of diagnostic material from patients with discordant MRD results confirmed the presence of the *BCR::ABL1* fusion in other hematopoietic cells, such as T lymphocytes, and other myeloid cells, confirming the involvement of a Ph⁺ pluripotent hematopoietic progenitor similar to CML (Figure 1).[22]

Whether these patients, referred to as having CML-like ALL, follow a distinct disease trajectory was explored in a larger cohort of 147 pediatric patients with Ph⁺ ALL.[23] Patients were defined as having CML-like disease (n = 48) if ≥1 MRD time point had >1 log discordance between *BCR::ABL1* and Ig/TCR-measured MRD.[23] There was no significant difference in the 5-year survival parameters, specifically event-free survival and OS, between patients with CML-like ALL and typical Ph⁺ ALL. However, the level of MRD in patients with typical Ph⁺ ALL appeared to correlate with event-free survival and OS, with higher levels of MRD (≥10^{-3}) indicating markedly inferior outcomes.[23] In comparison, the MRD level was less concerning and not informative for therapy adjustment in CML-like disease.[23] Hyperleukocytosis at diagnosis remains a poor prognostic feature in typical Ph⁺ ALL, whereas there was no association with outcome in CML-like ALL.[23] Further investigation is required to validate these findings on a larger scale, but given the trend to alter therapy in ALL based on rising MRD, there is clearly a subset of patients with CML-like disease in whom a rising level of *BCR::ABL1* may not have the same ominous implications.

Investigating advanced CML—the role of NGS

At the time of BP-CML, standard investigation to identify why these specific patients progressed involves cytogenetic analysis and investigation for kinase domain mutations, which remain the best understood mechanism of resistance. However, cytogenetic analysis does not often reveal karyotypic abnormalities in addition to the standard Ph translocation while kinase domain mutations are only identified in ~50% of patients.[24] Targeting the *BCR::ABL1* kinase domain via NGS has improved sensitivity and therefore detection of kinase domain mutations, observed in almost 80% of AP/BP-CML enrolled in the Next-in-CML study,[25] but not all patients are found to harbor these mutations.

With increasing availability of NGS, our appreciation of the contribution of additional genomic defects in BP-CML has rapidly expanded. While early investigation focused on single gene studies, more recent evaluation includes unbiased interrogation of the whole exome or transcriptome.[26,27] All patients at progression to BP-CML harbor additional genetic abnormal-



	BCR-ABL1 kinase domain	RUNX1	IKZF1 exon deletion	ASXL1	BCORL1	GATA2	TET2	WT1	DNMT3A	TP53	SETBP1	SETD1B	PHF6	BCOR	PTPN11	IDH1	IDH2	CBLB	JAK2	NRAS	KMT2D	CBL	IKZF1 SNV/indel	KRAS	EZH2
Diagnosis																									
No. of patients tested		349	49	518	109	109	439	224	348	237	209	109	209	209	209	489	489	209	323	224	333	224	333	224	348
No. with variants		9	3	50	1	0	4	0	8	4	0	3	0	0	1	1	0	0	5	0	4	2	4	0	2
%		**2.6**	**6.1**	**9.7**	**0.9**	**0**	**0.9**	**0**	**2.3**	**1.7**	**0**	**2.8**	**0**	**0**	**0.5**	**0.2**	**0**	**0**	**1.5**	**0**	**1.2**	**0.9**	**1.2**	**0**	**0.6**
AP/BC																									
No. of patients tested	89	191	106	199	58	143	165	105	66	206	58	58	66	66	67	285	285	98	111	191	66	145	126	191	67
No. with variants	35	35	17	30	5	12	11	6	3	8	2	2	2	2	2	8	6	2	2	2	1	2	1	2	0
%	**39.3**	**18.3**	**16.0**	**15.1**	**8.6**	**8.4**	**6.7**	**5.7**	**4.5**	**3.9**	**3.4**	**3.4**	**3.0**	**3.0**	**3.0**	**2.8**	**2.1**	**2.0**	**1.8**	**1.6**	**1.5**	**1.4**	**0.8**	**1.0**	**0**

Figure 2. Frequency of mutated cancer genes at diagnosis and AP/BP. The data from 15 studies of patients at diagnosis and 20 studies at AP/BP are reported where cancer genes were mutated in more than 1 patient at diagnosis and/or BP. Only genes listed in the COSMIC Cancer Gene Census are included. Adapted from Branford et al. with permission.[2]

ities, either involving cancer gene variants or rearrangements involving the Ph chromosome, although the Ph-associated rearrangements are present from diagnosis as opposed to being acquired at progression.[26-28] Genomic analysis to date suggests that there are only a relatively small number of clinically relevant genes recurrently mutated in CML, enabling targeted capture of select candidate genes.[2,29,30] Genes recurrently mutated in AP/BP-CML are *RUNX1*, *IKZF1*, and *ASXL1* in descending order, but others have been described (Figure 2).[2] Even when kinase domain mutations are identified, a high proportion of these cases is found to have co-occurring additional genetic abnormalities.[2] What is also clear is that the mutational subtypes observed in BP-CML are not limited to single nucleotide variants and small insertions and deletions but also involve larger gene deletions, aberrant splicing, and fusions.[27,31] Furthermore, the presence of additional genetic abnormalities at diagnosis of CP-CML was more frequent in patients who progressed to BP-CML compared to those with optimal outcomes.[26] Interestingly, the presence of genomic abnormalities at diagnosis of CP-CML also predicted for inferior survival and molecular response in patients treated with imatinib,[32] suggesting that genomic investigation at diagnosis of CP-CML has the potential to identify higher-risk patients, including those who with a high risk of progressing to BP-CML. However, recent interim data suggest that more potent 2G-TKIs can perhaps ameliorate the adverse impact of additional genetic abnormalities, although not completely negate their effect.[33] This adds weight for the inclusion of NGS to the repertoire of tests that could be performed at diagnosis in order to enable optimal TKI selection.

CLINICAL CASE 1 (continued)

While the bone marrow biopsy specimen confirmed the presence of $CD19^+$ $CD20^+$ $CD34^+$ lymphoblasts, fluorescence in situ hybridization confirmed the loss of 17p and TP53, and kinase domain mutation screening demonstrated emergence of T315I mutations. He was treated with hyperCVAD chemotherapy in combination with ponatinib 45 mg/d and was able to enter a second CP prior to proceeding to an unrelated donor transplant with MAC. Two years post-allograft, he remains in remission with 100% chimerism and undetectable *BCR::ABL1* transcripts. He has not been able to commence post-transplant TKI maintenance due to various cytopenias. Interestingly, retrospective NGS investigation demonstrated expansion of a low-level *IKZF1* deletion at progression that was detectable at diagnosis (Figure 3).

Discussion points

1. What is the optimal dose of ponatinib in this situation?

The MATCHPOINT study suggested that 30 mg/d ponatinib was the optimal dose in conjunction with chemotherapy to minimize associated toxicity based on the EffTox model. However, in this setting, the presence of the T315I mutation dictated the use of the higher ponatinib dose to maximize a response and enhance the prospects of achieving a second CP.

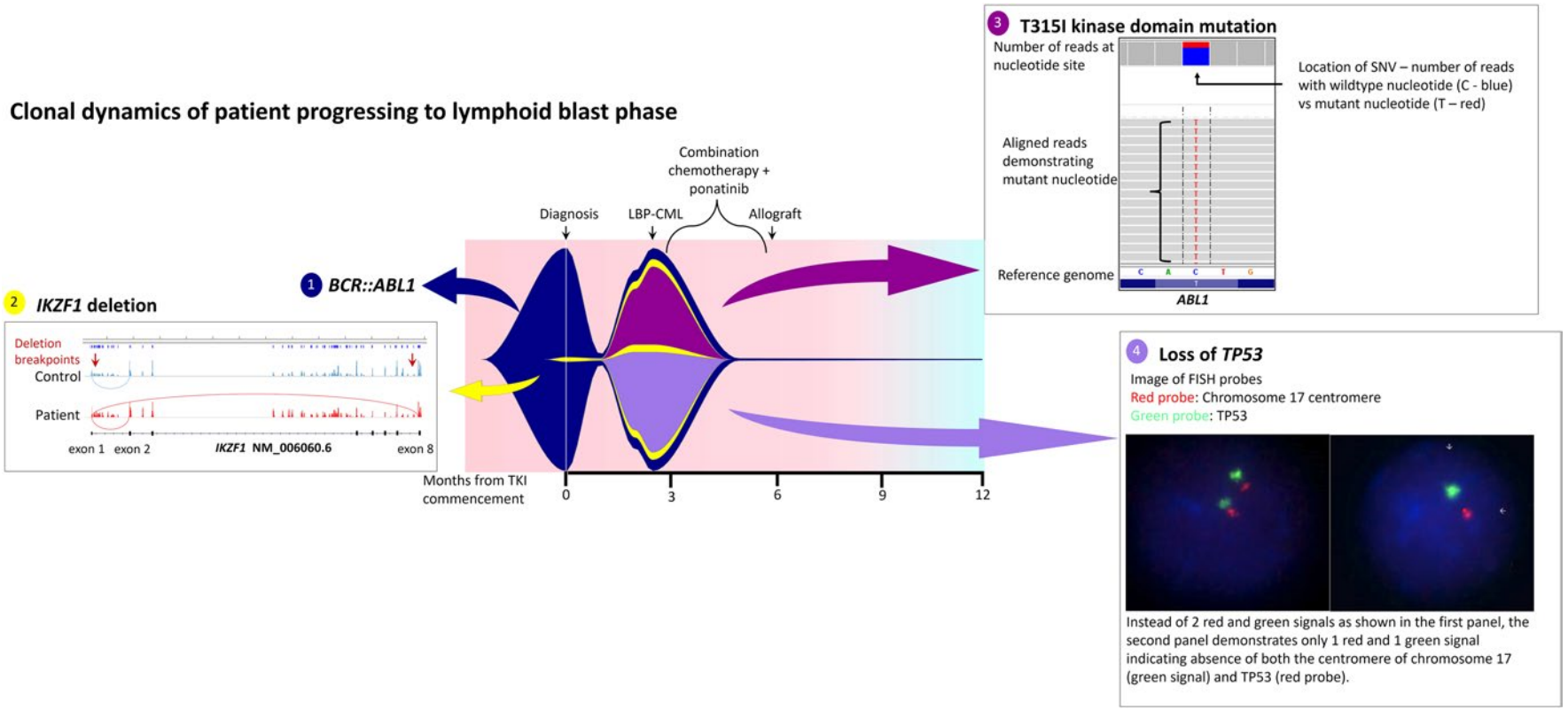


Figure 3. Fish plot illustrating mutation profile and clonal dynamics of patient 1. This patient progressed to lymphoid blast crisis within 3 months of diagnosis. This figure illustrates the complex clonal dynamics involved, with the fish plot highlighting the primary *BCR::ABL1* clone in addition to the 3 subclones, including expansion of the *IKZF1* subclone detectable at diagnosis in addition to the acquisition of T315I as well as loss of *TP53*. Detection of all variants involved NGS, Sanger sequencing, and cytogenetic analysis. The *IKZF1* deletion is indicated by aberrant splicing, with the breakpoints indicated by the red arrows. The T315I mutation is shown on NGS while the loss of TP53 is indicated via fluorescence in situ hybridization probes. Combination chemotherapy in addition to ponatinib enabled clearance of the leukemic clone; LBP, lymphoid blast phase.

2. Would identification of the *IKZF1* deletion at diagnosis trigger any alteration to the approach?

This patient was deemed high ELTS risk at diagnosis and so a 2G-TKI was selected for frontline therapy. While detection of a low-level *IKZF1* deletion would not necessarily alter the initial management of this patient at diagnosis beyond ensuring appropriate TKI selection (such as a 2G-TKI as opposed to frontline imatinib) and maintaining TKI intensity, the presence of the *IKZF1* clone, often seen in BP-CML (Figure 2), may have indicated the potential for progression to BP-CML despite the early response to TKI. It may have been prudent to perform early tissue typing, although there is no definitive evidence to support this.

3. Is there benefit to TKI maintenance after allograft?

TKI administration after allograft has been demonstrated to be beneficial in Ph$^+$ ALL, improving leukemia-free survival,[34] but similar utility in CML is less evident. A recent Center for International Blood and Marrow Transplant Research study analyzed clinical outcomes for 390 CML transplants, with 89 patients receiving TKI maintenance after allograft.[35] A range of TKIs were used posttransplant, including imatinib, nilotinib, and dasatinib. Outcome measures did not significantly differ between those who received maintenance compared with those who did not. For patients who had evaluable data following day +100, the 5-year OS was 61% respectively in the maintenance TKI group vs 57% if patients did not receive TKI posttransplant ($P = .61$).[35] Likewise, the 5-year leukemia-free survival did not differ between the 2 groups either.[35] This study carries inherent bias, as only patients who survived to day +100 were evaluable, and early relapses would not have been captured by the landmark analysis in addition to higher-risk individuals being selected for maintenance treatment. Furthermore, while this study did not demonstrate a benefit for maintenance TKI after allograft, specific additional considerations may influence this decision. The selection of conditioning regimen may be a factor as while there is no difference in OS between a MAC compared to a RIC protocol, there is a higher potential for early relapse with RIC.[20] Measurable *BCR::ABL1* and/or history of BP-CML prior to transplant can support TKI maintenance, whereas the presence of posttransplant complications, such as poor engraftment, infection, and graft-versus-host disease, may curtail the potential for TKI initiation altogether.

Novel therapeutic strategies in BP-CML

The primary goal of therapy in BP-CML, irrespective of whether the disease has progressed from CP or presents in de novo BP-CML, is to return to CP-CML once more and proceed to an allogeneic transplant if patients are eligible. However, there is no consistent strategy recommended to achieve this. The low frequency of de novo BP-CML but also transformed disease contributes to the difficulty of developing high-powered clinical trials to investigate therapeutic options in BP-CML. TKI alone is generally inadequate to revert BP-CML to CP[36] as only 31% of patients achieve a major hematologic response even with ponatinib monotherapy.[37] Multiagent chemotherapy in conjunction with TKI is required if patients can tolerate therapy intensity to maximize entering a second CP.[38] The chemotherapy regimen is generally dictated by the blast lineage, with more myeloid-directed combinations being used in myeloid BP-CML, whereas lymphoid BP-CML is generally treated with ALL-directed regimens, such as hyperCVAD. The choice and dose of TKI are not always clear, but combination therapy using more potent TKIs does correlate with improved outcomes, including relapse-free survival.[38] Consequently, a more efficacious and uniform treatment model is required.

CLINICAL CASE 3

A 70-year-old woman presents with marked leukocytosis (white blood cell count, 213×10^9/L) with circulating myeloblasts of 5%. There was associated splenomegaly, with the splenic edge extending 10cm below the costal margin. The bone marrow biopsy specimen confirmed 10% myeloblasts and a standard Ph-chromosome alone. She was diagnosed with CP-CML and commenced on nilotinib 300 mg twice daily on a clinical trial. She developed marked pancytopenia (hemoglobin <70 g/L, neutrophil count <0.2×10^9/L, and platelets <20×10^9/L) with nilotinib, and despite dose reduction and treatment interruption, this failed to resolve. She was withdrawn from the study and switched to imatinib with recurrence of pancytopenia, necessitating long treatment interruptions. Transitioning to 50 mg/d dasatinib had the same outcome, and her BCR::ABL1 slowly increased in the presence of persistent pancytopenia. She was transitioned to the phase 1 asciminib study where treatment intensity was maintained with aggressive transfusion support. However, following 6 months of asciminib with dose interruption and modification for pancytopenia, she progressed to myeloid BP with acquisition of trisomy 8 on cytogenetic analysis. By this stage, she was 72 years old and not fit for intensive chemotherapy, nor was she an allogeneic stem cell transplant candidate. Ponatinib 45 mg/d was commenced, and to maintain treatment intensity, she was once more supported aggressively with transfusions. She was able to enter a second CP within 6 months, achieving a complete cytogenetic remission for the first time and maintained a good response on ponatinib monotherapy for a further 3 years before progressing to a second myeloid BP, succumbing to her disease shortly after.

Discussion points

1. Maintaining TKI intensity in patients with marked pancytopenia

While this patient was high risk by ELTS score, treatment intensity could not be maintained due to the associated marked cytopenia. This would have contributed to the risk of progression. Patients with high-risk disease and cytopenia would ideally be considered for an allogeneic stem cell transplant at an early stage (Table 3) if fitness was adequate. However, this patient was not a transplant candidate, and so when progression to myeloid BP occurred, the preference was to maintain ponatinib dose intensity to maximize a response.

2. Role of transfusions and cytokines to enable dose intensity to be maintained

Maintaining dose intensity is vital to maximize response and minimize transformation potential. Managing grade 3 cytopenias may necessitate platelet and red cell transfusion support to permit adequate TKI intensity as opposed to dose interruptions. Judicious use of granulocyte-colony stimulating factor to manage neutropenia is also recommended. Early cytopenias are often secondary to eradication of the CML clones that are primarily responsible for most hematopoiesis in the bone

marrow, and not maintaining treatment intensity will essentially leave the CML inadequately treated. The benefits of maintaining treatment intensity need to be balanced with the competing risks of bleeding and infection. While this is largely an evidence-free zone, we used this strategy to manage this patient's BP and maximize ponatinib dosing.

MATCHPOINT

While ponatinib is certainly an attractive choice of TKI for use in BP-CML given potency and ability to overcome a number of highly resistant kinase domain mutations, optimal dosing and the ideal chemotherapy regimen to be combined with ponatinib needs clarity. The combination of ponatinib in addition to fludarabine, cytarabine, idarubicin chemotherapy was investigated in a phase 1/2 study that recruited across the United Kingdom. Recruited patients (n = 17) had myeloid, lymphoid, or mixed-lineage BP-CML and had a combination of de novo and progressed disease with a median age of 33 years (range, 16-64 years).[39] The aim of the study was to identify the optimal dose of ponatinib in combination with conventional chemotherapy and capitalized on an EffTox design, which is a Bayesian adaptive dose-finding schedule that rigorously investigates both efficacy and toxicity.[40] The optimal dose of ponatinib was identified to be 30 mg/d, and of the 16 patients evaluable for the primary outcome, 69% (n = 11) entered a second CP-CML following 1 cycle of treatment, including 5 patients achieving a *BCR::ABL1* ≤0.1%[IS].[39] Dose-limiting toxicity was observed in 4 patients, including 1 episode of fulminant cardiomyopathy and another with cerebral vein sinus thrombosis. Twelve patients were able to proceed to an allogeneic stem cell transplant with a median follow-up of 41 months. All 5 patients not transplanted died within 7 months of study entry, which included 3 of the 4 patients with dose-limiting toxicity.[39] Five of the transplanted patients also died, 2 from disease relapse and the remainder secondary to transplant-related complications.[39] While further investigation is required, this study demonstrates that the MATCHPOINT approach of combining 30 mg/d ponatinib with FLAG-Ida chemotherapy is a feasible strategy to salvage patients in BP-CML in order to bridge to an allogeneic stem cell transplant. However, the long-term OS remains <50% despite this intense treatment strategy.

Dasatinib and decitabine

Another recent study examined the combination of dasatinib and decitabine in advanced phase CML. Using a 3+3 design, doses of either 10 or 20 mg/m^2 decitabine for 10 days with either 100 or 140 mg dasatinib daily were investigated.[41] Thirty patients (including 19 in BP-CML, 7 AP-CML, and 4 Ph$^+$ AML) were enrolled with a median age of 51 years (range, 18-89 years).[41] Dose-limiting toxicity was observed in only 2 patients, but this was only with the higher dasatinib dose of 140 mg, one with grade 3 cardiac failure and another with a cardiac arrest following a myocardial infarction. Twenty-seven patients completed the minimum 2 cycles for response evaluation, and 19 patients achieved a hematologic response, whereas no response was observed in patients with Ph$^+$ AML.[41] A complete cytogenetic response and major molecular response were observed in 10 and 9 patients, respectively. Median OS was 13.8 months, with a superior survival among patients who achieved a hematologic response compared to nonresponders (median not reached vs 4.65 months, respectively; P<.001).[41] However, 6 of the 19 responders relapsed at a median of 1.4 months, including 5 patients with BP-CML who all succumbed to their disease.[41] Eight patients were successfully bridged to an allograft, and while <50% of responders proceeded to a transplant, there was a trend to improved OS if an allograft was performed. These preliminary data demonstrate that dasatinib combined with decitabine can be a safe and feasible option in advanced CML, even in older patients who may not be able to tolerate intensive chemotherapy.

Future directions

CML that presents or advances beyond the chronic phase remains the biggest challenge for CML clinicians, and frustratingly, very limited progress has been made in this setting. Unfortunately, very few clinical trials have been conducted to provide some level of consensus about the best approach. Ongoing genomic investigation in CML in all phases will continue to improve our understanding of the biology of BP-CML, hopefully eventually identifying a genetic signature for patients that is sufficiently high risk for progression to justify testing novel approaches designed to modify that risk. While more data are required, the preliminary findings from the studies investigating novel approaches will hopefully stimulate further innovative trials in the setting of blast phase aiming to make meaningful progress in improving outcomes in this challenging setting.

Conflict-of-interest disclosure

Naranie Shanmuganathan received research funding from Novartis and honoraria from Takeda.
Timothy P. Hughes has received research funding and honoraria from Novartis and Bristol-Myers Squibb and honoraria from Takeda.

Off-label drug use

Naranie Shanmuganathan: No off label drug use discussed.
Timothy P. Hughes: No off label drug use discussed.

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Resistance mutations in CML and how we approach them

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Among the variety of resistance mechanisms that may underlie a non-optimal response to tyrosine kinase inhibitor (TKI) therapy in chronic myeloid leukemia patients, secondary point mutations in the BCR::ABL1 kinase domain (KD) represent the only actionable one. Each of the 5 ATP-competitive inhibitors (imatinib, dasatinib, nilotinib, bosutinib, ponatinib) has a well-defined spectrum of resistance mutations. Growing clinical experience will soon allow to also elucidate the full spectrum of mutations conferring resistance to asciminib (that appear not to be confined to the myristate binding pocket). Regular molecular response (MR) monitoring is fundamental for evaluating treatment efficacy, catching early signs of relapse, and intervening promptly in case of confirmed failure. Whenever MR is not deemed satisfactory according to the European LeukemiaNet or the National Comprehensive Cancer Network definitions, BCR::ABL1 KD mutations testing should be performed. When needed, prompt and informed TKI switch can improve response and outcome and prevent the accumulation of mutations, including highly challenging compound mutations. Novel technologies like next-generation sequencing and digital polymerase chain reaction have recently been explored for BCR::ABL1 KD mutation testing; they have both advantages and disadvantages that are discussed in this article. This review also provides suggestions for interpretation and clinical translation of mutation testing results, which may not always be straightforward, particularly in cases of low-level or unknown mutations.

LEARNING OBJECTIVES

- Identify chronic myeloid leukemia patients who need testing for resistance mutations
- Weigh the role of BCR::ABL1 mutation status in clinical decision-making

Introduction

Selection of point mutations in the kinase domain (KD) of BCR::ABL1 can be observed in chronic myeloid leukemia (CML) patients who relapse on tyrosine kinase inhibitor (TKI) therapy or who do not achieve the target response. BCR::ABL1 KD mutations are not the most frequent mechanism of resistance to therapy—yet they remain the only actionable one.

CLINICAL CASE

A 62-year-old man is diagnosed with chronic phase (CP)-CML. *BCR::ABL1* transcript type is e13a2; both Sokal and EUTOS long-term survival score are intermediate. The patient is started on imatinib 400 mg/d. Monitoring of *BCR::ABL1* transcript levels by reverse transcription–quantitative polymerase chain reaction on peripheral blood yields the following results, expressed on the International Scale (IS):

- Baseline: 88%^{IS}
- 3 months: 12%^{IS}
- 6 months: 8.7%^{IS}
- 9 months: 6.1%^{IS}
- 12 months: 3.8%^{IS}

How would you manage this patient?

When should testing for BCR::ABL1 KD mutations be performed?

The clinical value of *BCR::ABL1* KD mutations testing is widely recognized, so that both the European LeukemiaNet (ELN)[1] and the National Comprehensive Cancer Network (NCCN)[2] recommend testing when response is not satisfactory. Both the ELN and NCCN base the evaluation of response on the stepwise achievement of key molecular response (MR) milestones at given checkpoints during therapy, with some slight differences in timing and levels. The ELN distinguishes nonoptimal responses into "failures"

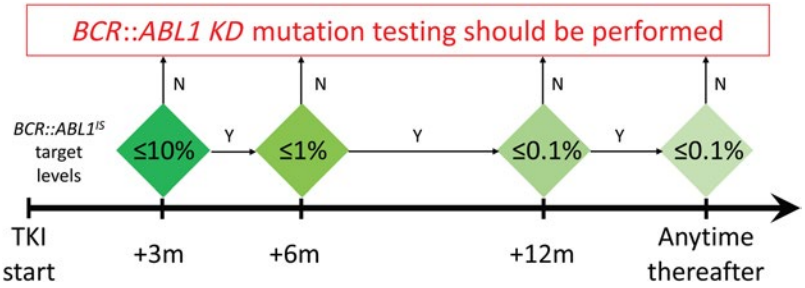

Figure 1. Molecular response milestones to be achieved and maintained in first- and second-line TKI therapy for optimal response to be defined according to the ELN. BCR::ABL1 KD mutation testing may provide useful information if such milestones are missed. KD, kinase domain; m, months; TKI, tyrosine kinase inhibitor.

(therapy must be changed) and "warnings" (". . . continuation or change should be carefully considered, depending on patient's characteristics, comorbidities and tolerance as well as on therapeutic endpoints"), while NCCN uses a traffic-light approach, with red corresponding to TKI-resistant disease and yellow corresponding to possible TKI resistance. In case of failure/red or warning/yellow, which mean that the expected MR milestone has not been achieved, or when a previously achieved milestone is lost, thorough investigation into the underlying reason(s) should be undertaken, bearing in mind that an unsatisfactory response may be due to reduced compliance, drug interactions (especially in elderly patients taking many concomitant medications), or truly resistant disease.

Reliable molecular monitoring by reverse transcription–quantitative polymerase chain reaction, performed regularly (every 3 months until major molecular response [MMR] is achieved and confirmed, and every 3 to 6 months thereafter)[1] is instrumental to check MR for evaluating treatment efficacy, to catch early signs of relapse, to trigger mutation testing when appropriate, and to intervene quickly in case of manifested failure. BCR::ABL1 kinase activity is thought to feed genetic instability, thus incomplete or inefficient BCR::ABL1 inhibition is insidious in that it might set the ground for acquiring mutations in the KD or elsewhere in the genome and for additional cytogenetic abnormalities. Beyond a certain threshold, accumulating genetic lesions may ultimately lead to disease progression, which remains a major concern and must be avoided.[3] The kinetics of *BCR::ABL1* increase ("doubling time") may help identify both disease recurrence in case of non-adherence and impending resistance due to mutation development. Shorter doubling times (median=9 days) have been associated with the former, whereas longer doubling times (median=48 days) have been associated with the latter.[4] However, the study was conducted using *BCR* as a control gene; validating these observations using the other control genes usually employed—*ABL1* and *GUSB*—would help incorporate the assessment of transcript kinetics into routine use.

BCR::ABL1 KD mutation testing is not recommended at diagnosis. Even when using a highly sensitive and reliable approach of single molecule sequencing, mutations could not be detected in de novo CP patients.[5] Hence, more "routine" methods would inexorably yield negative results. This is consistent with the fact that mutant clones require the selective pressure of treatment to outgrow: without such pressure, they are very unlikely to outcompete the unmutated clone. Accordingly, patients who lose MMR after attempting to discontinue treatment have not been reported to harbor mutations. For this same reason, samples for mutation testing should be taken before stopping or switching therapy in order to not modify the selective pressure—particularly when using Sanger sequencing, which, despite its limitations, remains the most widely employed method for testing, as discussed below.

Last but not least, we must bear in mind that mutation testing is technically feasible (regardless of the method used) only if *BCR::ABL1* transcript levels are >0.1% (that is, the threshold of MMR), and that reliability and reproducibility of results improve when the transcript levels are >1%.[6] Thus, patients in MMR (or better) should not be tested for mutations: this would not make sense clinically and would waste efforts and resources for the laboratory. A practical algorithm that can be used to assess whether mutation testing is advisable is shown in Figure 1.

CLINICAL CASE (continued)

The patient has failed all the MR milestones set to be achieved during the first 12 months of treatment. The patient swears he has been taking imatinib regularly. His physician ultimately sends a peripheral blood sample for BCR::ABL1 KD mutation testing, but Sanger sequencing shows no evidence of mutations. At month +15 (*BCR::ABL1*=4.2%^{IS}) the patient is switched to nilotinib 400 mg/twice a day, but MR does not improve: *BCR::ABL1* levels slowly but steadily increase to 7.2% at 18 months, 8.5% at 21 months, and 10.1% at 24 months. Sanger sequencing is repeated and an E255K mutation is detected. The patient is then switched to dasatinib 140 mg/daily, but he progresses to blast crisis after 3 months. BCR::ABL1 KD mutation testing shows evidence of an E255K mutation and a T315I mutation, both at 100%.

Compound mutations: the ultimate enemy

Each of the 5 ATP-competitive inhibitors (imatinib, dasatinib, nilotinib, bosutinib, ponatinib) have a well-defined spectrum of resistance mutations. Ponatinib is efficacious against every individual mutation among those conferring resistance to imatinib, dasatinib, nilotinib, and bosutinib, but its activity is compromised when a further nucleotide substitution at codon 315 turns a preexisting T315I into a T315M or -L mutation.

Table 1. Provisional list of mutations that might confer resistance to asciminib based on the currently available data

Reference	Type of study	Mutations
Wylie et al. (2017)[7]	In vitro screen in KCL-22 CML cells cultured in increasing concentrations of asciminib and IC_{50} data in luciferase-transformed murine Ba/F3 cells expressing native BCR::ABL1 as compared with various BCR::ABL1 mutants	*P223S*; K294E; *A337V*; *P465S*; *V468F*; *I502L*
Qiang et al. (2017)[28]	In vitro screen of asciminib-resistant CML cell lines cultured in increasing concentrations of asciminib	*C464W*; M244V/*A337V*
Eide et al. (2019)[11]	In vitro cell-based accelerated mutagenesis screen and IC_{50} data in murine Ba/F3 cells expressing native BCR::ABL1 as compared with various BCR::ABL1 mutants	*A344P*; F359V; F359I; F359C; *P465S*; G671R (based on outgrowing mutants) T315L; T315M; T315I/G250E; T315I/Y253H; T315I/E255V; T315I/M351T; T315I/H386R; T315I/E453K; E255V/299L; V299L/F317L; F317L/F359V (based on IC_{50} data in a selected panel of single mutations and CMs of interest)
Hughes et al. (2019)[29]	Clinical trial (phase 1, CP and AP)	G109D*; Y115N*; *A337T*; *G463S*; *G463D**; *P465S**; *V468F*; *I502L*
Mauro et al. (2023)[30]	Clinical trial (phase 1; 4-year update of T315I-negative CP patients)	M244V; *G463D*; *G463S*; *V468F; I502L*
Hochhaus et al. (2023)[31]	Clinical trial (phase 3 asciminib vs bosutinib [ASCEMBL])	M244V; E355G; F359V; T315I; *A337T*; *P465S*
Cortes et al. (2020)[32] (ASH meeting abstract)	Clinical trial (phase 1, T315I-positive patients)	F359I; T315I/F359I; *A337T*/F359V; T315I/M244V; T315I/M351T; T315I/E453Q_

Mutations have either been recovered from in vitro studies where resistant cell lines were obtained using various strategies or identified as newly emerging mutations in patients enrolled in the clinical trials. Myristate-binding site mutations are highlighted in italic. The asterisk denotes mutations detected in patients with a variant frequency <10%, mostly in combination with other myristate-binding pocket or catalytic site mutations.

More recently asciminib, an allosteric inhibitor specifically targeting the myristate-binding pocket rather than the ATP-binding pocket, has been approved for the treatment of patients resistant to at least 2 previous TKIs. Given that asciminib binds to a region different and distant from the region targeted by ATP-competitive inhibitors, the molecule was initially predicted to have a nonoverlapping spectrum of resistance mutations.[7] However, accumulating evidence from clinical trials indicates that even well-known imatinib- or 2G TKI-resistant mutations like E355G and F359V may be identified at the time of asciminib failure (Table 1). Yet mutation data from trials are scarce, and "real-life" clinical experience with asciminib is still limited. Thus, time is needed before the full spectrum of asciminib-resistant mutations will be conclusively elucidated.

While single mutations can be tackled by one or more of the currently available TKIs, compound mutations (CMs; 2 mutations in *cis* on the same *BCR::ABL1* transcript) have emerged as a major concern. Data of in vitro IC_{50} (ie, the intracellular concentration of drug required to inhibit by 50% the growth of a cell line engineered to express the given mutant oncoprotein), corroborated by an increasing number of clinical reports, indicate that the most frequent mutation combinations (that are usually T315I-inclusive CMs) are resistant to all available TKIs, including, in some cases, even ponatinib and asciminib (Table 1).[5,8-11] CMs usually result from sequential TKI failures, although they have also been documented in a few patients whose switch to another TKI was delayed despite persisting failure: if the original mutant clone is not rapidly and fully eradicated, it may acquire a "second hit" that may lead to a highly challenging CM.[5,12] At least in vitro, a combination of asciminib and ponatinib at clinically achievable concentrations is capable of counteracting (as well as preventing) several CMs.[11,13] Combination of ponatinib with hydroxyurea or with the CDK4/6 inhibitor palbociclib has also shown promising activity in vitro against T315I-inclusive CMs.[14] It is likely that other combinations exploiting specific vulnerabilities of CMs or synthetic lethality may be devised in the future. However, given the difficulty of exploring off-label combinations in vivo, the best strategy is currently to prevent, rather than to counteract, CMs.

Sanger sequencing vs newer techniques: pros and cons

Sanger sequencing enables scanning of the entire KD for any nucleotide substitution. Given the multitude of mutations associated with imatinib resistance, it naturally became the gold standard for BCR::ABL1 KD mutation testing[15] and remains such after 2 decades. Mutation screening of *BCR::ABL1* transcripts by Sanger sequencing is relatively fast and easy but suffers from the inherent poor sensitivity of the technique (ranging between 10% and 20%, that is, 10 to 20 mutant transcripts in 100 total *BCR::ABL1* transcripts), meaning the technique can only identify major or dominant mutant clones. It is thus not surprising that, in recent years, several retrospective studies and two prospective studies have explored the use of targeted next generation-sequencing (NGS) that improves the detection limit to 1% to 5%.[16-20] These studies have shown that Sanger sequencing may miss minor additional subclones at the time of treatment failure, as well as emerging ones in patients with warning responses. Moreover, NGS may more easily and robustly identify CMs (see below), although it is important to correct results for the likelihood of polymerase chain reaction (PCR)–mediated recombination[21] that may artifactually bring in *cis* 2 mutations sitting on different molecules.[22] However, despite wider and wider availability of benchtop NGS instruments, implementing routine NGS for BCR::ABL1 KD mutation testing is facing some challenges. First is the need to set up and internally validate a laboratory-developed test.

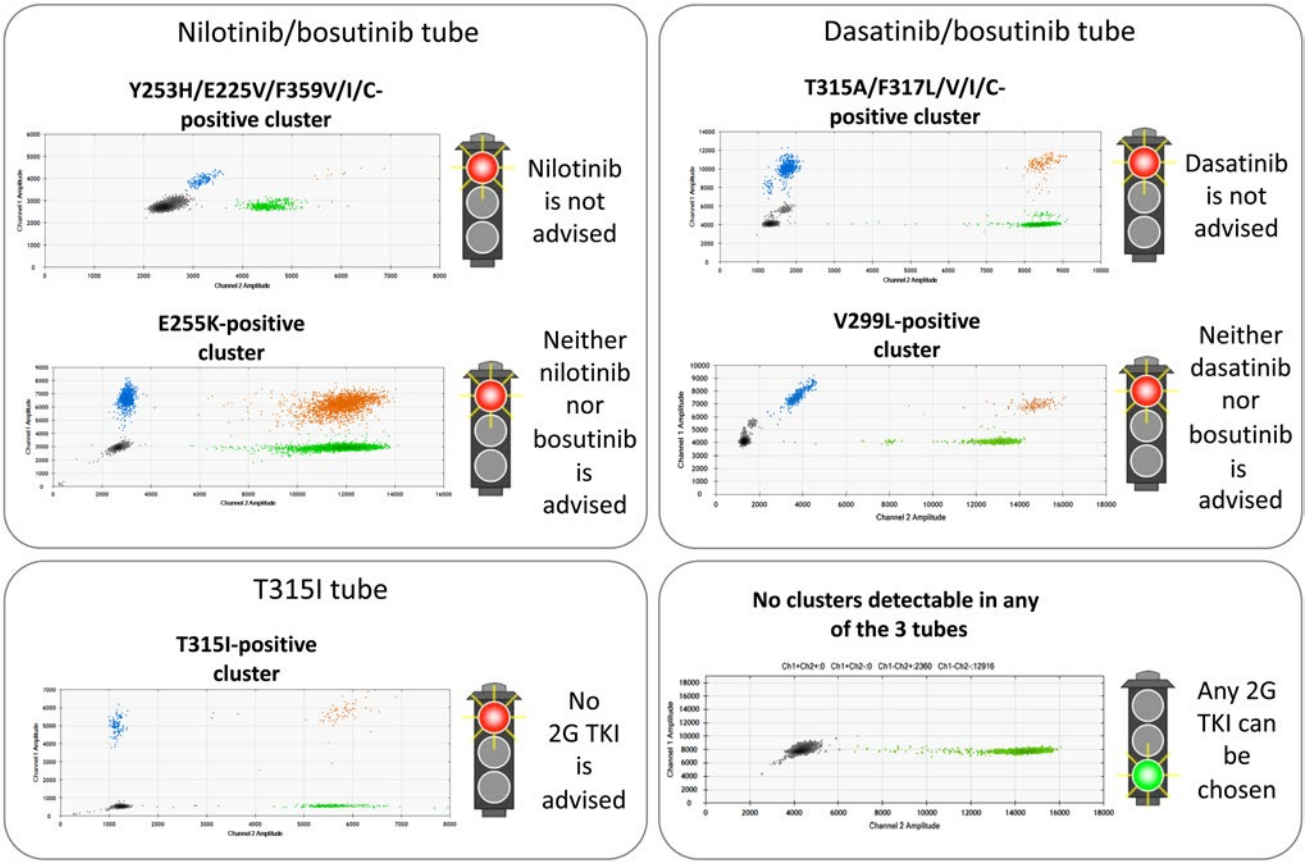

Figure 2. Example of a ddPCR-based strategy for detecting 2G TKI-resistant mutations at the time of switch. Representative 2-dimensional plots (in which channel 1 fluorescence [FAM] is plotted against channel 2 fluorescence [HEX]) expected for mutations conferring resistance to one or more 2G TKI. Black clusters at the bottom left corner represent FAM/HEX double-negative droplets. Green clusters at the bottom right corner correspond to droplets positive for e13a2 or e14a2 *BCR::ABL1* fusion transcripts (detected by HEX-conjugated probes). Blue clusters at the top left corner correspond to droplets positive for the indicated BCR::ABL1 KD mutations or mutation subgroups (detected by FAM or FAM/HEX-conjugated probes). Orange clusters at the top right corner correspond to double-positive droplets. One tube is specific for the pan-resistant T315I mutation; one tube detects dasatinib-resistant mutations (with the nucleotide substitution leading to the V299L mutation clustering separately from the others since they also confer resistance to bosutinib); and one tube detects nilotinib-resistant mutations (with the E255K mutation clustering separately from the others since it also confers resistance to bosutinib). Results are expressed as percentage of mutant transcripts over total *BCR::ABL1* transcripts. 2G TKI, second-generation tyrosine kinase inhibitor.

Second is the high throughput of the instruments as compared with the dimensions of the library, which requires pooling several samples in each sequencing run. Consequently, only sample centralization in regional or national reference laboratories could balance cost-effectiveness and turnaround time. Last but not least of the challenges is the higher error rate as compared with Sanger sequencing.

Mutation-specific approaches taking advantage of mass spectrometry (MS) or digital PCR can be more sensitive and less error-prone than NGS. MS was indeed the first high-sensitivity (0.05% to 0.5%, depending on the mutation) approach to be explored. A multiplex strategy of primer extension followed by MS-based identification of the extended nucleotide developed for a panel of 31 imatinib-resistant mutations was applied to a relatively large cohort of imatinib-resistant patients who were switched to dasatinib or nilotinib.[23] The study provided the first robust evidence that detection of low-level mutations at the time of TKI switch may offer critical information to guide subsequent therapy selection, and that if an "inappropriate" TKI is selected, there is a high risk of treatment failure with clonal expansion of the resistant mutant.[23] A more recent development is a droplet digital PCR (ddPCR)–based strategy multiplexing the detection and quantitation of 2G TKI-resistant mutations in a 3-tube format.[24] The first tube contains primers and probes to detect the T315I mutation; the second tube is specific for dasatinib- and bosutinib-resistant mutations, and the third tube detects nilotinib- and bosutinib-resistant mutations. The appearance of a positive cluster of droplets and where such cluster sits in the 2D plot directly indicate which 2G TKI(s) should be excluded from the decision algorithm at the time of switch (Figure 2). All the approaches discussed so far look for mutations in *BCR::ABL1* transcripts, because at the genomic level the selective analysis

Table 2. Advantages and disadvantages of the main methods currently available for *BCR::ABL1* KD mutation testing

Method	Lower limit of detection	Mutation-specific?	Advantages	Disadvantages
Sanger sequencing	10%-20%	N	Enables TKD-wide screening; easy workflow and data analysis; relatively short turnaround time	Poorly sensitive
Mass spectrometry	0.05%-0.5%	Y	Accurate; highly sensitive	Not widely available; high throughput; longer turnaround time; no commercial kits available; can be implemented for a limited number of mutations; difficult identification of the T315L and T315M mutations (that are caused by a double nucleotide substitution)
NGS	1%-5%	N	Enables TKD-wide screening; sensitive	High throughput; longer turnaround time; requires specialized personnel and bioinformatic competences; relatively high error rate; no commercial kits available
ddPCR	0.1%-0.5%	Y	Accurate; highly sensitive; relatively easy workflow and data analysis; short turnaround time	Can be implemented for a limited number of mutations

ddPCR, droplet digital polymerase chain reaction; N, no; NGS, next generation sequencing; TKD, tyrosine kinase domain; Y, yes.

of the translocated allele would be impossible due to the width of the region to be amplified (17 kb). Nevertheless, an allele-specific ddPCR strategy using genomic DNA to estimate mutated cells and follow their clonal evolution over time has recently been described.[25] The approach quantitates the mutated clone as the percent ratio between the copy number of mutated *ABL1* and the copy number of the *BCR-ABL1* fusion assessed at the DNA level with patient-specific primers. Comparison with NGS conventionally performed using RNA as input nucleic acid showed very good concordance between the level of mutation at the transcript and genomic level, although, as expected, ddPCR featured greater sensitivity. Routinely implementing a DNA-based strategy would be impractical due to the need to determine each patient's exact sequence of the *BCR::ABL1* breakpoint at diagnosis, yet this study further highlights the advantages of ddPCR in terms of rapidity and sensitivity.

The main advantages and disadvantages of current technologies for BCR::ABL1 KD mutation testing are summarized in Table 2.

Despite the limitations highlighted here, Sanger sequencing and NGS are the only ways to obtain a detailed snapshot of *BCR::ABL1* mutation status. Thus, they are the most informative methods in (1) imatinib-resistant patients when the causes of an unsatisfactory response are to be investigated, (2) multi-TKI-resistant or advanced-phase patients where two or more mutations (and CMs) can be expected, and (3) patients on asciminib. When a TKI switch is already planned, ddPCR might instead be useful to rapidly inform rational selection of 2G TKIs or ponatinib.

CLINICAL CASE (continued)

The patient provides written informed consent for his samples to be retrospectively reanalyzed by NGS. NGS reveals that the E255K mutation was already detectable in 6.7% and 12.1% of *BCR::ABL1* transcripts at 9 and 12 months, respectively. Being E255K resistant to nilotinib, this mutation was selected by subsequent nilotinib treatment. Moreover, NGS showed that the acquisition of the T315I, generating the T315I/E255K CM, occurred during nilotinib treatment (Figure 3).

How mutation results should (and shouldn't!) be used

BCR::ABL1 KD mutation testing supports clinical decision-making whenever response is unsatisfactory. In case of failure, a change of therapy is mandatory and the main aim of BCR::ABL1 KD mutation testing is to help exclude the TKI(s) unlikely to be effective. A handful of mutations have been robustly associated with resistance to 2G and 3G TKIs: these are listed in Table 3. What about the others? For many (yet not for all) mutations, IC_{50} data are available and have been used to generate multicolored tables using a traffic-light code to indicate whether the mutation is expected to be sensitive, moderately resistant, or highly resistant to a given TKI.[9,10,26] However, different tables sometimes report conflicting data—which highlights how different experimental conditions impact the raw IC_{50} data obtained. Therefore, although it is tempting to use a pocket version of one of these tables as a sort of "at-a-glance" guide to the TKI choice, caution should be exerted: measuring in vitro antiproliferative activity is an artificial way to rank inhibitors for their antileukemic activity in vivo in a much more complex system. Thus, for mutations other than those listed in Table 3, it is wiser to let clinical considerations regarding comorbidities, risk factors, and therapeutic endpoints specific to the patient prevail.

In case of warning, a gray area where continuation or change are equally allowed, detecting resistance mutations tilts the balance toward a change of therapy. But what does resistance mutation mean? From a clinical standpoint, interpreting BCR::ABL1 KD mutation testing results may not always be straightforward. What about mutations for which there are no

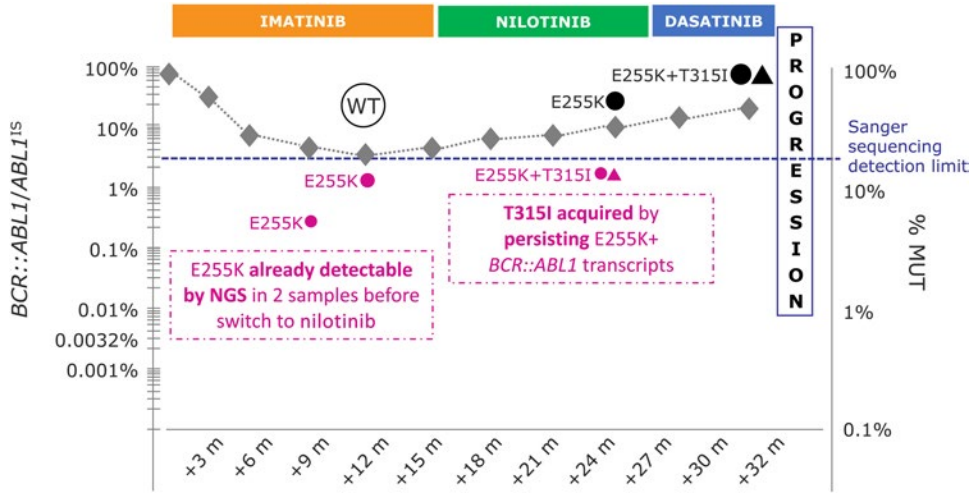

Figure 3. Graphical summary of the clinical case herein presented. m, month(s); % MUT, percentage of mutant; NGS, next-generation sequencing; WT, wild type.

IC_{50} data whatsoever? There is not necessarily a biunivocal link between resistance and mutations: some mutations might just be innocent bystanders. Comprehensive databases of mutations detected in TKI-resistant patients have never been built, so for less frequently occurring mutations, physicians often need to perform cumbersome literature searches for whether the mutation has ever been reported in any published study. And what about mutations detected at low levels, eg, by NGS? In case of warning, there is usually not a compelling urgency to intervene: when the relation between the mutation detected and the inadequate response is uncertain, it may be wise to repeat testing in 1 to 3 months. True resistance mutations are expected to expand, or at least persist, if therapy is not changed. Anyway, mutations are likely to be a gauge of the degree of genetic instability: thus, regardless of their actual role in resistance, positivity for any mutation identifies higher-risk patients requiring more careful monitoring.[27]

In general, when the clinical relevance of a detected mutation is uncertain (irrespective of whether this is due to the lack of sensitivity data or to a low mutational burden), closer monitoring of *BCR::ABL1* transcript kinetics may help in deciding whether therapy should be switched.

Table 3. Mutations impacting the selection of the subsequent-line 2G TKI or of ponatinib

Mutation	Contraindicated TKI(s)
Y253H	Nilotinib
E255K	Nilotinib, bosutinib
E255V	Nilotinib
V299L	Dasatinib, bosutinib
T315I	Dasatinib, nilotinib, bosutinib
T315A	Dasatinib
T315L	Ponatinib (asciminib?)
T315M	Ponatinib (asciminib?)
F317L	Dasatinib
F317V	Dasatinib
F317I	Dasatinib
F317C	Dasatinib
F359V	Nilotinib (asciminib)
F359I	Nilotinib (asciminib)
F359C	Nilotinib (asciminib)

List of single mutations that, when detected, should exclude one or more 2G TKIs or ponatinib from the decision algorithm. All mutations except V299L, T315A, T315L, and T315M are also resistant to imatinib. Asciminib has been included, in brackets, for some mutations based on in vitro or in vivo data. This list will grow once asciminib resistance mutations are fully elucidated.

Take-home message from the clinical case

In the case presented above, using NGS could have been beneficial. The E255K is well known to confer resistance to both imatinib and nilotinib. Thus, detecting a low-level E255K mutation would have prevented the unfortunate choice of a TKI (nilotinib) not active against this mutation. The case also exemplifies that if a mutant clone is not rapidly cleared, and if inefficiently inhibited BCR::ABL1 persists at high levels (as mirrored by the high levels of transcript), further acquisition of mutations, giving rise to CMs, may occur.

Conflict-of-interest disclosure

Simona Soverini has received speaker fees from Incyte Biosciences and Novartis.

Off-label drug use

Simona Soverini: There is nothing to disclose.

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Atypical CML: diagnosis and treatment

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Atypical chronic myeloid leukemia (aCML) is included in the group of myelodysplastic/myeloproliferative neoplasms by the International Consensus Classification and has been renamed as MDS/MPN with neutrophilia by the fifth edition of World Health Organization classification. It is always characterized by morphologic identification of granulocytic dysplasia with >10% circulating immature myeloid cells, 2 distinguished features that differentiate this disease among the others. Somatic mutations may help to diagnose but are not specifically pathognomonic of the disease, with the most detected including ASXL1, SETBP1, NRAS, KRAS, SRSF2, and TET2 and with low-frequency CBL, CSF3R, JAK2, and ETNK1. The genomic landscape of aCML has been recently unravelling, revealing that SETBP1 and ETNK1 are usually not ancestral but secondary events associated with disease progression. Unfortunately, until now, no consensus on risk stratification and treatment has been developed: Mayo Clinic prognostic score identified as adverse events age >67 years, hemoglobin level <10 g/dL, and TET2 mutations. Although some possible genetic markers have been identified, allogeneic transplant remains the only curative strategy.

LEARNING OBJECTIVES
- Evaluate the diagnostic algorithm
- Characterize the somatic mutations landscape
- Analyze prognostic features
- Summarize possible treatments

Introduction

Atypical chronic myeloid leukemia (aCML) is a clonal hematopoietic disease previously identified as a neoplasm with mixed dysplastic/myeloproliferative (MDS/MPN) features in the absence of monocytosis and eosinophilia.[1] It was introduced in the fourth edition of the World Health Organization classification in the subgroup of mixed MDS/MPN together with other entities, such as juvenile myelomonocytic leukemia, MDS/MPN with ring sideroblasts and thrombocytosis, and MDS/MPN unclassifiable.[2] In the recent 2022 World Health Organization revision, aCML was retained as terminology with a clear distinction between this disorder and myeloid neoplasms with monocytosis and eosinophilia.[3] The 2022 International Consensus Classification replaces the term *aCML* with *MDS/MPN with neutrophilia*, avoiding the possible confusion with typical chronic myeloid leukemia (Ph+ CML).[4]

CLINICAL CASE

A 62-year-old patient was admitted to the hospital for intense asthenia and widespread pain. Complete blood count showed mild anemia (10.7 g/dL), hyperleukocytosis (155×10^9/L), and absolute neutrophil count of 136×10^9/L. Splenomegaly was noted on physical examination (4 cm below costal margin), and peripheral blood smear showed neutrophilia (64% with >20% dysplastic features), in the absence of monocytosis. The absence of an abnormal karyotype on cytogenetic analysis, as well as the absence of BCR::ABL1 and other rearrangements in MPN driver genes, allowed to exclude the diagnosis of CML or Ph-negative MPNs. Bone marrow analysis showed dysplastic and markedly expanded granulopoiesis (myelo-erythroid ratio 9:1) and 3% of $CD34^+$ blast cells, consistent with the morphologic suspect of aCML.

Table 1. Diagnostic criteria for atypical chronic myeloid leukemia

World Health Organization criteria	International Consensus Classification criteria
PB leukocytosis (WBC count ≥13×10⁹/L) because of increased numbers of neutrophils and their precursors with prominent dysgranulopoiesis	Leukocytosis ≥13×10⁹/L, due to increased numbers of neutrophils and their precursors (promyelocytes, myelocytes, and metamyelocytes), the latter constituting ≥10% of the leukocytes
Neutrophil precursors (promyelocytes, myelocytes, metamyelocytes) ≥10% of leukocytes	Dysgranulopoiesis, including the presence of abnormal hyposegmented and/or hypersegmented neutrophils±abnormal chromatin clumping
	Cytopenia (anemia, hemoglobin <13 g/dL in males, <12 g/dL in females; neutropenia, absolute neutrophil count <1.8×10⁹/L; thrombocytopenia, platelets <150×10⁹/L)
Less than 20% blasts in the PB and BM	Blasts <20% of the cells in PB and BM
No or minimal absolute monocytosis; monocytes usually <10% of leukocytes	No or minimal absolute monocytosis; monocytes constitute <10% of the PB leukocytes
Minimal absolute basophilia; basophils usually <2% of leukocytes	No eosinophilia; eosinophils constitute <10% of the PB leukocytes
Hypercellular BM with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages	Hypercellular BM with granulocytic proliferation and granulocytic dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages
No Ph chromosome or *BCR::ABL1* fusion gene and not meeting criteria for PV, ET, or PMF	No *BCR::ABL1* or genetic abnormalities of M/L-Eo with TK gene fusions. The absence of MPN-associated driver mutations and the presence of *SETBP1* mutations in association with *ASXL1* provide additional support for a diagnosis of aCML.
No evidence of *PDGFRA*, *PDGFRB*, *FGFR1* rearrangement, or *PCM1::JAK2*	

BM, bone marrow; ET, essential thrombocythemia; M/L-Eo, myeloid/lymphoid neoplasms with eosinophilia; PB, peripheral blood; PMF, primary myelofibrosis; PV, polycythemia vera; TK, tyrosine kinase; WBC, white blood cell.

Clinical and morphologic features

aCML is a rare disease with an incidence of 1% to 2%; it affects elderly patients with a median age ranging between 60 and 70 years, with a male predominance.[5-7] The disease is associated with a poor outcome, with a median overall survival (OS) of 10 to 28 months[8] and a high rate of leukemic transformation (>15%–20% at 5 years).[9] It is always characterized by leukocytosis with white blood cell count >13 × 10⁹/L with dysplastic features in neutrophils and their precursor for >10% of the whole leukocyte population[10] (Table 1). Dysplasia includes abnormal chromatin clumping, hypersegmented or Pelger-Huet forms, and cytoplasmatic hypogranularity.[10] Monocytes must be <10% of total leukocytes, and the new International Consensus Classification includes also cytopenia defined as in MDS by anemia (hemoglobin level <13 g/dL in males and 12 g/dL in females), neutropenia with absolute neutrophil count <1.8 × 10⁹/L, and thrombocytopenia with a platelet count <150 × 10⁹/L.[4] The upper limit of blast cells in all MDS/MPN entities is <20%. Bone marrow morphologic analysis showed hypercellularity, with a predominance of granulocytes showing marked dysplasia; more than half of patients showed also erythroid and some degree of megakaryocytic dysplasia. Variable degrees of increased reticulin fibrosis have been also reported.[3,4,10] The differential diagnosis of aCML includes the following:

1. BCR1/ABL-positive CML, distinguished by not only the absence of specific t(9;22) but also the presence of dysgranulopoiesis and the almost normal basophil count (<2%) in aCML.
2. Chronic neutrophilic leukemia (CNL), including the presence of >10% of immature myeloid precursors and dysplasia, which are unique distinctive features of aCML. Mutational features may allow distinction with the CSFR3 mutation most frequently observed in CNL but not restricted only to this disease.
3. Chronic myelomonocytic leukemia (CMML), with the increased monocyte count exceeding more than 10% of the leukocyte count.
4. Prefibrotic myelofibrosis, in which the availability of myeloproliferative gene markers (JAK2, CALR, and MPL) may allow the possible distinction between the 2 forms. More difficult is the distinction in triple-negative myelofibrosis[3,4] (Figure 1).

Genetic features

The reported frequency of chromosomal abnormalities in aCML is widely variable, ranging from 20% to 88% in different reports.[5,6,11,12] The most common abnormalities reported were trisomy 8 or 9, del 20(q), −7/7(q), and isochromosomes 17q. Less frequently, aberrations in chromosomes 12, 13, 14, 19, and 21 have been described.[5,6,11,12] Mutations are usually detected in all aCML cases: higher frequency for ASXL1, SETBP1, NRAS, KRAS, SRSF2, and TET2 has been reported, whereas CBL, CSF3R, JAK2, and ETNK1 were revealed with low frequency (<10% of cases).[13] SETBP1 may support aCML diagnosis: identified in one-fourth of patients, it can be also found in MDS/MPN unclassifiable patients, in some CMML, occasionally in juvenile CMML, and in some secondary acute myeloid leukemia arising from MDS/MPN.[14,15] SETBP1 can be associated with specific baseline features such as higher leukocyte count, low platelet count, and a worse prognosis.[14-16] The mutation maps on chromosome 18q21.1 and encodes for SET binding protein, a negative regulator of the tumor suppressor protein phosphatase 2A (PP2A) with increased repression of activity and cellular proliferation.[17,18] SETBP1 interacts with SET, protecting it from cleavage and allowing the creation of a complex (SETBP1/SET/PP2A) that increases proliferation and expansion of the leukemic clone.[19-26] Piazza and colleagues[14,27] clearly demonstrated that most SETBP1 somatic mutations cluster in a mutational hotspot within the SKI-homologous region of the

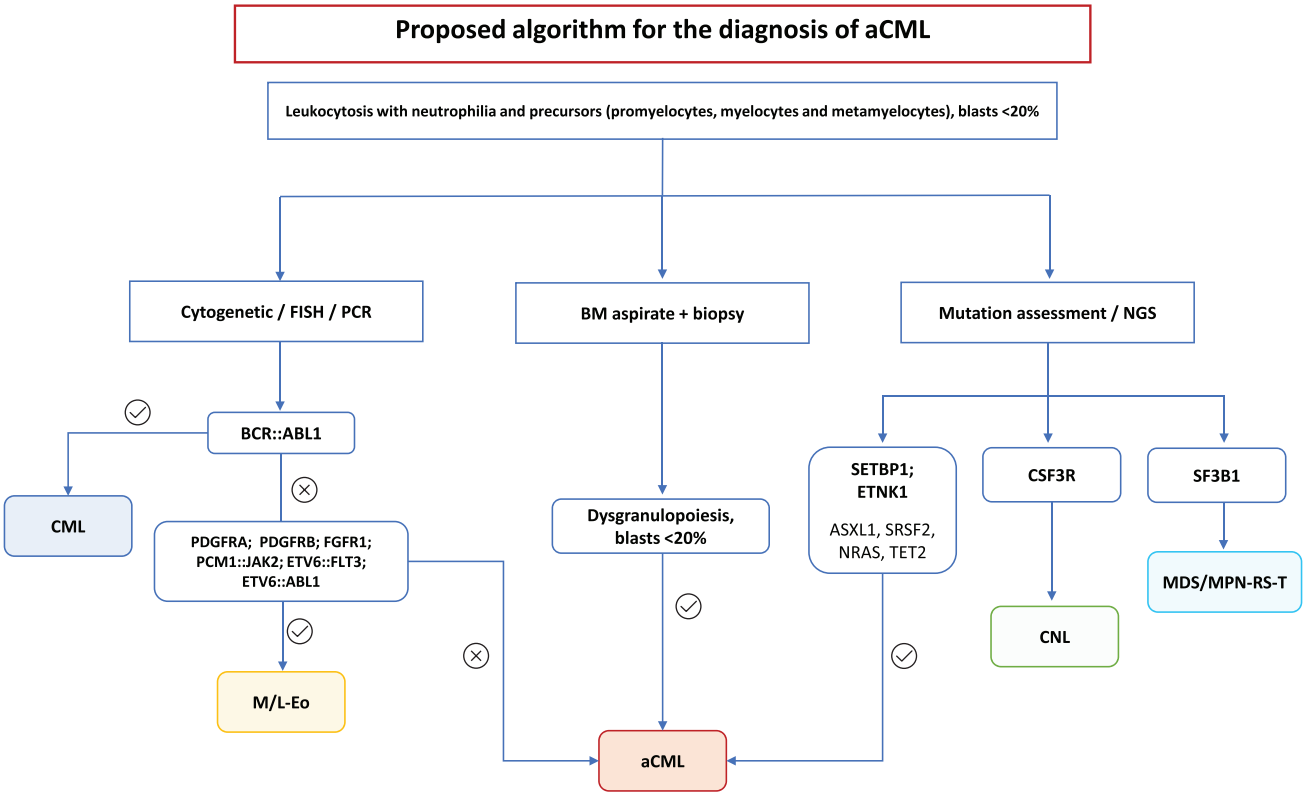

Figure 1. Algorithm proposed for the diagnosis of aCML. FISH, fluorescence in situ hybridization; MDS/MPN-RS-T, myelodysplastic/myeloproliferative neoplasms with ring sideroblasts and thrombocytosis; M/L-Eo, myeloid/lymphoid neoplasms with eosinophilia; NGS, next-generation sequencing; PCR, polymerase chain reaction.

protein, conferring a proliferative advantage to the mutated cells and protecting the mutation from ubiquitination. Most SETBP1 mutations are located within a 14-amino-acid stretch (codons 858-871), which is also mutated in Schinzel-Giedion syndrome, a rare genetic disease characterized by congenital malformations, mental retardation, and frequent epithelial tumors.

SETBP1 mutations have shown a strong association with ASXL1 and CBL mutations and are mutually exclusive of JAK2 and TET2 mutations.[14,28] SETBP1 also has a complex role as a transcriptional modulator, considering its ability in direct interaction with genomic DNA (recruitment of HCF1, KMT2A, PHF8, PHF6), the possible binding capacity to MDS1 and EVI1 complex locus protein EVI1 or MECOM (upregulation of several genes involved in stem cell proliferation and myeloid differentiation), self-renewal of myeloid progenitors, and RUNX1 downregulation.[27,29] A study conducted on 71 patients showed that most were ASXL1 positive (92%), and SETBP1 was detected in 38%. Clonal hierarchy was identified, and ASXL1 was acquired early, whereas SETBP1 was never reported as being ancestral but always secondary in disease progression[11] (Figure 2).

Recurrent mutations in ETNK1 have emerged as being relatively specific for aCML (up to 9% of cases) and CMML (3% of cases) and not found in other myeloid diseases.[30] The ETNK1 gene maps on chromosome 12p12.1 and encodes a protein known also as ethanolamine kinase, which acts as catalyzer for the biosynthesis of phosphatidylethanolamine through the Kennedy pathway, responsible for the de novo synthesis of membrane phospholipids.[31,32] These mutations cluster in a small region of the kinase domain, encoding for H243Y and N244S (1/8 H243Y; 7/8 N244S) and G245V/A, all as heterozygous and present in the dominant clone.[33] ETNK1 mutations decrease the activity of the enzyme, reducing the synthesis of phosphoethanolamine, increasing the mitochondrial activity with final increased reactive oxygen species production, DNA damage, and genomic instability.[34,35] On the hierarchical scale, it seems that ETNK1 mutation may precede ASXL1 and SETBP1.[34,35]

In summary, studies on the clonal architecture of aCML showed that ETNK1 and ASXL1 are ancestral mutations, with RAS, CBL, TET2, SRSF2, and SETBP1 as secondary events; CBL mutations have a tendency to reach homozygosity through somatic uniparental disomy.[10] CCND2 mutations also have been recently suggested in patients with aCML. Some groups[36,37] reported CCND2 mutations at low variant allelic frequencies: Khanna et al[36] showed, in a cohort of 116 patients with Ph-negative MPN, CCND2 mutations concomitant in 1 case to SETBP1 and in 1 case to SRSF2 mutation. CCND2 mutation in the P281 codon resulted in the accumulation of degradation-resistant cyclin in D2, with predominant staining in nuclear localization regardless of the cycle cell phase. This peculiar localization seems to be responsible for prolonged cell survival without conferring growth factor independence.[38] Recently, Carreño-Tarragona and colleagues[39] compared the clinical and genomic profile of aCML and CNL: they suggested that apart from CSFR3 more commonly mutated

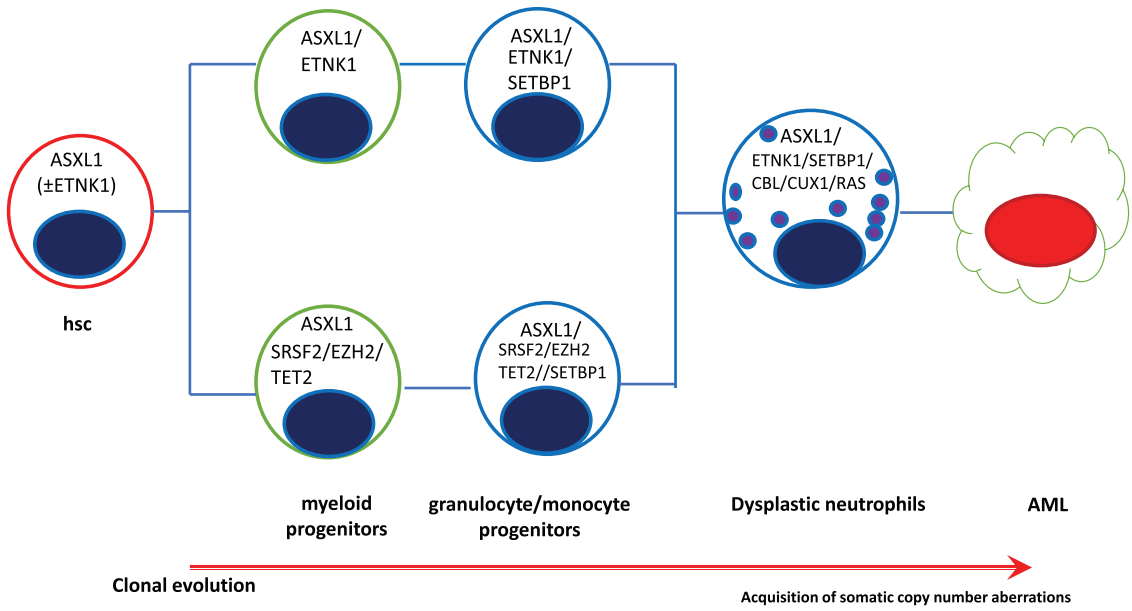

Figure 2. Clonal hierarchy in aCML showing the progressive acquisition of somatic mutations. AML, acute myeloid leukemia.

in CNL, as well as EZH2 and TET2 more commonly mutated in aCML, no differences were found in the pathways affected, suggesting that CNL and aCML are a continuum of the same disease.

Different groups analyzed gene expression profiling in aCML: a significant change in the expression levels of SETBP1, CDKN2A, GATA2, MPL, TMEM14C, CSF3R, and FLT3 genes was observed in a group of 26 patients compared to 59 patients with CMML.[40] Indeed, Zhang and colleagues[41] reported 3 different clusters in 158 samples of different myeloid disorders, including CNL, aCML, MDS/MPN unclassifiable, MDS/MPN, and CMML, showing dissimilarity of different combinations of mutant patterns without specific clusterization within any one diagnosis. Fontana et al[42] showed 2 different aCML groups based on gene expression with overexpression of 3 different genes (PARP1, DNPH1, and GFI1B) associated with a worse prognosis.

CLINICAL CASE (continued)

Next-generation sequencing was performed, and mutations were identified in ASXL1 (29% VAF), CBL (4% variant allele frequency), SETBP1 (44% VAF), and SRSF2 (43% VAF), with a compound mutation in CCND2 (c.841C>T, 28% VAF and c.842C>G, 4% VAF). In this case, in consideration of the mutational profile detected on next-generation sequencing, an indication for allogenic transplant has been made and human leukocyte antigen typing is being carried out, with a search for possible bone marrow stem cell donors.

Risk stratification and prognosis

Considering the rarity of the disease, no consensus on a possible risk stratification has been reported. Two studies showed that age >65 years, female sex, hemoglobin <10 g/dL, leukocyte count >50 × 10^9/L, and immature circulating precursors were adverse prognostic factors.[6,9] Wang et al[7] reported that leukocytosis and immature myeloid cells but not the dysgranulopoiesis maintained the negative role associated with shorter OS in a large series of patients, including 65 patients with aCML with a median OS of 12.4 months.

In 2017, Mayo Clinic developed a model based on a small data set of 25 patients. In this proposed model, advanced age, low hemoglobin, and TET2 mutations were considered of significance in multivariate analysis. Two categories of patients were identified (low, with 0-1 risk factor, and high risk with ≥2 risk factors) considering 1 point each for age >67 years, hemoglobin level <10 g/dL, and detection of the TET2 mutation. The median OS observed was 18 months for the low-risk category and 7 months for the high-risk group.[5] Palomo et al[11] explored the effects of mutations as prognostic indicators: while ASXL1 did not retain a prognostic impact considering that most patients were positive, RUNX1, CUX1, and NRAS were associated with a shorter OS and SRSF2 and SETBP1 with positive outcome.

Treatment

No standard of care exists for the treatment of aCML. In addition, no consensus recommendations or risk-based treatment algorithms exist to help guide a watch-and-wait approach vs initiation of therapy. The most common administered therapy is hydroxyurea (HU), typically used to control leukocytosis or symptomatic splenomegaly. There have been multiple reports of HU inducing complete and partial hematologic responses in aCML, but the duration of response is usually limited to a few months.[6,9,28,43] Interferon α (IFNα) has also been associated with partial or sometimes complete hematologic response but also with discontinuation due to toxicity.[28,43-45] A phase 2 study of pegylated-IFNα-2b was shown to have improved tolerability over standard IFNα in BCR-ABL1–negative MPNs.[46] This long-acting formulation is associated with a better toxicity profile and offers a treatment option for those ineligible for clinical

trials. Both drugs are usually used in a palliative setting, in the absence of a possible allogeneic transplant (hematopoietic stem cell transplant) procedure strategy.

Progressive anemia with development of transfusion dependence is common in aCML, contributing to increased morbidity. Splenectomy is generally not recommended in the management of this disease given its limited clinical response, anecdotal risk of accelerated neutrophilia, and relatively high perioperative morbidity already known in patients with MPN.[43,44] Erythroid-stimulating agents also have limited data in aCML, with only 1 study reporting a poor response.[47] Immunomodulating agents, such as thalidomide and lenalidomide, were also tested with scarce responses.[28] Even if not considered a standard of care, experiences with hypomethylating agents (HMAs) were described: the Mayo Clinic reported a limited experience in 5 patients with a 40% rate of stable disease.[5] Decitabine was reported in several other anecdotical cases with a complete remission rate after almost 4 cycles.[48-51]

Rarely, CSF3R mutation can be detected in aCML with constitutive activation of JAK-STAT signaling due to T615A, T618I, and T640N mutations. The potential benefit of ruxolitinib in CSF3R T618I–mutated disease was first demonstrated in a patient with CNL with CSF3R T618I who achieved a marked reduction in neutrophilic leukocytosis and improvement of anemia and thrombocytopenia.[52] Subsequently, a patient with hydroxyurea-refractory aCML treated with ruxolitinib escalated from 10 to 20 mg twice daily resulted in similar hematologic improvements (reduced splenomegaly and peripheral blood myeloid immaturity, reverted weight loss, and improved symptom scores, without a change in CSF3R mutant allele frequency).[53] A phase 2 trial enrolled 44 patients (23 with aCML and 21 with CNL). Of the aCML cohort, 6 patients had a CSFR3 mutation: only 2 patients obtained a partial response, and grade 3 anemia and thrombocytopenia were observed in 34% and 14% of patients, respectively.[54]

Allogeneic transplant remains the only potential curative strategy: a large series of 42 patients was described by Onida et al.[55] Sixty-four percent of patients underwent a matched sibling donor transplant, with reduced conditioning in 24% of them. The 5-year relapse-free survival was 36%, transplant-related mortality was 24%, and the relapse rate was 40%. Age and European Society for Blood and Marrow Transplantation score were the identified independent prognostic factors for OS. Contrasting results were reported by other small studies: Koldehoff et al[56] described favorable outcomes with over 80% OS at 5 years in 21 patients who had received allogeneic HSCT, whereas significantly worse outcomes were described by Mittal et al[57] due to high transplant-related mortality from graft-vs-host disease and sepsis.

Other actionable targets have been identified and small-molecule inhibitors tested: trametinib, a MEK1/2 inhibitor, inhibits the extracellular signal–regulated kinase directly downstream from the mitogen-activated protein kinase pathway. A single patient with aCML harboring a NRAS mutation attained a near-complete hematologic response with 14 months of disease control with trametinib.[42] Another patient obtained a 3-month improvement of leukocytosis and splenomegaly.[58]

Dasatinib, a dual inhibitor of BCR::ABL1 and SRC family kinase, could have potential therapeutic value in aCML: in vitro studies of cell lines with CSF3R-truncated mutations have demonstrated dysregulation in the SRC family–TNK2 kinases with sensitivity to dasatinib.[52] No in vivo reports have been published so far. Venetoclax also could be a possible option in the future associated with HMA in this setting.[59] The ABNL-MARRO (A Basket Study of Novel Therapy for Untreated MDS/MPN and Relapsed/Refractory Overlap Syndromes) is an ongoing recruiting phase 1/2 study that tests novel treatment combinations in MDS/MPNs. The exploratory objectives of the study include investigating genetic biomarkers of response and characterizing molecular responses in this setting of patients. The first open arm includes the JAK1 inhibitor itacitinib, as well as oral decitabine plus cedazuridine (ASTX727).[60]

Conclusions

aCML is a rare myelodysplastic/myeloproliferative neoplasm characterized by increasing leukemic cell burden, organomegaly, anemia, and bone marrow failure. Some negative prognostic factors associated with a shorter survival have been identified, such as age >65 years, female sex, leukocytosis >50×10^9/L, and SETBP1 mutations. The molecular pathogenesis of aCML is heterogeneous, with mutations involving SETBP1, CSF3R, ASXL1, and ETNK1 the most studied to date. The clonal hierarchy in the mutational landscape has been recently proposed.

Unfortunately, no consensus on treatment has been reported until now, and allogeneic transplant remains the only valid option. Palliative chemotherapy (HU, IFNα) is the most common treatment in patients not eligible for the transplant procedure. HMAs can be a bridge to the transplant approach, but the results reported, based on small cohort of patients, are not encouraging. Targeted therapies with JAK2 inhibitor (ruxolitinib), SRC kinase inhibitor (dasatinib), and MEK inhibitor (trametinib) in patients with aCML with actionable target mutations have shown some interesting responses, but large cohorts of patients are needed to understand if they could represent a future therapeutic strategy.

Conflict-of-interest disclosure

Massimo Breccia received honoraria by Novartis, Incyte, Pfizer, BMS, Abbvie, AOP, and Jazz.

Off-label drug use

Massimo Breccia: All of the new actionable target treatments are off-label.

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Hodgkin lymphoma treatment for older persons in the modern era

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There has been a renewed effort globally in the study of older Hodgkin lymphoma (HL) patients, generating a multitude of new data. For prognostication, advancing age, comorbidities, altered functional status, Hispanic ethnicity, and lack of dose intensity (especially without anthracycline) portend inferior survival. Geriatric assessments (GA), including activities of daily living (ADL) and comorbidities, should be objectively measured in all patients. In addition, proactive multidisciplinary medical management is recommended (eg, geriatrics, cardiology, primary care), and pre-phase therapy should be considered for most patients. Treatment for fit older HL patients should be given with curative intent, including anthracyclines, and bleomycin should be minimized (or avoided). Brentuximab vedotin given sequentially before and after doxorubicin, vinblastine, dacarbazine (AVD) chemotherapy for untreated patients is tolerable and effective, and frontline checkpoint inhibitor/AVD platforms are rapidly emerging. Therapy for patients who are unfit or frail, whether due to comorbidities and/or ADL loss, is less clear and should be individualized with consideration of attenuated anthracycline-based therapy versus lower-intensity regimens with inclusion of brentuximab vedotin +/− checkpoint inhibitors. For all patients, there should be clinical vigilance with close monitoring for treatment-related toxicities, including neurotoxicity, cardiopulmonary, and infectious complications. Finally, active surveillance for "postacute" complications 1 to 10 years post therapy, especially cardiac disease, is needed for cured patients. Altogether, therapy for older HL patients should include anthracycline-based therapy in most cases, and novel targeted agents should continue to be integrated into treatment paradigms, with more research needed on how best to utilize GAs for treatment decisions.

LEARNING OBJECTIVES

- Describe prognostic factors associated with inferior outcomes for older Hodgkin lymphoma patients in population-based and clinical studies
- Examine contemporary clinical trial results for older Hodgkin lymphoma patients with emphasis on integration of targeted treatment agents
- Discuss treatment-related toxicities, including lethal events, with attention to cardiac disease and postacute survivorship considerations

CLINICAL CASE

A 70-year-old Hispanic man presents with increasing generalized fatigue, low back pain, and unintentional 20-pound weight loss over the preceding 4 months. Physical examination revealed large nontender adenopathy in the left axilla. The patient's hemoglobin was 9.5 g/dL with mean corpuscular volume (MCV) 98 fL, percent transferrin saturation 8%, ferritin 780 ng/mL, normal B12 and folate, and an absolute reticulocyte count of 45 000.

The patient has a history of coronary artery disease status post stent 5 years prior, well-controlled hypertension, type 2 diabetes on oral therapy, prior smoker (30 packs per year), hiatal hernia, and past history of basal cell cancer (Cumulative Illness Rating Scale-Geriatric [CIRS-G] score = 10). He is a retired mechanic and lives alone; he performs all self-care and instrumental activities of daily living (ADLs) without restriction, but ECOG performance status was 2.

Excisional lymph node biopsy of a left axillary node showed effacement by a mixed population of lymphocytes, plasma cells, eosinophils, neutrophils, and histiocytes. Scattered large cells stained positive for CD15, CD30, PAX5, and EBER. The diagnosis was consistent with

classic Hodgkin lymphoma, mixed cellularity type with Epstein-Barr virus positivity by EBER in situ hybridization.

A staging positron emission tomography (PET) scan for the patient showed hypermetabolic disease in the left axilla node (3.5×5.9cm) with standardized uptake volume max of 24, precaval nodal region with standardized uptake volume max of 19, a discrete liver lesion, and diffuse bony uptake (axial and appendicular skeleton). The baseline cardiac left ventricular ejection fraction was 55% with a global longitudinal strain of −27%.

Introduction

Older patients ages ≥60 years represent approximately 20% to 25% of all classic Hodgkin lymphoma (HL) cases diagnosed in Western and European countries.[1-5] Survival rates for older HL patients have historically been inferior compared with younger patient populations[2,6-9] and age- and sex-matched controls.[5,10] The poorer outcomes for older HL patients are likely multifactorial, including comorbidities, poor performance status, histologic differences (eg, mixed cellularity and Epstein-Barr virus–related disease), frequent advanced-stage disease, inability to tolerate chemotherapy at full dose and schedule, and increased incidence of severe treatment-related toxicity. Previous underrepresentation of older patients in HL clinical trials has compounded these factors.[6,11-13] In addition, the unique bimodal age distribution in HL results in an uncommon comparison of outcomes of individuals primarily in their 20s versus those in their 70s, which disproportionately magnifies the survival disparity.

More recently, there has been a renewed effort across the world to study older HL patients, resulting in a multitude of new data, including the prognostic impact of geriatric measures and the importance of anthracyclines for patient survival. Additionally, multiple recent prospective clinical studies have emphasized the integration of novel targeted therapeutic agents into frontline treatment paradigms. Collectively, outcomes appear to have improved for older HL patients in the contemporary era.[14-17] However, unlike other aggressive lymphoma subtypes, a standard treatment paradigm has been mostly absent, and treatment-related toxicity remains a critical issue to navigate, especially bleomycin lung toxicity (BLT), infectious complications, and neurotoxicity. In this review, we examine contemporary real-world and clinical trial treatment data for older HL patients with an emphasis on prognosis, geriatric measures, treatment intensity, anthracycline use, tolerability, integration of targeted therapeutic agents, and postacute survivorship.

Prognostication

The International Prognostic Score (IPS) included ages >45 years as an adverse covariate.[18] Of note, only 9% of patients were >55 years in the IPS, and no patients aged >65 years were included. Several analyses have shown that the IPS was not prognostic in older HL patients,[9,19,20] while 2 recent population studies from British Columbia and Sweden identified a correlation with survival.[15,17] Increasing age beyond 30 years (continuous variable) was an adverse factor for survival on the recently published advanced-stage Hodgkin Lymphoma International Prognostic Index, but only a minority of patients were >60 years.[21]

Race/ethnicity

There are intriguing racial differences seen in older HL patients. In a US Surveillance, Epidemiology, and End Results (SEER) analysis, incidence rates for older HL patients (ie, ages >64 years) were highest among Hispanics, followed by non-Hispanic Whites and Blacks (Supplementary Figure S1).[22] Furthermore, 5-, 10- and 15-year overall survival (OS) rates were inferior for Hispanics and Blacks compared with non-Hispanic Whites and Asian/Pacific Islanders, which persisted on multivariable analyses. In a contemporary SEER analysis of older HL patients treated across 2 time periods (2006-2010 and 2011-2015), Shah et al. documented improvement in OS across the 2 cohorts. However, this was primarily seen in non-Hispanic Whites (Table 1).[16] Furthermore, survival disparity persisted across the study periods between non-Hispanic Whites and Hispanics, while other factors associated with worse OS were increasing age, male sex, stage III-IV, unmarried status, and lack of chemotherapy.

Functional status

The impact of geriatric assessments (GA) in older patients with cancer is well recognized,[23] and a multitude of analyses have documented the frequent occurrence and prognostic importance of GAs in older HL patients (Supplementary Table S1).[20,24-28] Retrospective Chicago-based real-world evidence (RWE) of older HL patients treated from 2000 to 2009 found that 61% of patients had at least 1 severe comorbidity, 26% were "unfit" (using the original simplified GA tool[29]), 17% had a geriatric syndrome, and 13% had a loss of self-care activities of daily living (ADLs) at diagnosis.[20] Loss of any ADL was strongly prognostic in this data set. A recent SEER-based prediction model for 1-year mortality of older HL patients treated with curative intent was developed and validated (Table 1).[30] In addition to the presence of B-symptoms, advanced stage, and older age at diagnosis, increased comorbidities via the Charlson Comorbidity Index correlated with inferior survival.

In a multicenter phase 2 clinical trial, older HL patients were treated with 2 initial doses of single-agent brentuximab vedotin (BV), followed sequentially by adriamycin, vinblastine, dacarbazine (AVD) for 6 cycles, with subsequent consolidative single-agent BV.[19] Two-year progression-free survival (PFS) rates for HL patients with a low CIRS-G comorbidity score (ie, <10 vs ≥10) were 100% vs 45%, respectively ($P < 0.0001$). Furthermore, patients with no loss of instrumental ADLs vs a loss of *any* instrumental ADL at baseline had 2-year PFS rates of 94% vs 25% ($P < 0.0001$). A recent US multicenter RWE analysis of older HL patients treated from 2010 to 2018 confirmed the significance of ADLs on survival (Table 1 and Figure 1A/B).[28] Taken together, these studies support the prognostic importance of baseline GAs in older patients with HL.

However, more research is needed to delineate the optimum classifications of *fit* vs *unfit* vs *frail* for older HL patients based on functional status and advancing age. For example, the elderly prognostic index from the Fondazione Italiana Linfomi (FIL) group for older diffuse large B-cell lymphoma (DLBCL) patients defined a "modified simplified GA" based on model building in multivariable analysis that better accounted for age and varying levels of comorbidities,[31] and a large Norwegian analysis used the Charlson Comorbidity Index, ADLs, ages ≥85 years, and a nutritional index to delineate fitness and frailty

Table 1. Contemporary real-world data for older Hodgkin lymphoma patient outcomes

Citation	Study type	Population (study years)	Study objectives	Findings
Moccia et al. 2020[35]	Retro	Ages ≥60, N = 269 (2000–2017)	5-yr survival analyses and toxicity evaluation	BLT 17%; 5-yr PFS 53%, OS 64%, and CSS 86%; survival poorer ages >70 vs 60–70 yrs
Rodday et al. 2020[38]	SEER	Ages ≥65, N = 2825 (1999–2014)	Factors associated with first-line tx: full (25%), partial (36%), single-agent/RT (13%), no tx (26%)	Less-aggressive tx, frailty, heart disease, advanced stage, and treatment in Southern US associated with not receiving full chemotherapy
Kumar et al. 2021[30]	SEER	Ages ≥65, N = 1315 (2000–2013)	Prediction model of 1-yr mortality on standard chemotherapy	Final OS model: CCI, B-symptoms, advanced-stage disease, and older age
Orellana-Noia et al. 2021[28]	Retro	Age ≥60, N = 244 (2010–2018)	Predictors of survival based on GA and chemotherapy tx	BLT 18%; TRM 3.3%; inferior PFS and OS with ADL loss and with alternative tx vs conventional chemotherapy
Wahlin et al. 2021[17]	Registry	Age >60, N = 691 (2000–2014)	Survival by period, age, stage, sex, and ABVD vs CHOP	OS improved: 2010–2014 vs 2000-2009, with ABVD vs CHOP, and ages ≤70 vs >70 yrs
Rodday et al. 2021[37]	SEER	Ages ≥65, N = 2686 (2000–2013)	Survival by tx and stage with Cox regression, competing risk, and propensity	HL-specific survival for full tx vs partial tx (for advanced stage): HR 3.26 and "other cause" survival HR 1.76
Overgaard et al. 2022[40]	Retro	Ages ≥60, N = 1554 (2000–2021)	Survival by stage and tx with multivariable analyses	5-yr OS: AVD 64% vs ABVD 63% vs CHOP 46% (multivariable: AVD and ABVD > CHOP)
Cheng et al. 2022[15]	Registry	Ages ≥60, N = 744 (1961–2019); N = 401 (2000–2019)	5-yr survival by decade and age with toxicity analyses	BLT 21%; survival improved by decade; post 2000: 5-yr PFS 60%, OS 65%, and DSS 76%; improved survival <70 vs ≥70 yrs
Shah et al. 2022[16]	SEER	Ages ≥60, N = 4957 (2006–2015)	Comparison 2006–2010 vs 2011–2015 and by race and clinical factors	Median OS by period 4 yr vs 4.8 yr, respectively; 5-year OS inferior among Hispanics
Goh et al. 2023[39]	Registry	Ages >60, N = 195 (2011–2020)	Survival analyses, including by treatment (with Cox regression)	TRM 5.2%; 2-yr PFS 64%, OS 71%; 2-yr PFS with anthracycline 70% vs 33% without; Cox OS model: CCI and anthracycline use

ADL, activities of daily living; AVBD, doxorubicin, vinblastine, bleomycin, dacarbazine; AVD, doxorubicin, vinblastine, dacarbazine; BLT, bleomycin lung toxicity; CCI, Charlson Comorbidity Index; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone; CR, complete response; CSS, cause-specific survival; DSS, disease-specific survival; GA, geriatric assessments; HR, hazard ratio; N, number; OS, overall survival; PFS, progression-free survival; pts, patients; retro, retrospective; RT, radiation therapy; SEER, Surveillance, Epidemiology, and End Results registry; TRM, treatment-related mortality; tx, treatment; US, United States; yrs, years.

for untreated DLBCL (Supplementary Tables S2 and S3).[32] Most reports describing patient fitness for older HL patients have been retrospective analyses and have utilized the Tucci classification published for DLBCL.[29] It is essential that prospective studies for older HL patients include objective measures of GA and other measures of fitness in part for consistency and to aid cross-study interpretation as well as to assist in identifying potential fitness-based therapeutic recommendations. Notably, objective GAs were shown to be more effective than subjective clinical judgment in identifying older B-cell lymphoma patients likely to benefit from aggressive, curative therapy.[33,34]

Advancing age

Advancing age within the older HL population is associated with inferior survival.[3,9,15-17,20,27,30,35] In the Chicago RWE series, ages ≥70 years and loss of any self-care ADLs were the dominant prognostic factors.[20] Moreover, patients with both factors present at diagnosis had a 3-year OS of 0%. In recent British Columbia RWE,[15] survival correlated with increasing age (ie, ≥70 vs 60-69 years), and similar data were reported from Swedish[17] and Swiss[35] RWE (Table 1). However, despite the use of multivariable analyses, it is not clear if advancing age alone is an independent risk factor for inferior survival among older patients vs a proxy for increased comorbidities with decreased functional status and/or use of less-intensive chemotherapy treatment, especially anthracyclines. In the US RWE, patients aged 70 to 79 years who received conventional anthracycline-based regimens had comparable survival with patients aged 60 to 69 years ((Table 1).[28]

Dose intensity and anthracyclines

Dose intensity and use of anthracyclines have been cornerstones of HL treatment for decades.[36] Landgren et al. reported that

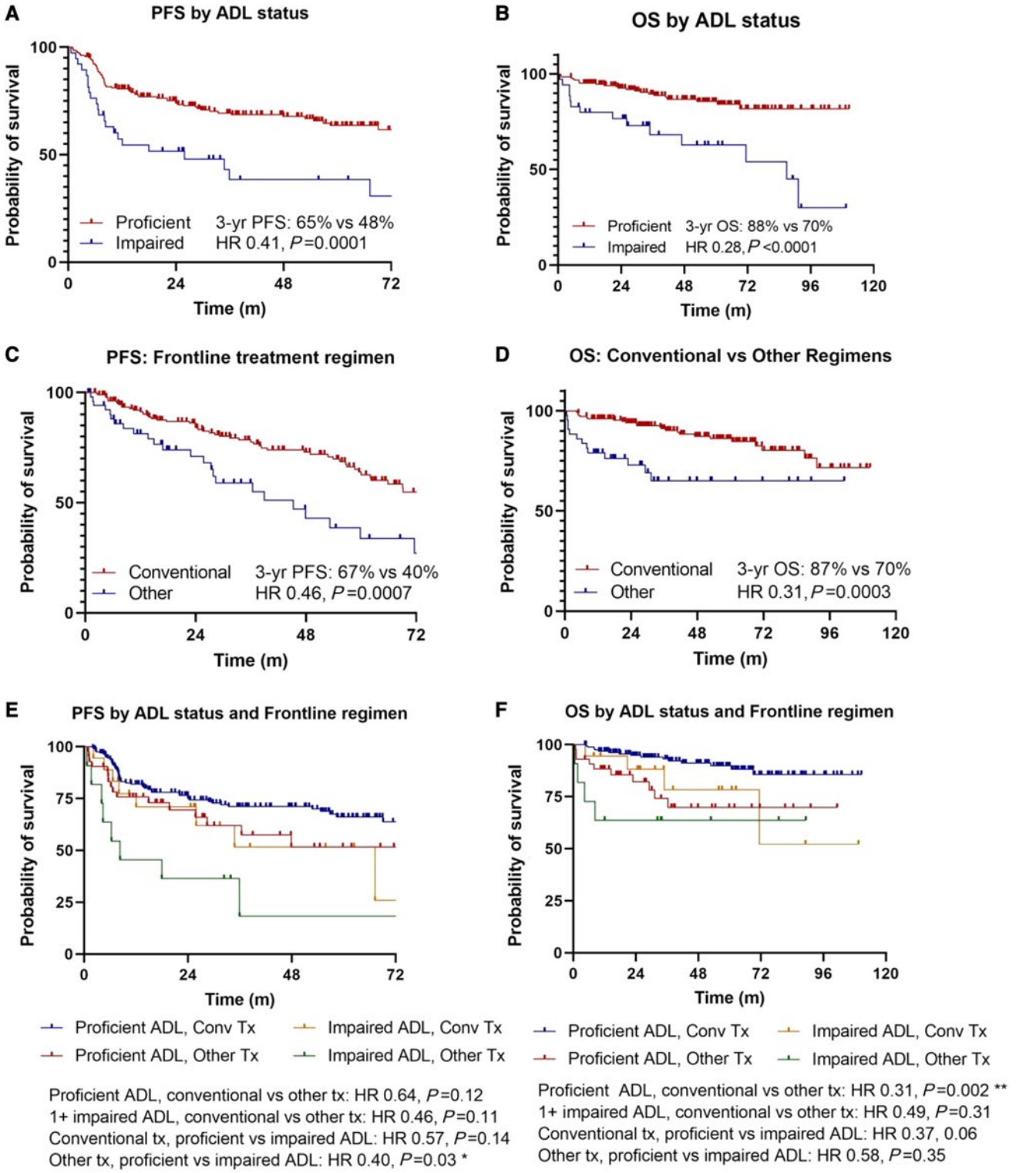


Figure 1. Outcomes of older HL patients based on functional status and conventional therapy. PFS and OS by geriatric fitness measures in stage II to IV disease. Time is listed in months for all figures. (A) PFS by ADL status. (B) OS by ADL status. (C) PFS by frontline treatment regimen. (D) OS by treatment regimen. (E) PFS by ADL status and frontline regimen. (F) OS by ADL status and frontline regimen. Tx, treatment. Reprinted with permission.[28]

older HL patients treated with ABVD-based chemotherapy from 1973 to 1994 had significantly improved OS with relative dose intensity (RDI) of >65%.[3] In a large German Hodgkin Study Group (GHSG) analysis of older HL patients treated on 5 consecutive clinical trials from 1988 to 1998, reduced RDI was a major factor associated with inferior outcomes.[6]

RWE supports the importance of treatment intensity with the use of conventional combination chemotherapy, in particular anthracyclines (Table 1).[16,28,37-39] In a SEER-Medicare study of 2686 older HL patients, only 49% received a full regimen (defined as conventional multiagent chemotherapy for a minimum of 2 cycles).[37] In advanced-stage, treatment with a full regimen was associated with markedly improved OS and HL-specific survival vs partial treatment. In a related SEER analysis, Medicaid dual eligibility, marital status, frailty, cardiac comorbidity, prior cancer, advanced-stage disease, B-symptoms, and residence in the Southern US were independently associated with not receiving full chemotherapy regimens.[38] Recent Australian RWE identified that the use of anthracycline was associated with superior PFS and OS after adjusting for comorbidities, age, and performance status (Table 1).[39] Previous data from the Nebraska Group compared ChlVPP with ChlVPP/ABV in a nonrandomized study of previously untreated HL patients.[8] In older patients treated with ChlVPP, the 5-year EFS and OS rates were 24% and 30% vs 52% and 67%, respectively, for patients treated with ChlVPP/ABV.

Several recent analyses in older HL patients have also shown improved survival with the use of classic HL regimens (ie, ABVD/AVD) over CHOP.[17,28,40] In a Nordic RWE study, older patients who received CHOP had significantly poorer outcomes than those treated with ABVD or AVD (Table 1).[40] Additionally, there were no apparent survival differences identified in patients who received AVD vs ABVD.

Overall, it remains unclear if improved outcomes for older HL patients treated with anthracyclines are due to selection bias for patient fitness and more robust functional status. In the aforementioned Norwegian study, the use of R-CHOP (attenuated or full-dose) was associated with superior survival vs anthracycline-free regimes in unfit and frail DLBCL patients.[32] In the US RWE HL analysis, the use of conventional anthracycline-based therapy was associated with improved PFS and OS, which persisted after adjustment for ADL status (Figure 1).[28]

Therapy for newly diagnosed patients

Pre-phase treatment

In DLBCL, Pfreundschuh et al. showed that the use of steroids as a pre-phase at least 1 week before the start of therapy improved patient performance status as well as reduced therapy-associated deaths.[41] We advocate a similar pre-phase paradigm for most newly diagnosed older HL patients utilizing a short course of pulse steroids (eg, prednisone 60-100mg daily for 5 days), which was done in the aforementioned sequential BV-AVD-BV study.[19] At a minimum, this ameliorates disease-related symptoms and improves functional status while testing and approval processes are being completed. The use of a "pre-phase" therapy before the start of definitive therapy needs to be better studied in HL.

Early stage

Most published early-stage HL studies have uncommonly included older patients. In the GHSG HD8 trial, patients with early unfavorable stage were randomized to 4 courses of chemotherapy cyclophosphamide, vincristin, procarbazine, prednisone (COPP) and doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) and involved field radiotherapy (IFRT) or extended field radiotherapy.[42] The 5-year freedom from treatment failure (FFTF) and OS were lower in older patients (FFTF 64% vs 87%; $P<0.001$ and OS 70% vs 94%; $P<0.001$). Moreover, older patients had poorer outcomes when treated with extended field radiotherapy vs IFRT (5-year FFTF 58% vs 70%, respectively, $P=0.034$; and 5-year OS 59% vs 81%, respectively, $P=0.008$).[43]

An analysis of older patients within the GHSG HD10 and HD11 trials included 117 older early-stage HL patients treated with 2 to 4 cycles of ABVD followed by IFRT.[44] Mean delay of treatment was twice as high in the older patients (2.2 vs. 1.2 weeks), and WHO grade 3 and 4 toxicity was also more frequent in this group (68% vs 50%) than in younger patients, which resulted in a higher treatment-related mortality (TRM) in older patients. Boll et al. analyzed the outcomes of older HL patients treated in the GHSG HD10 and HD13 trials.[45] In patients receiving 2 cycles of ABVD, respiratory adverse events were uncommon; however, the incidence of BLT was 10% (including several related deaths) for early-stage patients who received 4 cycles of ABVD. Other studies have analyzed other chemotherapy regimens for older early-stage HL patients (eg, VEPEMB or CHOP followed by IFRT)[24,46] or IFRT alone.[47,48]

Advanced stage

Historical data. Three-to-five-year PFS rates for advanced-stage older HL patients treated with ABVD therapy range from 28% to 55%, with OS rates of 31% to 67% (Supplementary Table S4).[4,9,11,13,49,50] Efforts to improve outcomes for older HL patients have included the development of chemotherapy platforms with decreased intensity and regimens with individualized dosing to mitigate toxicity.[4,8,9,50-54] A non-anthracycline regimen studied in an advanced-stage HL ECOG study, BCVPP (carmustine, cyclophosphamide, vinblastine, procarbazine, and prednisone), was well tolerated and associated with good outcomes.[55]

Proctor et al. reported results from the Study of Hodgkin in the Elderly/Lymphoma Database (SHIELD) project consisting of a prospective trial and RWE component.[4] For prognostication, achievement of CR strongly predicted survival. Factors associated with CR were comorbidity score and ADLs. In the observational group of advanced-stage patients treated according to physician discretion (most often ABVD), the overall response rate (ORR) was modest and the TRM was 18%. Furthermore, all 13 frail HL patients died (12 from HL).

Contemporary data with chemotherapy +/− targeted therapy. Targeted therapeutic agents have been incorporated into frontline treatment for older HL patients (Table 2). In the study of BV given before and after AVD chemotherapy for untreated older HL patients,[19] the choice of sequential therapy was predicated on the following: (1) initial single-agent BV would improve performance status, establish early disease control, and increase the likelihood of tolerability to chemotherapy; (2) to minimize overlapping neurotoxicity with vinblastine; and (3) consolidation would decrease the risk of relapse. The ORR and CR rates after the initial 2 lead-in doses of BV were 82% and 36%, respectively, and 95% and 90%, respectively, after AVD. Survival was robust (Figure 2). The most common grade 3/4 adverse events were

Table 2. Contemporary clinical trials for newly diagnosed older HL patients*

Author, year	N	Therapy	Median age (years)	Baseline GA and patient fitness	Outcomes	Febrile neutropenia	Peripheral neuropathy (≥ grade 3)	Treatment-related mortality rate
Anthracycline-based chemotherapy +/− targeted therapy								
Evens 2018[19]	48	Brentuximab vedotin *sequentially* with AVD	69	Median CIRS-G 7 (31%≥10); 14% pts loss IADL	2-yr PFS 84% 2-yr OS 93%	8%	4%	2%
Boll 2018[61]	49	Brentuximab vedotin + CAP (*concurrent*)	66	Limited GA; all pts CIRS-G ≤6	1-yr PFS 74% 1-yr OS 93%	27%	0%	2%
Boll 2019[60]	25	Lenalidomide + AVD (*concurrent*)	67	Limited GA; all pts CIRS-G ≤7 (mean 2)	3-yr PFS 70% 3-yr OS 84%	4%	NR	NR
Salvi 2019[57]	47	MBVD	75	Limited GA; CIRS-G grade 3/4 in 11%	3-yr PFS 43% 3-yr OS 70%	6.3%	NR	6.4%
Ghesquieres 2021[58]	89	PVAB	68	Limited GA; median CIRS-G 3	4-yr PFS 50% 4-year OS 69%	NR	NR	NR
Evens 2022[59]	84	A+AVD (*concurrent*)	68	ND	2-yr PFS 70% 2-yr OS ~85%	37%	18%	3.6%
	102	ABVD	66	ND	2-yr PFS 71% 2-yr OS ~85%	17%	3%	5.1%
Torka 2023[64]	40	N+AVD (*concurrent*)	66	Median scores: ADL 83, IADL 14, TUG 11.5sec	2-yr PFS 86% 2-yr OS 96%	8%	0%	0%
Wilson 2023[62]	41	ACOPP**	74	Limited GA; median CIRS-G 5	2-yr PFS 73% 2-yr OS 94%	15%	0%	2%
Targeted therapy +/− low-intensity chemotherapy								
Forero-Torres 2015[65]	27	Brentuximab vedotin	78	7% loss IADL; 30%≥1 fall; TUG >13.5sec in 48%	2-yr PFS ~25% 2-yr OS ~70%	0%	26%	NR
Friedberg 2017[66]	21	Brentuximab vedotin + DTIC	69	20% loss IADL; 26%≥1 fall; TUG >13.5sec in 70%	2-yr PFS ~45% 2-yr OS ~90%	5%	27%	0%
Gibb 2020[67]	35	Brentuximab vedotin	77	Limited GA, median CIRS-G 6	2-yr PFS 7% 2-yr OS 42%	0%	~10%	3%
Yasenchak 2020[66]	42	Brentuximab vedotin + nivolumab	72	NR	2-yr PFS ~60% 2-yr OS ~90%	NR	33%	0%
Cheson 2020[68]	46	Brentuximab vedotin + nivolumab	72	ND	2-yr PFS ~40% 2-yr OS NR	0%	11%	2%
Lazarovici 2021[69]	64	Nivolumab +/− vinblastine	78	Median CIRS-G 10; median G8 score 12.5	2-yr PFS ~20% 2-yr OS 77%	0%	0%	3%
Dickinson 2023[70]	25	Pembrolizumab	77	Limited GA; median CIRS-G 7	2-yr PFS ~15% 2-yr OS 83%	0%	0%	0%

* Since 2018; minimum 20 patients; **retrospective.

A+AVD, brentuximab vedotin, doxorubicin, vinblastine, dacarbazine; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; ACOPP, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisolone; ADL, activities of daily living; AVD, doxorubicin, vinblastine, dacarbazine; CAP, cyclophosphamide, doxorubicin, prednisone; CIRS-G, cumulative illness rating score-geriatric; DTIC, dacarbazine; ECOG, Eastern Cooperative Oncology Group performance status; G8, geriatric 8 score; GA, geriatric assessment; IADL, instrumental activities of daily living; KPS, Karonfsky performance scale; MBVD, nonpegylated liposomal doxorubicin (myocet), bleomycin, vinblastine, dacarbazine; mPFS, modified progression-free survival; N+AVD, ; ND, not done; NR, not reported; OS, overall survival; PFS, progression-free survival; pts, patients; PVAB, prednisone, vinblastine, doxorubicin, bendamustine; PVAG (prednisone, vinblastine, doxorubicin, and gemcitabine); sec, seconds; TUG, timed up and go.

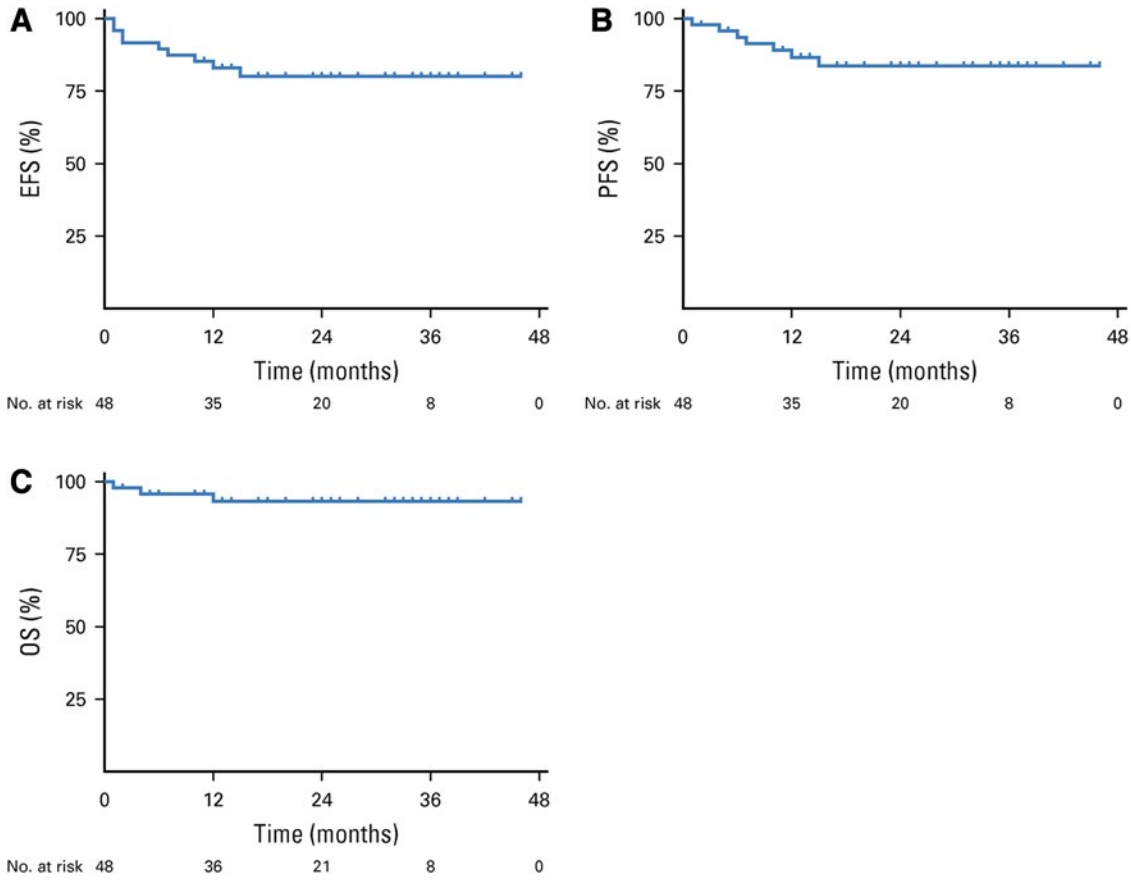


Figure 2. Survival among older patients treated on the frontline HL study with sequential brentuximab vedotin and AVD chemotherapy. Kaplan-Meier curves at 2 years for (A) event-free survival (EFS; 80%; 95% CI, 65% to 89%), (B) progression-free survival (PFS; 84%; 95% CI, 69% to 92%), and (C) overall survival (OS; 93%; 95% CI, 80% to 98%) for all 48 patients. In addition, patients with a Cumulative Illness Rating Scale-Geriatrics (CIRS-G) score <10 had 2-year event-free survival (EFS), PFS, and OS rates of 100% (95% CI, 100% to 100%), and patients who had preserved functional status without loss of instrumental activities of daily living (IADL) at baseline had corresponding 2-year EFS, PFS, and OS rates of 89% (95% CI, 73% to 96%), 94% (95% CI, 79% to 99%), and 97% (95% CI, 82% to 99%), respectively. Reprinted with permission.[19]

neutropenia (44%); febrile neutropenia and pneumonia (8%); and diarrhea (6%). Response to the initial 2 doses of BV (ie, CR/PR vs not) was strongly associated with survival (2-year PFS 100% vs 50%, respectively, $P = 0.007$).

Response-adapted therapy has not been well studied in older HL patients, but if ABVD therapy is utilized, bleomycin should be eliminated for all patients with an interim negative PET-2 scan vis-à-vis the RATHL study design.[56] In fact, there should be great caution in giving any older HL patient more than 2 full cycles of bleomycin. Additionally, therapy should *not* be intensified to bleomycin, adriamycin, cyclophosphamide, vincristine, procarbazine, and prednisone (BEACOPP) therapy with positive PET-2 as older patients do not tolerate this regimen, as highlighted below. Changing therapy for early nonresponders to initial ABVD/AVD to a non-cross-resistant regimen (or added targeted agents) needs to be further tested in prospective studies.

For older and nonfit patients with HL, attempts have been made to mitigate toxicity of conventional doxorubicin by substituting it with liposomal doxorubicin. In a phase 2 trial of 47 older HL patients with median age of 75 years, patients were treated with bleomycin, vinblastine, dacarbazine, nonpegylated liposomal doxorubicin (MBVD).[57] Two patients had cardiac events and 49% had grade 3 or higher neutropenia, with 15% of patients having ≥ grade 3 infections. Overall tolerability to MBVD was poor in advanced-stage patients, with 38% of patients prematurely discontinuing treatment. An alternative approach included modification of the prednisone, vinblastine, doxorubicin, and gemcitabine (PVAG) regimen substituting gemcitabine with bendamustine in older HL patients.[58] The majority of patients completed 6 treatment cycles (88%), but TRM was seen in 4 patients and 32% had at least one serious adverse event.

Outcomes were analyzed across ages in phase 3 ECHELON-1 study (BV + doxorubicin, vinblastine, and dacarbazine [A+AVD] vs ABVD in untreated advanced-stage HL patients), which included 186 patients ≥60 years (Table 2).[59] The mean RDI for older patients who received BV+AVD chemotherapy was 92% to 97%. With a median follow-up of 61 months, the 5-year PFS rates per investigator with A+AVD vs ABVD were 67.1% vs 61.6%, respectively. Pulmonary adverse events were higher with ABVD vs A-AVD (13% vs 3%, respectively), and the incidence of any-grade and severe

peripheral neuropathy was higher in the A+AVD arm. However, rates of resolution or improvement in peripheral neuropathy were similar in patients treated with A+AVD and ABVD (80% vs 83%, respectively).

A phase 1 study added lenalidomide concurrently with AVD chemotherapy for older HL patients.[60] Dose-limiting toxicities were mainly hematologic but also included 3 thromboembolic events despite documented aspirin prophylaxis. The ORR was 79% for evaluable patients and 86% in patients treated with at least 20mg of lenalidomide. The GHSG and the Nordic Lymphoma Group presented data using BV concurrently with cyclophosphamide, doxorubicin, and prednisone (B-CAP) for fit older HL patients with CIRS-G ≤6.[61] Among eligible advanced-stage patients, the ORR was 98% (CR rate 65%). Notably, there was no grade 3 neuropathy and TRM was low (Table 2).

A recent multicenter retrospective review from the United Kingdom examined the elimination of bleomycin and etoposide from BEACOPP for older, less-fit HL patients. The analysis included 41 older HL patients who received ACOPP with dose-reduced cyclophosphamide.[62] Best overall response was 95% (CR 83%) and survival was strong. While there was 1 TRM, treatment was generally tolerated without severe side effects, though nearly 60% of the population required hospitalization at one point during the treatment process.

Checkpoint inhibitor therapy combined with multiagent anthracycline-based chemotherapy has been studied in the frontline setting for older HL patients. There were 4 patients ages ≥60 years treated in a study using sequential pembrolizumab before AVD chemotherapy.[63] The PFS and OS were 100% for all patients in the study, and there were no unexpected toxicities seen in

Table 3. Select ongoing or planned clinical trials for newly diagnosed older HL patients

Trial title	Trial phase	Study number	Disease stage	GA-based inclusion/ GA-directed therapy	Study design
Phase II Trial of Individualized Immunotherapy in Early-Stage Unfavorable Classical Hodgkin Lymphoma *(INDIE)*	2	NCT04837859	IA-IIB	Yes (CIRS-G)/no	2 cycles tislelizumab; PET neg: 4 cycles tislelizumab +30 gy ISRT; PET pos: 4 cycles T-AVD +30 gy ISRT
Response Adapted Incorporation of Tislelizumab Into the Front-line Treatment of Older Patients With Hodgkin Lymphoma *(RATiFY)*	2	NCT05627115	I-IV	No/no	3 cycles tislelizumab; PET neg: 2 cycles T +/− RT followed by tislelizumab until PD or toxicity for fav ES or 2–4 cycles T+AVD +/− RT for unfav ES and AS; PET pos: 4–6 cycles T+AVD +/− RT for ES and AS
Fitness-Adapted, Pembrolizumab-Based Therapy for Untreated Classical Hodgkin Lymphoma Patients 60 Years of Age and Above	2	NCT05404945	II-IV	Yes/yes (CIRS-G + ADLs)	Pembro + BV followed by repeat GA/ fitness; fit induction: 3 cycles Pembro q6w + 4 cycles AVD; unfit induction: 3 cycles Pembro q6w + 3 cycles BV; consolidation for all: Pembro +2 doses BV
A Study of Brentuximab Vedotin With Hodgkin Lymphoma (HL) and CD30-Expressing Peripheral T-cell Lymphoma (PTCL)	2	NCT01716806	II-IV	Yes/yes (CIRS-G + ADLs)	Cohorts E and F: single-agent BV for patients unsuitable or unfit for initial conventional combination chemotherapy by GA (ie, CIRS-G ≥10 and/or loss of any instrumental ADL)
BrEPEM-LH-22017 for Older Patients With Untreated Hodgkin Lymphoma (HL)	1/2	NCT03576378	IIB-IV	No/no	6 cycles BV-EPEM
HD21 for Advanced Stages Treatment Optimization Trial in the First-line Treatment of Advanced Stage Hodgkin Lymphoma; Comparison of 6 Cycles of Escalated BEACOPP With 6 Cycles of BrECADD (elderly extension)	2	NCT02661503	IIB with LMM or EN, III/IV	Yes (CIRS-G)/no	2 cycles BrECADD; PET neg: 2 cycles BrECADD; PET pos: BrECADD 4 cycles +/− RT
Immunotherapy (Nivolumab or Brentuximab Vedotin) Plus Combination Chemotherapy in Treating Patients With Newly Diagnosed Stage III-IV Classic Hodgkin Lymphoma *(S1826)**	3	NCT03907488	III/IV	No/no	6 cycles Nivo + AVD vs 6 cycles BV + AVD

* Approximately 10% of patients in the study population were ages 60 years and above.

ADL, activities of daily living; AVD, adriamycin, vinblastine, dacarbazine; AS, advanced stage disease; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone; BrECADD, brentuximab vedotin, etoposide, cyclophosphamide, doxorubicin, dacarbazine, dexamethasone; BV, brentuximab vedotin; CIRS-G, cumulative illness rating score-geriatric; EN, extranodal disease; EPEM, cyclophosphamide, procarbazine, prednisone, etoposide, mitoxantrone; ES, early-stage disease; fav, favorable; GA, geriatric assessment; gy, gray; ISRT, involved site radiation therapy; LMM, large mediastinal mass; neg, negative; Nivo, nivolumab; PD, progressive disease; Pembro, pembrolizumab; PET, positron emission tomography; pos, positive; q3w, every 3 weeks; q6w, every 6 weeks; RT, radiation therapy; T, tislelizumab; unfav, unfavorable.

older patients (personal communication, Dr. Jane Winter, June 5th, 2023). A single-arm phase 2 multi-institutional study of concurrent nivolumab and AVD (N-AVD) was recently reported.[64] Of 33 evaluable patients, the ORR was 100%, with a 97% CR rate. At 37-month median follow-up, survival rates were robust (Table 2). There was no correlation between baseline GAs and outcome, although most patients were deemed fit. We eagerly await additional data from recently completed studies that incorporated nivolumab concurrently with AVD chemotherapy (Table 3).

Targeted therapy +/− low-intensity chemotherapy. A host of varied phase 2 clinical studies have leveraged targeted therapeutic agents with or without low-intensity chemotherapy (Table 2). The clinical intent in these trials was to target patients who had increased comorbidities and/or compromised functional status. However, delineation of patient fitness was not consistently performed, and ineligibility for standard anthracycline-based therapy was typically determined subjectively by the investigator.

In a prospective phase 2 study of single-agent BV for older untreated HL patients deemed ineligible for frontline conventional combination chemotherapy in the investigator's judgment, the ORR was 92% (CR 72%).[65] However, the relapse rate was high (Table 2). The study was amended to combine concurrent bendamustine or dacarbazine.[66] The bendamustine arm closed prematurely due to unexpected toxicity, including several treatment-related deaths. An additional treatment arm added nivolumab to BV and the initial data is encouraging with median PFS and OS not reached. Rates of grade 3 peripheral neuropathy were 25% to 35% across the 4 treatment arms (Table 2).

A United Kingdom study examined single-agent BV for HL patients ages ≥60 years or ages <60 years and considered unfit or ineligible for combination chemotherapy by cardiac ejection fraction <50%, significant cardiac morbidity, and/or compromised lung function (Table 2).[67] Therapy was tolerable but response and durability were modest, with a CR rate of 26% and median PFS of 7.3 months. Additionally, 29% of patients permanently stopped treatment due to unacceptable toxicity, 8 due to sensory neuropathy.

A single-arm US trial studied frontline BV and nivolumab for 8 cycles in older patients unsuitable for standard chemotherapy due to cardiac ejection fraction <50%, pulmonary diffusion capacity <80%, 0.5 to 1.0 mL/s, or those who refused chemotherapy.[68] The ORR was 64% (CR rate 48%) and the median PFS was 18.3 months. However, the study did not meet its prespecified activity criteria. The Lymphoma Study Association examined untreated older HL patients with CIRS-G score ≥6 using nivolumab +/− vinblastine, all administered for a maximum of 18 cycles.[69] At end of therapy, the ORR was only 47% (CR rate 29%) and it similarly did not meet the prespecified efficacy endpoint. Additionally, adverse events led to treatment discontinuation in 30% of patients, with immune-related adverse events noted in 34%, including 3 pneumonitis, 1 myocarditis, 1 encephalitis, and 1 colitis.

A recent phase 2 Australian study of single-agent pembrolizumab in patients ≥65 years or who were considered unfit to receive frontline ABVD was presented.[70] Ineligibility for ABVD-based therapy was subjectively determined at provider discretion. Of the 27 who enrolled, 25 patients received a median of 11 cycles of pembrolizumab. The ORR was 72% (CR 32%) with a median duration of response of 10.6 months. The PFS was modest with most patients experiencing progression, though 2-year OS was 83%.

Treatment recommendations: newly diagnosed advanced-stage disease

Figure 3 depicts overarching treatment recommendations for untreated advanced-stage older HL patients, highlighting the importance of baseline GAs, comorbidity management, and pre-phase therapy. There are published guidelines on the GA testing and screening options (Supplementary Table S1),[23] but there is not "one right" tool for screening older HL patients who warrant referral for more detailed geriatric consultation/intervention. We assess at least comorbidities via CIRS-G and self-care and instrumental ADLs at baseline diagnosis.[71] Additionally, we offer most patients pre-phase therapy whether single-agent prednisone (eg, 60-100 mg/daily for 5 days) +/− single-agent brentuximab vedotin as published.[19] If brentuximab vedotin is not available, single-agent vinblastine 3-6 mg/m^2 may also be utilized. Furthermore, we recommend reassessment of functional status after pre-phase therapy as the initial physical determinant and debilitation may be due to tumor burden.

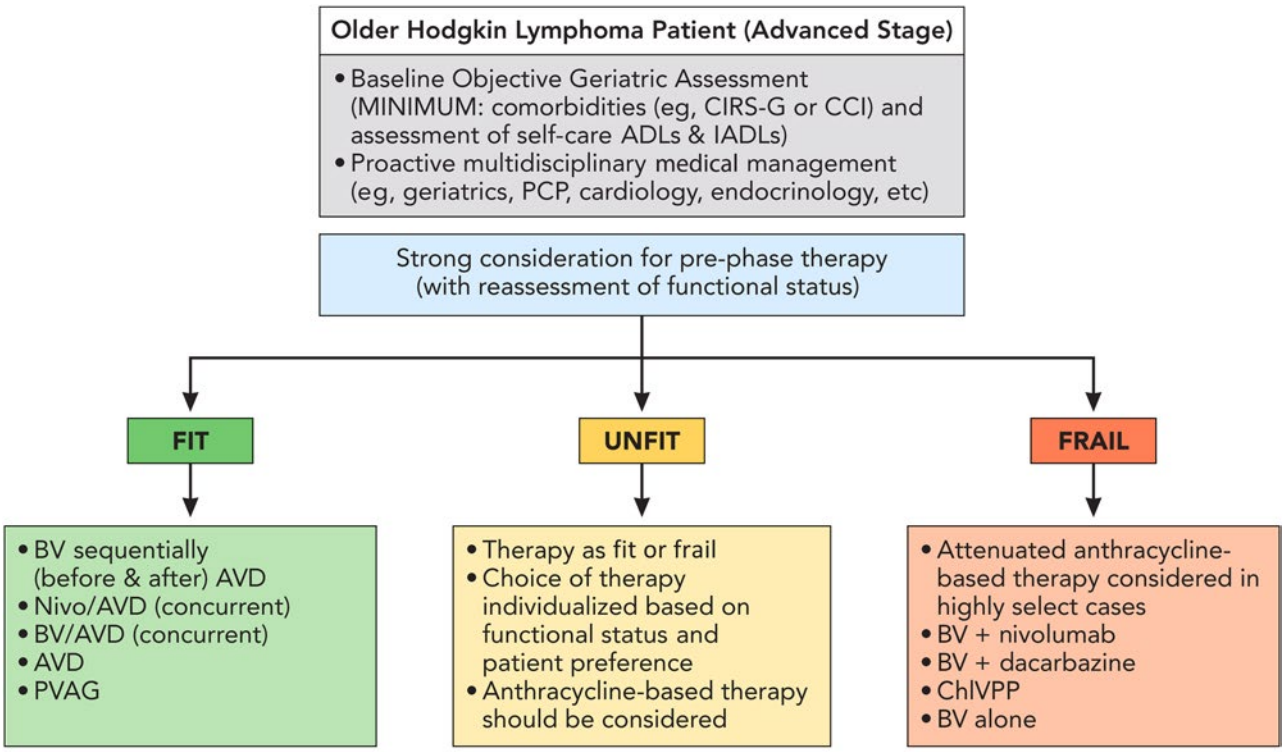
For primary therapy, anthracycline-based chemotherapy platforms are associated with the most robust outcomes for older HL patients, especially fit patients. Optimum therapy for patients objectively classified as unfit or frail are less clear. Unfit HL patients and highly select frail patients may be considered for anthracycline-based therapy, with reduced dosing and/or number of treatment cycles. There remains an unmet need to identify effective and tolerable HL treatment regimens with attenuated anthracycline dosing. In addition, we advocate aggressive supportive care measures for all older HL patients, including weekly office visits for assessments of fluid status and basic blood count and chemistry laboratory studies and concurrent comanagement with other disease specialties (eg, cardiology, endocrinology, primary care, etc). This often proves essential to determining and managing individualized treatment tolerability.

There are several ongoing and planned prospective studies for untreated older HL patients (Table 3). These span all disease stages with studies incorporating BV and/or checkpoint inhibitors into treatment regimens. It is crucial to incorporate *objective* GAs into prospective clinical studies, and similar tools and measures of fitness should be utilized across studies. In addition to establishing consistency of data and more accurate measurement of treatment effect across varying studies, there remains a critical need to identify HL-specific GA models that can aid in therapy decision-making. This includes understanding which older HL populations should receive anthracycline-based combination chemotherapy (full-dose or dose-attenuated), including unfit or highly select frail patients, and who may benefit most from frontline targeted therapeutic approaches (with or without low-intensity chemotherapy).

Therapy for relapsed disease

Prospective studies have not specifically evaluated the treatment of relapsed older HL patients. Therefore, treatment recommendations in this setting are largely based on small subset analyses or retrospective studies. Small single-center studies have suggested that high-dose chemotherapy followed by autologous stem cell support is effective for selected older patients with relapsed HL.[72]

A large GHSG analysis examined 105 older relapsed/refractory HL patients.[73] Different second-line treatment strategies were used, including intensified salvage regimens in 22%, conventional



Criteria	Fit	Unfit	Frail
ADL	6	5	≤4
IADL	8	6-7	≤5
CIRS-G	0 score of 3-4 and <5 of score 2	0 score of 3-4 and 5-8 of score 2	≥1 score of 3-4 and/or >8 of score 2
Age	<80 years	≥80 years FIT	≥80 years UNFIT

Figure 3. Treatment algorithm for newly diagnosed, advanced-stage older Hodgkin lymphoma (HL) patients. All patients should undergo a geriatric assessment to determine fitness before initiation of treatment, which should include at least an evaluation of ADLs, comorbidities, and calculation of noncancer expected survival (https://eprognosis.ucsf.edu/leeschonberg.php). The associated table of geriatric risk categories is adapted from Tucci et al. (with permission).[29] Scoring for ADL and IADL indicate number of *residual functions*. There should also be consideration of pre-phase therapy before initiation of definitive therapy, especially in unfit or frail and/or symptomatic patients with high tumor burden. Furthermore, patient fitness should be reassessed *following pre-phase therapy*. Aggressive supportive care measures should be pursued, including increased office evaluations (eg, weekly fluid assessments) and intentional comanagement with other disease specialists. Treatment options are based on published data and investigator experience (listed by order of preference); a clinical trial should always be considered. Treatment for unfit and frail patients is highly individualized. Anthracyclines may be considered for unfit patients with minor fitness limitations and preserved cardiac function; dose-attenuated anthracyclines may be considered for select fit patients ages ≥80 years or highly select frail patients ages <80 years with close monitoring of cardiac function (eg, comanagement with cardiology with assessment of ejection fraction q 2 cycles, etc). ADL, activities of daily living; AVD, doxorubicin, vinblastine, dacarbazine; BV, brentuximab vedotion; CCI, Charlson Comorbidity Index; ChlVPP, chlorambucil, vinblastine, procarbazine, prednisone; CIRS-G, Cumulative Illness Rating Scale-Geriatric; IADL, instrumental activities of daily living; PCP, primary care provider; PVAG, prednisone, vinblastine, doxorubicin, and gemcitabine. Scoring for ADL and IADL indicates the number of residual functions.

polychemotherapy and/or salvage radiotherapy with curative intent in 42%, and palliative approaches and best supportive care in 31%. A prognostic score applied the risk factors (RFs) of early relapse, clinical stage III/IV, and anemia. The median OS for the entire cohort of relapsing older HL patients was 12 months. Survival varied within different risk groups (ie, ≤1 RF: 3-year OS, 59%; ≥2 RFs: 3-year OS, 9%). In low-risk patients, the impact of therapy on survival was significant in favor of the conventional polychemotherapy approach.[73] There is a continued need to evaluate the safety, efficacy, and optimal timing (ie, sequential or concurrent) of established and experimental therapeutic compounds specifically in older patients with relapsed/refractory HL.

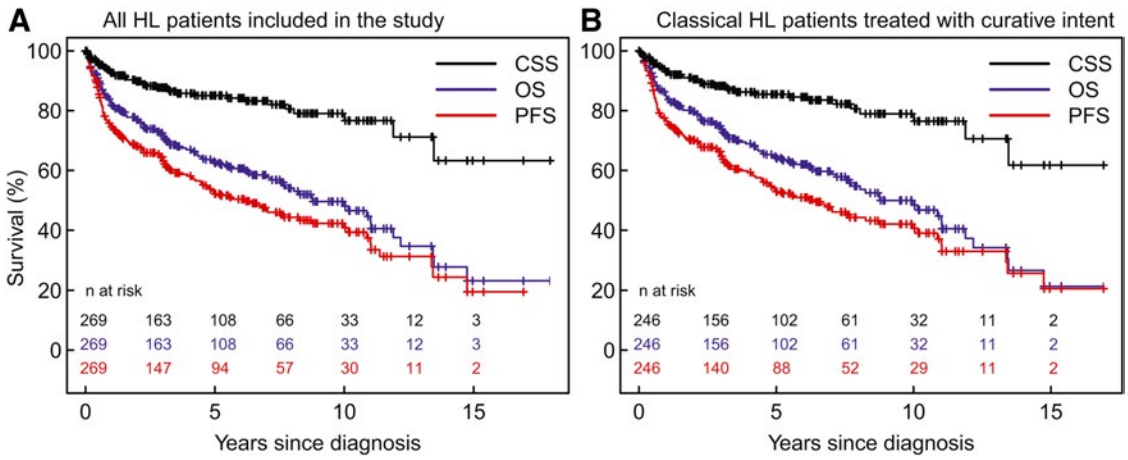


Figure 4. Outcomes analyzing cause-specific survival in a large older Hodgkin lymphoma Swiss cohort. Kaplan-Meier estimate of cause-specific survival (CSS), overall survival (OS), and progression-free survival (PFS) of the entire cohort (A) and of patients treated with curative intent (B). Reprinted with permission.[35]

Therapy-associated toxicity

Conventional chemotherapy in older HL patients may result in reduced tolerability with severe toxicities, including treatment-induced fatalities.[2,3,7,8,35,47,51,74] The most common toxicities for patients treated with ABVD-based therapy are hematologic, neuropathic, infectious, and cardiopulmonary.[6,9,15,20,28,35,39,49,75] Severe hematologic and other toxicities were significantly more frequent in older vs younger HL patients treated on the randomized E2496 study (ABVD vs Stanford V)[13] as well as the recent ECHELON-1 study.[75]

In the Swiss RWE, the 5-year PFS, OS, and cause-specific survival (CSS) rates were 53%, 64%, and 86%, respectively, for older HL patients treated with curative intent (Figure 4).[35] The prominent difference in CSS and PFS highlights the impact that tolerability and toxicity have on outcomes in older patients. Similarly, competing risk analyses within the E2496 study demonstrated that the age-related survival disparity between older and younger patients was due primarily to non-HL-related causes (Supplementary Figure S2).[10]

Providers should remain vigilant with close clinical monitoring and full supportive care measures for all patients, and proactive, multidisciplinary management of coexistent comorbidities (eg, cardiology, endocrinology, primary care, etc) is highly encouraged throughout all phases of therapy.

Treatment-related mortality

Acute toxic deaths in older HL patients are commonly reported in the literature. In the randomized study comparing baseline BEACOPP regimen with COPP-ABVD ($HD9_{elderly}$), the treatment-related mortality (TRM) rates among advanced-stage HL patients aged 66 to 75 years were 21% and 8%, respectively (Table 1).[49] TRM rates across ABVD studies have ranged from 8% to 23%.[4,9,11,13,49,50] Contemporary chemotherapy-based analyses have suggested a lower incidence of TRM (3%-8%) for older HL patients.[15,28,35,39]

Bleomycin lung toxicity

The incidence of BLT in most studies of older HL patients ranges from 5% to 32% with associated mortality rates of 10% to 25%.[13,15,20,28,35,39,44,45,76] The primary risk factor for BLT is age, with an exponential increase in risk with rising age due in part to declining creatinine clearance as bleomycin is primarily metabolized renally.[77] In a Veterans Administration analysis of >800 HL patients, the incidence of BLT by ages ≤49, 50 to 59, 60 to 69, and ≥70 years were 3%, 7%, 13%, and 24%, respectively.[78] Rates of BLT in the Swiss series for HL patients ages 60 to 69 and ≥70 years were 12.6% and 25%, respectively (Table 1).[35]

The incidence of BLT in the Chicago RWE series was 32%, with an associated mortality rate of 25%.[20] The incidence was 38% vs 0% among patients who received colony-stimulating factor (G-CSF) vs not, respectively ($P<.0001$), which has been noted in other series.[78] The incidence of BLT among older HL patients treated on E2496 was 24% with an associated death rate of 18%[13]; the vast majority of cases occurred with ABVD. Data supporting the number of doses of bleomycin as a risk factor comes in part from GHSG HL data in older adults showing that BLT was uncommon in early-stage patients who received 2 cycles of ABVD but occurred in 10% who received 4 ABVD cycles, including several lethal events.[44] However, recently reported data from BC RWE identified an overall BLT incidence of 21% (TRM 14%), and 38% of cases occurred during the first 2 cycles of treatment.[15]

Neuropathy

In contemporary studies, neurotoxicity has been more closely examined, especially studies incorporating BV (Table 2). In the prospective study of extended dosing of single-agent BV followed by cohorts combining either bendamustine or dacarbazine for older HL patients as described before,[65,66] the incidence rates of grade 3 neuropathy were high (Table 2). In the above-mentioned study utilizing sequential and more limited dosing of BV with AVD, the risk of grade 3 neuropathy was 4%.[19] Importantly, clinically significant neurotoxicity is also seen in patients who receive ABVD as was seen in the ECHELON-1 study with grade 2 and 3 peripheral neuropathy rates of 13% and 3%, respectively.[75] Continual surveillance and repeated examination of patients with individualized dose reductions are important to mitigate anti-tubulin-related neurotoxicity.

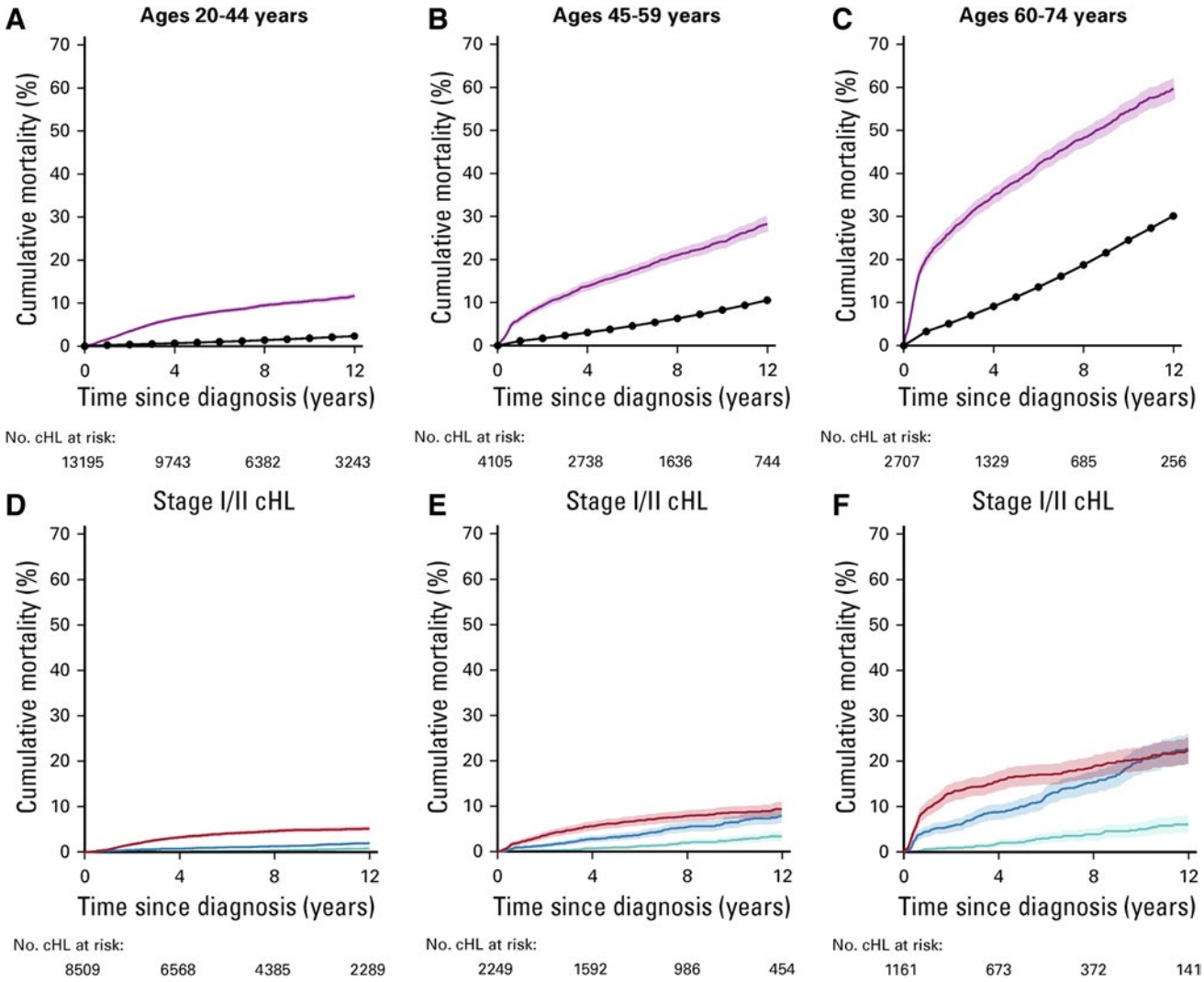


Figure 5. Cumulative mortality among a simulated general US population and 20 007 individuals diagnosed with cHL at ages 20–74 years and treated with initial chemotherapy. 17 SEER cancer registry areas, 2000–2015 (followed through 2016). (A-C) Cumulative mortality from all causes in the general population and classical Hodgkin lymphoma (cHL) population according to age group. (D-F) Cumulative mortality from lymphomas, noncancers, and other neoplasms among patients diagnosed with stage I/II cHL according to age group. (G-I) Cumulative mortality from lymphomas, noncancers, and other neoplasms among patients diagnosed with stage III/IV cHL according to age group. Shaded areas (and error bars) represent the upper and lower bounds of the 95% CI for cumulative mortality. Reprinted with permission.[96]

Cardiac

Older patients with preexisting structural heart disease or multiple cardiac risk factors have an elevated risk of heart failure (HF) with anthracyclines.[79] Among patients with preexisting HF or cardiomyopathy, HL-related mortality was fourfold higher than cardiovascular mortality (37% vs 8%, respectively) in a SEER-Medicare analysis, with 1-year all-cause mortality >60%.[80] This high HL-related mortality was likely in part due to the lower use of anthracyclines amongst those with preexisting HF or cardiomyopathy.

In patients with established HF or cardiomyopathy, optimization of HF guideline-directed medical therapy[81] and consideration of infusional cardioprotective strategies such as dexrazoxane, liposomal doxorubicin, or continuous infusion doxorubicin may be considered to allow patients with well-compensated HF or cardiomyopathy to receive anthracycline-containing regimens. In randomized trials in adults with solid tumors or children with hematologic malignancies, dexrazoxane before doxorubicin administration,[82-85] the substitution of doxorubicin with liposomal doxorubicin,[86,87] or continuous infusion of doxorubicin[88] were associated with a decrease in clinical HF events with preserved oncologic efficacy. However, the safety and efficacy data of these strategies in older adults with HL are limited.[58,89] Close collaboration with cardiology and shared decision-making with the patient, oncology, and cardiology are necessary.

In addition to infusional strategies, cardiovascular medications should be optimized to improve cardiovascular outcomes. There are 4 foundational medications recommended for the treatment of HF with reduced ejection fraction: (1) beta-blockers; (2) the angiotensin receptor–neprilysin inhibitor, sacubitril-valsartan;

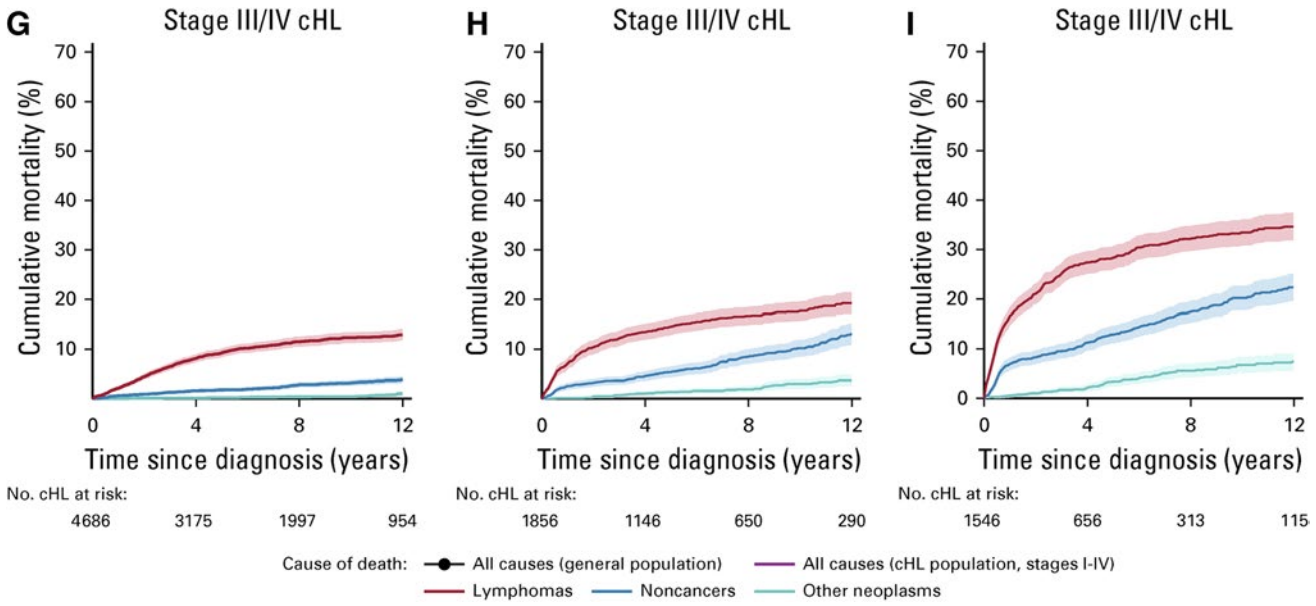


Figure 5. Continued

(3) aldosterone antagonists; and (4) sodium-glucose cotransporter 2 inhibitors. These are associated with improved survival, functional capacity, and left ventricular ejection fraction.[81] Neurohormonal antagonist therapy with beta-blockers and/or angiotensin II receptor blockers /angiotensin-converting enzyme inhibitors may be cardioprotective in patients receiving anthracyclines and should be used in all patients with reduced left ventricular ejection fraction (LVEF). They can also be considered for cardioprotection in patients with a normal baseline echocardiogram but with cardiac risk factors, especially hypertension.[90] Atorvastatin reduced the incidence of LVEF declines in a randomized trial of patients with lymphoma receiving anthracycline-based chemotherapy,[91] although another study in patients with breast cancer and lymphoma did not demonstrate a benefit in LVEF at 2 years.[92]

Guidelines from the European Society of Cardiology (ESC), American Society of Clinical Oncology, and the National Comprehensive Cancer Network endorse the optimization of prevalent cardiovascular disease and cardiac risk factors, use of screening echocardiograms in patients at high risk for HF with anthracycline after therapy, and referral to cardiology or cardio-oncology in patients with cardiovascular symptoms, abnormal cardiac testing, or inadequately managed cardiac risk factors.[81,90,93-95] According to the 2022 ESC risk stratification schema, patients receiving anthracycline-chemotherapy are at "very high risk" in the setting of preexisting HF or cardiomyopathy and "high risk" with any of the following high-risk factors: valvular heart disease, coronary artery disease, angina, LVEF <50%, age >80 years, prior anthracycline or chest radiation exposure, or >5 moderate risk factors (eg, age 65 to 79 years, LVEF 50%-54%, hypertension and diabetes).[90]

Survivorship

Survivorship in the "postacute" period 1 to 10 years post therapy is clinically relevant for older individuals. A large SEER analysis of 20,007 HL survivors diagnosed between ages 20 and 74 years treated with initial chemotherapy in US population-based cancer registries during 2000 to 2015 was reported.[96] With a mean follow-up of 8 years, all-cause mortality exceeded the general population, with noncancer cumulative mortality comprising a substantial number of total deaths, especially those ages 60 to 74 years at diagnosis (Figure 5). There were strikingly elevated risks among HL survivors in the 60-to-74-year group, with excess deaths as a result of heart disease (excess absolute risk [EAR] stage III/IV, 59.6), interstitial lung disease (EAR 36.9), infections (EAR 31.3), adverse events (EAR 33.0), and solid tumors (EAR 24.6). Notably, excess non-HL mortality started within 1 year of completion of therapy compared with the general population. Similar findings of increased noncancer mortality were documented in another SEER analysis that included competing risks.[97] In a separate SEER-Medicare analysis of older patients treated with anthracycline-based chemotherapy who were free from HF at the time of HL diagnosis, the cumulative incidence of HF was 15% at 1 year and 25% at 4 years.[98] Older age, cardiac risk factors such as diabetes and hypertension, intrinsic heart disease, and vascular disease are associated with increased risk of incident HF.[79,98]

For cardiac disease, the American Society of Clinical Oncology and NCCN survivorship guidelines suggest a screening echocardiogram 6 to 12 months after completing anthracycline therapy in those at elevated risk for anthracycline cardiotoxicity.[94,95] The ESC guidelines recommend a screening echocardiogram 1 year after completing anthracycline therapy in all anthracycline-treated patients and more frequent screening in patients at high or very high risk for heart failure (echocardiograms after 2 cycles, 3 months, and 1, 3, and 5 years after therapy completion).[90] Cardiac risk factors such as hypertension, hyperlipidemia, and diabetes should be aggressively managed according to standard guidelines.[90,94,99]

CLINICAL CASE (continued)

The patient received pulse steroids 100 mg po daily for pre-phase therapy and had rapid improvement in his B-symptoms, bone pain, and performance status. He was subsequently treated with

sequential brentuximab vedotin (BV) for 2 cycles. He had grade 2 diarrhea after the second cycle of BV that was treated with supportive care measures. Therapy was otherwise tolerated well; he achieved a partial remission by CT imaging, and he proceeded with full-dose AVD chemotherapy.

After cycle 2 of AVD, the patient had grade 1 sensory neuropathy that evolved to grade 2 after cycle 3 (eg, difficulty using the phone). Vinblastine was decreased by 1 dose level and the neuropathy improved to grade 1. He achieved metabolic complete remission after cycle 3 AVD. The patient had increasing fatigue and asthenia after the fifth AVD cycle, and chemotherapy was stopped. He proceeded with 4 sequential, attenuated dosed BV cycles and remained disease-free for 2+ years.

However, 28 months post diagnosis, he had dyspnea on exertion, orthopnea, and lower extremity edema. An echocardiogram showed a mildly dilated left ventricle with left ventricular ejection fraction of 35%. He was referred to cardiology and started on sacubitril-valsartan, carvedilol, spironolactone, and dapagliflozin, which resolved his heart failure symptoms. A coronary angiogram showed the prior stent was patent with nonobstructive coronary artery disease, and statin and aspirin were continued. He completed cardiac rehabilitation. A repeat echocardiogram 4 months later showed normal left ventricular size and improvement in left ventricular ejection fraction to 55%.

Conclusions

Outcomes have improved in the modern era for older HL patients, in part due to the integration of targeted agents. Additionally, the importance of dose intensity and the inclusion of anthracycline therapy strongly correlates with optimized survival in the contemporary era. Clinical factors that drive prognosis include advancing age and GAs, the latter of which should be objectively measured in all studies at baseline and include assessment of ADLs and comorbidities. However, more research is needed to delineate which GAs are most relevant and prognostic for older HL patients.

Collectively, treatment for fit older HL patients should be given with curative intent that includes anthracycline for most patients, and bleomycin should be minimized (ie, maximum 2 cycles) or avoided altogether, especially in patients ages ≥70 to 75 years. Proactive multidisciplinary management of comorbidities is strongly recommended, and the use of pre-phase treatment should be considered for most patients. Therapy for early-stage disease should follow similar treatment paradigms to younger patients using more limited cycles of ABVD (or AVD) therapy followed by IFRT. In advanced-stage disease, BV given sequentially before and after AVD chemotherapy for untreated older HL patients is highly effective and well tolerated, and we eagerly await emerging data incorporating checkpoint inhibitors into frontline chemotherapy platforms.

Therapy for patients who are unfit or frail, whether due to comorbidities or ADL loss, is less clear and should be individualized with consideration of attenuated anthracycline-based therapy vs lower-intensity treatment with inclusion of BV +/− checkpoint inhibitor therapy. For all patients, there should be continual vigilance with close clinical monitoring of treatment-related toxicities, with attention to dehydration, neurotoxicity, cardiopulmonary, and infections.

In addition, all-cause mortality is significantly elevated in older HL individuals during the postacute period 1 to 10 years after treatment, which includes a prominent fraction of non-HL causes (especially cardiac). Older patients should be evaluated in survivorship clinics and referred to clinically pertinent disease specialists for optimum management of comorbidities, with attention to excess secondary cancers and cardiac, pulmonary, and infectious complications. Finally, more research is needed to delineate HL-specific GA-based clinical prognostic models that can aid in treatment decisions, including fit and unfit patients who should receive anthracycline-based combination chemotherapy vs patient populations who may benefit most from targeted therapeutic approaches, with or without low-intensity chemotherapy.

Conflict-of-interest disclosure

Andrew M. Evens: advisory board or educational forum (with honorarium): Bayer, Seattle Genetics, Affimed, Verastem, Pharmacyclics, Research to Practice, and Physician Education Resource; research support: Takeda, Seattle Genetics, Merck, NIH/NCI, Leukemia and Lymphoma Society, and ORIEN.
Marshall McKenna: no competing financial interests to declare.

Yun Kyoung Ryu Tiger: no competing financial interests to declare.

Jenica N. Upshaw: no competing financial interests to declare.

Off-label drug use

Andrew M. Evens: frontline use of checkpoint inhibitors.
Marshall McKenna: frontline use of checkpoint inhibitors.
Yun Kyoung Ryu Tiger: frontline use of checkpoint inhibitors.
Jenica N. Upshaw: frontline use of checkpoint inhibitors.

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Management of limited-stage Hodgkin lymphoma

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Hodgkin lymphoma (HL) is a rare type of B-cell malignancy with bimodal age distribution targeting young adults and elderly. Prognostic models are available to identify risk of recurrence and response to treatment. Currently, positron emission tomography scanning is most useful in optimizing therapy. Outcomes are generally excellent with standard chemotherapy or combined modality therapy. Balancing efficacy and the risk of late effects in Hodgkin lymphoma is essential, including early detection of potential complications. Incorporation of novel therapies such as brentuximab vedotin and checkpoint inhibitors are being explored in the frontline setting, having already demonstrated improved survival and tolerable toxicity in advanced HL. Furthermore, the addition of these agents have the potential to transform treatment paradigms for early-stage HL and may result in improved outcomes with decreased risks of late toxicities that continue to afflict long-term survivors. However, the patient population, sequencing, and combinations with cytotoxic chemotherapy all remain still standing questions as results of current and upcoming randomized trials are awaited. In this article, we discuss the current data on the approach to initial treatment of early-stage classical HL, review toxicity profiles, and examine upcoming novel therapy trials.

LEARNING OBJECTIVES

- Review the impact of prognostic scoring in early-stage Hodgkin lymphoma
- Examine the role of chemotherapy alone and combined with radiotherapy; explore novel agent combinations in early-stage Hodgkin lymphoma
- Discuss late effects stratified by treatment modality and early detection strategies in survivorship

Introduction

Hodgkin lymphoma (HL) is a rare malignancy that has a bimodal distribution with increased incidence in young adults as well as in patients aged 55 and older.[1,2] Its unique biology includes an inflamed microenvironment, dysfunctional immune response, and relatively low presence of malignant cells (pathognomonic Reed-Sternberg cells).[3-6] Clinically, disease often presents as supradiaphragmatic lymphadenopathy that spreads contiguously and is occasionally bulky; extranodal involvement at initial presentation is unusual. Patients may be asymptomatic, develop symptoms related to mass effect on surrounding tissue or constitutional symptoms like night sweats, fever, and weight loss (which are referred to as "B symptoms"), and play a role in risk stratification.[7]

While the treatment of early-stage HL continues to evolve, current approaches are based on multiple large, randomized controlled studies that differ in the designation of pretreatment risk factors, eligibility criteria, and the definition of response based on interim positron emission tomography (PET). In the current era, well more than 70% of advanced-stage patients are cured while advancements in therapy have increased cure rates above 90% for early-stage HL.[2] Unfortunately, many survivors continue to experience late effects of radiation and chemotherapy, including second primary malignancies, cardiovascular disease, and endocrine dysfunction, despite reductions and advancements in therapy. Consequently, modern treatment algorithms need to weigh the competing risks of excellent therapeutic efficacy in addition to decreasing late toxicity.

We review the management of newly diagnosed limited/early-stage HL, including the role of combined modality treatment (CMT), chemotherapy, and radiation therapy (RT), and discuss data incorporating brentuximab vedotin and/or checkpoint inhibitors.[8-10] We do not discuss the rare subtype of nodular lymphocyte predominant HL.

CLINICAL CASE

A 21-year-old previously healthy woman presented with progressive shortness of breath over weeks with intermittent chest pressure and a new palpable lump on the left side of the neck. Excisional biopsy of the neck node reveals classical HL, nodular sclerosing subtype. On PET/CT imaging, a 8.5 cm mediastinal mass is seen with standardized uptake value (SUV) of 12, in addition to a left posterior cervical node of 2.1×1 cm with SUV of 7.3 and a right supraclavicular lymph node measuring 1.2 cm SUV 6.7. No intra-abdominal or splenic uptake was noted. Her laboratory testing demonstrated a white blood cell count of 8000/microliter, hemoglobin of 11.9 g/dL, platelet count of 371 000, and an elevated erythrocyte sedimentation rate of 60 mm/h. She therefore has nonbulky stage IIA classic HL.

Risk stratification in early-stage HL

The current management approach in early HL is risk adaptive by using several known prognostic factors.[11-13] The most important step is accurate disease staging, and HL follows the Ann Arbor staging system for lymphoma that divides patients into four stages depending on where malignant nodes lie in relation to the diaphragm and if extranodal organs are involved.[14,15] Recently, data have demonstrated that bone marrow biopsies may be omitted if PET imaging is available.[16] Most guidelines approach stage I and II in the same manner, and they are referred to as limited or early-stage disease and further split into two groups: favorable and unfavorable, based on age, presence of bulky lesions (usually defined as 10 cm or more in size), presence of B symptoms, erythrocyte sedimentation rate (ESR), and number of nodal areas involved (Table 1). Different cooperative groups have used varying combinations of these risk factors, which makes comparisons across studies challenging. Nevertheless, the most commonly used prognostic scores are the European Organization of Research and Therapy in Cancer (EORTC/LYSA) score, the German Hodgkin Study Group (GHSG) score, and the National Comprehensive Cancer Network (NCCN) score (Table 1). Generally speaking, ESR >50, presence of B symptoms, and ≥3 involved nodal areas are considered high-risk features in these scoring systems. It is important to highlight that these prognostic scores have limitations, such as their arbitrary delineations developed in older HL studies, and they are rarely used in current studies to stratify patients. Furthermore, while more modern prediction models have been validated in advanced HL, such as the advanced-stage Hodgkin lymphoma International Prognostic Index, no such model is available for early-stage HL.[17] In the modern era, interim and end-of-treatment PET results carry the highest level of prognostic yield, with the caveat that PET-based adaptive approaches were developed before targeted drugs were incorporated into frontline therapy.[18-22] Studies are needed to assess if PET- based adaptive methodologies are still valid prognostication tools for patients who receive chemoimmunotherapy.

Role of chemotherapy vs combined modality treatment

The general treatment approach in early-stage disease has historically been 2-4 cycles of ABVD (doxorubicin, bleomycin, vinblastine, and dacarbazine) followed by consolidative involved-field radiotherapy (IFRT) ideally using a PET-based risk-adapted strategy. The actual number of cycles and even the dose of IFRT depend on whether the disease is favorable or not and the results of interim PET. The role of radiation after chemotherapy continues to prompt debate in the field, and many trials have attempted to explore this issue. It appears that omitting radiation may slightly increase recurrence risk but without clear detriment in overall survival. Many oncologists prefer chemotherapy alone approaches to avoid distant toxicity from radiation, such as an increased risk of second primary malignancies and cardiovascular disease; however, it is important to highlight that current radiation modalities are safer, which may partially mitigate risk of toxicity.[23,24]

Treatment of favorable vs unfavorable disease

Patients with favorable early-stage HL have an excellent prognosis and benefit from a shorter course of chemotherapy when combined with radiation. Several large prospective studies using PET-adapted approaches have evaluated the number of cycles of therapy and the role of RT, including UK RAPID, EORTC H10, and GHSG HD16 in Europe and the US-led CALGB 50604.[19,25-27] Of note, European studies considered a Deauville score (DS) of 1-2 as negative, while CALGB considers a score of 3 as negative as well, which is in line with most clinical practice. Table 2 contains selected clinical trials in early-stage HL of both PET-adapted and non-adapted approaches.

The EORTC H10 study is the largest PET-adapted study in early-stage HL to date and enrolled 1950 patients with either

Table 1. Unfavorable risk factors in early-stage HL per cooperative groups

GHSG	EORTC/LYSA	NCCN
Bulky mediastinal mass MMR >0.33	Bulky mediastinal mass MTR >0.35	Bulky mediastinal mass MMR >0.33
Extranodal site	Presence of B symptoms	Adenopathy 10 cm in size or more
Nodal sites >2	Nodal sites >3	Nodal sites >3
ESR >50 (or >30 if B symptoms)	ESR >50 (or >30 if B symptoms)	ESR ≥50 or any B symptoms
	Age >50	

EORTC, European Organization for Research and Treatment of Cancer; ESR, erythrocyte sedimentation rate; GHSG, German Hodgkin Study Group; LYSA, Lymphoma Study Association; MMR, mediastinal mass ratio, maximum width of mass/maximum intrathoracic diameter; MTR, mediastinal thoracic ratio, maximum width of mediastinal mass/intrathoracic diameter at T5–6; NCCN, National Comprehensive Cancer Network.

Table 2. Selected therapeutic trials in early-stage HL

Trial	Trial design	PET negative definition	Disease stage/ characteristics	N	Median follow-up	PFS	OS
RAPID[25]	ABVD×3 – > PET neg: no further treatment or 30 Gy IFRT PET pos: ABVD×1 plus 30 Gy IFRT	DS 1–2	Stage IA or IIA Nonbulky	602	5 yrs	3-yr 90.8% 3-yr 94.6% 3-yr 83%	3-yr 99.0% 3-yr 97.1% 3-yr 87.6%
EORTC H10[19]	Favorable ABVD×2- > ABVD×1 plus INRT Or ABVD×2 – > PET PET neg: ABVD×2 PET pos: escBEACOPP×2 plus INRT Unfavorable ABVD×2- > ABVD×2 plus INRT Or ABVD×2- > PET PET neg: ABVD×4 PET pos: escBEACOPP×2 plus INRT	DS 1–2	Stage I or II Favorable or unfavorable	1950	4.5 yrs	(F) PET neg control = 5-yr 99% (F) PET neg trial = 5-yr 87.1% (U) PET neg control = 5-yr 92% (U) PET neg trial = 5-yr 89.6% (F/U) PET pos control = 5-yr 77.4% (F/U) PET pos trial = 5-yr 90.6%	5-yr 100% 5-yr 99.6% 5-yr 96.7% 5-yr 98.3% 5-yr 89.3% 5-yr 96.0%
CALGB 50604[27]	ABVD×2 – > PET PET neg: ABVD×2 PET pos: escBEACOPP×2 plus IFRT	DS 1–3	Stage I or II Nonbulky	164	3.8 yrs	3-yr 91% 3-yr 66%	
GHSG HD16[26]	CMT arm: ABVD×2 plus 20 Gy IFRT ABVD×2 – > PET neg: no further treatment PET pos: 20 Gy IFRT	DS 1–2	Stage I or II – Favorable Nonbulky	1150	3.8 yrs	5-yr 93.4% 5-yr 86.1% 5-yr 88.4%	5-yr 98.1% 5-yr 98.4% 5-yr 97.9%
GHSG HD17[28]	CMT arm: escBEACOPP/ABVD×4 plus 30 Gy IFRT PET-4 neg: no further treatment PET-4 pos: 30 Gy IFRT	DS 1–2	Stage I or II – Unfavorable Bulky	1100	3.9 yrs	5-yr 97.7% 5-yr 95.9% 5-yr 94%	5-yr 98.7% 5-yr 98.8% 5-yr 99.2%
CALGB 50801[39]	ABVD×2 – > PET PET neg: ABVD×4 PET pos: escBEACOPP×4 plus 30 Gy ISRT	DS 1–3	Stage IA-IIB Bulky only	94	5.5 yrs	3-yr 89.7% 3-yr 92%	3-yr 94.4% 3-yr 97.7%
RATHL[29]	ABVD×2 – > PET PET neg: ABVD×4 or AVD×4 PET pos: BEACOPP×4	DS 1–3	Stage IIB-IV or IIA with adverse features	1203	3.4 yrs	3-yr 85.7%, ABVD 3-yr 84.4%, ABVD- > AVD 3-yr 67.5%	3-yr 97.2% 3-yr 97.6% 3-yr 87.8%
HD10[40]	ABVD×4 plus 30 Gy IFRT ABVD×4 plus 20 Gy IFRT ABVD×2 plus 30 Gy IFRT ABVD×2 plus 20 Gy IFRT	Not PET adapted	Stage I or II Favorable	1370	7.5 yrs	8-yr 88.4% 8-yr 90.0% 8-yr 85.4% 8-yr 86.5%	8-yr 94.4% 8-yr 94.7% 8-yr 93.6% 8-yr 95.1%

ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone; CMT, combined modality treatment; DS, Deauville score; escBEACOPP, escalated BEACOPP; F, favorable; IFRT, involved-field radiotherapy; INRT, involved nodal radiotherapy; ISRT, involved site radiotherapy; neg, negative; OS, overall survival; PFS, progression-free survival; pos, positive; U, unfavorable; – >, followed by.

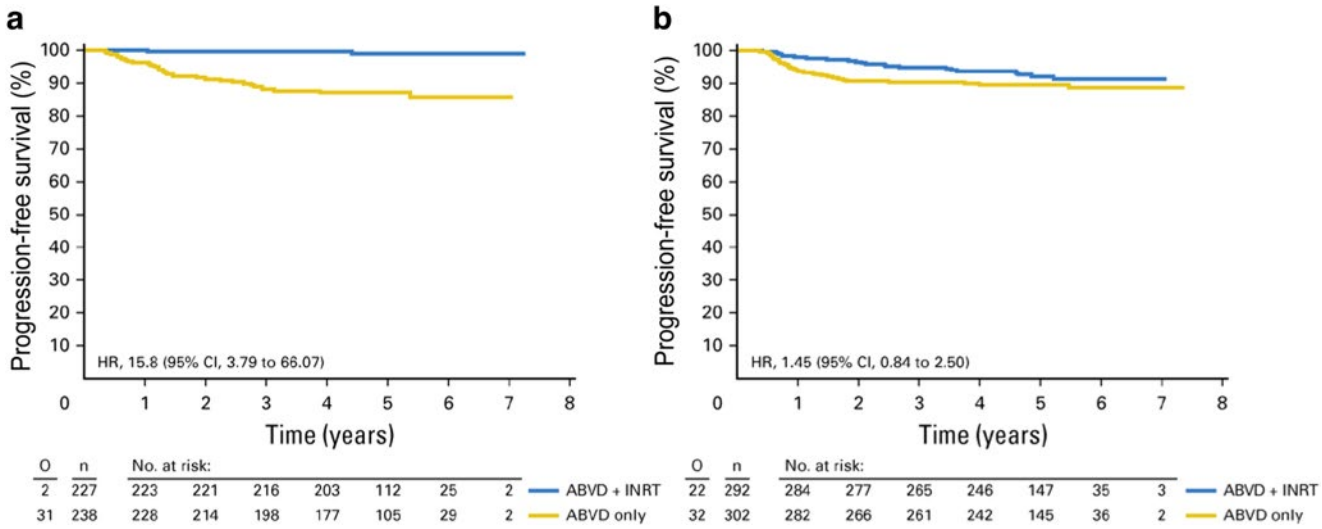

Figure 1. Progression-free survival of 1059 early PET-negative patients who were treated according to the initial protocol. Shown are the rates of progression-free survival of the (a) favorable (F) groups of patients randomly assigned to doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) plus involved-node radiotherapy (INRT; *n* = 227) or ABVD only (*n* = 238) and of the (b) unfavorable (U) groups randomly assigned to ABVD plus INRT (*n* = 292) or ABVD only (*n* = 302). HR = hazard ratio; O = observed; *n* = number of patients. Previously published in: André MPE, Girinsky T, Federico M, et al. Early positron emission tomography response-adapted treatment in stage I and II Hodgkin lymphoma: final results of the randomized EORTC/LYSA/FIL H10 trial. *J Clin Oncol.* 2017;35(16):1786–1794.[19] doi: 10.1200/JCO.2016.68.6394. Reproduced with permission from the American Society of Clinical Oncology.

stage I or II (754 had favorable, and 1196 had unfavorable disease). In the control arms, treatment consisted of 3 (favorable) or 4 (unfavorable) ABVD cycles and involved nodal radiotherapy (INRT), regardless of PET results. Patients in the experimental arm received 2 cycles of ABVD followed by PET imaging, and subsequently, if PET was negative (DS 1-2), they received either 2 cycles of ABVD for favorable or 4 cycles if unfavorable. Patients with positive PET received a more intensified BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone) regimen in addition to 30 Gray (Gy) INRT. In the favorable disease group with negative interim PET, 5-year progression-free survival (PFS) was 87% with ABVD×4 vs 99% with ABVD×3 plus 30 Gy INRT while 5-year overall survival (OS) was 99.6% and 100%, respectively.[19] Of note, the EORTC H10 study was closed prematurely after exceeding the boundary limit of 10% relapse or progression in the non-RT arm. (Figure 1)

The RAPID and GHSG HD16 studies demonstrated CMT improved PFS compared to chemotherapy alone, but the PFS benefit was small without corresponding improvement in OS and thus might not be clinically significant when factoring in late toxicities from RT. In the CALGB study, 164 patients were enrolled and received ABVD×2 cycles followed by PET imaging. If PET was negative, patients received 2 additional cycles of ABVD without radiation; for PET-positive disease, therapy was changed to BEACOPP followed by 30 Gy consolidative RT. In this study, 91% of patients had a negative interim PET and received 2 additional cycles of AVBD resulting in a 3-year PFS of 91%. The 3-year PFS in interim PET-positive group after escalation of therapy was 66%.[27]

Patients with one or more of the risk factors cited above are considered to have unfavorable disease and were treated with a multimodality approach with CMT. Generally speaking, 4 cycles of ABVD followed by 30 Gy of RT is recommended for these patients based on results of the H10 study, where 1196 out of 1950 patients had unfavorable disease. It also demonstrated those achieving negative PET after 2 cycles of ABVD had a 5-year PFS of 89.6% with ABVD×6 cycles vs 92% with ABVD×4 cycles plus 30 Gy consolidative RT, while 5-year OS was 98.3% and 96.7%, respectively. Other studies have attempted to omit RT. For example, in the GHSG HD17 phase 3 study, 1100 patients with unfavorable stage I/II HL were randomized to either 2 cycles of escalated BEACOPP in addition to 2 cycles of ABVD (2+2) followed by 30 Gy consolidative RT (standard CMT arm) or the RT was omitted if patients achieved a negative PET (DS 1-2) after 4 cycles of chemotherapy (experimental arm). Five-year PFS was 97.3% in the standard CMT arm vs 95.1% in the chemotherapy-only arm, representing a 2.2% difference that excluded their noninferiority margin of 8%.[28] Another strategy is the PET-adapted approach following the response-adjusted therapy for advanced Hodgkin lymphoma (RATHL) study, in which 40% of patients in both ABVD and AVD arms had stage II disease. The omission of bleomycin if interim PET-CT was negative (DS 1-3) after 2 cycles of ABVD did not affect outcomes, with a 3-year and a 7-year PFS in the AVD group of 84.4% and 79.2%, respectively.[29,30] While the RAPID, EORTC H10, and GHSG HD16 trials all failed to show noninferiority in PFS with PET-adapted omission of RT compared with CMT, particularly in favorable patients, there was no OS benefit with inclusion of radiation. Alternatively, CALGB 50604 demonstrated an inferior PFS of 77% in patients treated with chemotherapy alone with a DS 3 on interim PET.

The outcomes of patients with poor response on interim PET (DS 4 or 5) are significantly worse, which represents an unmet need, and studies have explored intensification of therapy to improve response rates, such as changing ABVD to BEACOPP and adding RT as in the CALGB study. In the H10 study, patients with

positive interim PET either received ABVD×4 and 30 Gy INRT, and their 5-year PFS and OS were 77.4% and 89.3%, respectively or for patients who received ABVD×2 followed by BEACOPP×2 and 30 Gy INRT, 5-year PFS and OS were 90.6% and 96%, respectively.

The use of RT should be discussed with the patient and a multidisciplinary team highlighting the potential late effects specific to that particular patient and their inherent risk factors (i.e., a different discussion may take place for a young woman with stage IIB anatomically central disease as compared with a singular node in the periphery). While there is no OS benefit observed across these studies for patients with negative interim PET scans, consideration of toxicity and patient preference should guide treatment decisions. Particularly in early-stage HL, it is crucial to include nuclear medicine colleagues in the discussion because many trials used DS 1-2 as their PET-negative arms in contrast to clinical practice, where DS 1-3 is considered negative. Within the confines of the various trials and specific eligibility, for early-stage disease we recommend CMT with the above caveats. In particularly favorable disease, clinicians could consider the HD10 approach of ABVD×2 plus 20 Gy IFRT, although given the ubiquitous use of PET2 imaging, we generally use PET results when presenting patients with therapy choices. In our practice, we do not escalate to BEACOPP in patients with a positive interim PET scan because of the potential risks of infertility and second primary malignancies with the regimen; instead, we repeat the biopsy to confirm disease and proceed to salvage chemoimmunotherapy followed by consolidative autologous stem cell transplant in chemosensitive disease, particularly given the impressive response rates with modern salvage regimens.[31-33]

Bulky disease

While patients with bulky mediastinal masses are generally treated with CMT, there are more studies that suggest that radiation can be eliminated in PET-negative patients without compromising outcomes. Older studies demonstrated that 4 cycles of combination chemotherapy with IFRT have improved local control.[34-36] A large Canadian study omitted RT in patients with early-stage bulky HL who were PET negative (DS 1-3); 84% achieved PET negativity and did not receive radiation. In PET-negative patients with bulk ($n = 112$), 5-year freedom from treatment failure was 89% compared with 88.5% for PET-negative nonbulky disease ($n = 152$).[37] CALGB 50801 treated 101 bulky stage I to II HL patients with 2 cycles of ABVD, and those with PET-negative (DS 1-3) disease received 4 additional cycles of ABVD and no radiation, while PET-positive patients received 4 cycles of escalated BEACOPP and 30 Gy involved-site RT (ISRT). In this study, 78% were PET negative with a 3-year PFS of 93.1% compared with 89.7% for PET-positive patients, and the majority of patients were spared of radiation exposure.[38] Additionally, the RATHL study included 500 patients with bulky or high-risk stage II HL and demonstrated a 90.9% PFS in these patients if PET was negative after 6 cycles of ABVD.[29] In practice, we generally prefer CMT for bulky disease based on the data available; however, it is also reasonable to omit radiation in patients with an interim negative PET scan after a multidisciplinary team meeting with radiation oncology colleagues for a discussion of risk vs benefit.

Older patients

Older patients, commonly defined as ≥60 years of age, historically have lower survival rates compared with younger patients, which may be due to a different disease biology, including increased incidence of mixed cellularity histology, Epstein-Barr virus–related, and advanced-stage disease.[41] Additionally older patients are more likely to be unable to tolerate chemotherapy at full dose and schedule and have increased treatment-related toxicity (including bleomycin pulmonary toxicity) and mortality.[42] Novel agents have been studied in older patients with early-stage disease, and given the toxicity profile and improvement in outcomes, our practice is generally to treat with sequential Bv-AVD in robust patients whereas we use Bv-AD, Bv-DTIC, immunotherapy in combination, or single agent in more frail patients.[43-45] (Table 3)

The role of brentuximab vedotin and checkpoint inhibitors

Brentuximab vedotin (Bv) and checkpoint inhibitors (CPI) have revolutionized the treatment of relapsed HL, and they are increasingly incorporated in the frontline trials.[46] Replacing bleomycin with Bv (CD30-directed monoclonal antibody) demonstrated improved PFS and OS in patients with advanced HL in the ECHELON-1 study and is considered the standard for advanced-stage HL.[47,48] Moreover, results of a recent randomized phase 3 study combining nivolumab with AVD revealed improvement in PFS compared to Bv-AVD on interim analysis and is poised to become the new standard.[49]

Investigators have incorporated these agents in the treatment of early-stage HL with encouraging results (Table 3).[50] Allen and colleagues conducted a single-arm phase 2 study with sequential administration of 3 cycles of pembrolizumab followed by AVD including unfavorable stage II and advanced-stage HL. The overall response rate after single agent pembrolizumab was 67% in early-stage disease, including 42% complete metabolic response but an impressive complete metabolic response (CMR) of 100% after AVD.[51] GHSG conducted a randomized phase 2 study of nivolumab with AVD given either concomitantly or sequentially followed by consolidative RT with a dose of 30 Gy in early-stage unfavorable HL with excellent outcomes; PFS was 100% in the concomitant group and 98% in the sequential group.

Kumar et al published a multicenter phase 2 study with unfavorable stage I/II HL in which 117 patients received 4 cycles of Bv-AVD and, if PET negative, were assigned to one of four cohorts with different doses of consolidative RT, except cohort 4, which excluded RT. Two-year PFS ranged from 90% to 97%, and the addition of RT did not lead to further benefit.[52] European investigators conducted a phase 2 study wherein patients with unfavorable early-stage HL were randomized to either Bv-AVD×4 or standard ABVD×4 and 30 Gy consolidative RT. The primary endpoint was the rate of negative PET after 2 cycles that was met (82% in Bv-AVD group vs 75% in ABVD group), while 2-year PFS was 97% with Bv-AVD and 93% with standard therapy.[53] Collectively, these studies, though small with short follow-up, are very promising, although not without their own toxicity profile. Bv is generally well tolerated but with higher rates of peripheral neuropathy, and more febrile neutropenia/sepsis is observed when combined with chemotherapy. CPI have unique immune-mediated toxicities, including hypothyroidism, pneumonitis, and colitis.[54,55] In our current practice, outside of unique situations, including older patients or those ineligible for standard chemotherapy, we do not treat patients with early-stage disease with novel agents off clinical trials.

Table 3. **Selected clinical trials incorporating Bv and immunotherapy in early-stage HL**

Trial	Trial design	Disease stage	N	Median follow-up	Outcomes	PFS	OS
Pembrolizumab followed by AVD[51]	Pembro×3 ⟶ AVD×4–6 (4 cycles for early stage, 6 cycles for advanced-stage or early-stage bulky)	Stage I/II unfavorable Stage III/IV	30	22.5 months	CMR 55% with pembro alone, but reached 100% after AVD×2	Median PFS not reached, 2-year PFS 100%	Median OS not reached, 2-year OS 100%
Nivolumab and AVD[54]	Nivo-AVD×4 plus 30 Gy ISRT Sequential therapy: nivo×4 doses ⟶ nivo-AVD×2 ⟶ AVD×2plus 30 Gy ISRT	Early-stage unfavorable	109	13 months	CMR Group 1: 83% Group 2: CR 84%	12-month PFS: Group 1: 100% Group 2: 98%	12-month OS 100% in both groups
Bv-AVD vs ABVD, followed by 30 Gy INRT[53]	Bv-AVD×4 or ABVD×4⟶30 Gy INRT	Early-stage unfavorable	170	45 months	CMR Bv-AVD 86.7% ABVD 78.9%	2-year PFS: 97.3% with Bv-AVD vs 92.6% with ABVD	Median not reached
Bv-AD[45]	Bv-AD×2 ⟶ PET 1. If PET neg, then Bv-AD×2 (total 4) 2. If PET pos, then Bv-AD×4 (total 6)	Non-bulky early-stage favorable or unfavorable	34	53 months	CMR 97%	Estimated 5-year PFS of 91%	Estimated 5-year OS of 96%
Bv-AVD +/− RT (4 cohorts)[52]	BV-AVD×4 ⟶ PET If PET neg, then 1. 30 Gy ISRT 2. 20 Gy ISRT 3. 30 consolidation volume radiotherapy 4. No radiotherapy	Early-stage unfavorable	117	45.6 months	CMR 1. 93% 2. 100% 3. 93% 4. 97%	Overall 2-year PFS 94% 2-year PFS for 4 cohorts 1. 93.1% 2. 97% 3. 90% 4. 97%	Overall 2-year OS 99.1%
ABVD followed by Bv consolidation[56]	ABVD×2 ⟶PET Favorable and PET neg, then BV consolidation Favorable and PET pos or unfavorable and PET neg, then ABVD×2 plusBV consolidation Unfavorable and PET pos, then ABVD×4 plusBV consolidation	Nonbulky early-stage favorable and unfavorable	41	47 months	CMR 95%	3-year PFS of 92% 3-year PFS 100% for PET neg after Bv	OS was 97% 3 year OS 100% for PET neg after Bv
Trials with novel agents including patients older than 60							
Bv followed by AVD followed by Bv consolidation[43]	Bv×2 ⟶ AVD×6 ⟶ Bv×4	Stage II-IV (age 60 or older)	48 (9 stage II)	23 months	CMR 90% after Bv-AVD	2-year PFS of 84%	2-year OS of 93%
Bv[57]	Bv monotherapy×16 cycles with PET after 4 cycles	Unfit for chemo Stage IIB or bulky, stage III/IV	38 (7 stage II)	36 months	CMR 25.8%	Median PFS 7.3 months	Median OS 19.5 months
Bv-DTIC vs Bv-bendamustine[44]	Bv-DTIC×12 Bv-benda×6* *closed early due to toxicity	Stages I-IV	42	21.6 months	CMR Bv-DTIC 62% Bv-benda 88%	Median PFS 17.9 months	Not reached
Pembrolizumab followed by AVD[51]	Pembro×3 ⟶ AVD×4–6 (4 cycles for early stage, 6 cycles for advanced-stage or early-stage bulky)	Stage I/II unfavorable Stage III/IV	30 (4 pts >60)	22.5 months	CMR 55% with pembro alone, but reached 100% after AVD×2	Median PFS not reached, 2-year PFS 100%	Median OS not reached, 2-year OS 100%
BVplus nivolumab[58]	Bv-nivo every 21 days×8 cycles	Stages I, II, III, IV, ≥60 years or <60 and unsuitable for standard chemo	46	21.2 months	CMR 48%	Median PFS 18.3 months	Median OS not reached

ABVD, doxorubicin hydrochloride, bleomycin, vinblastine sulfate, dacarbazine (DTIC); Bv, brentuximab vedotin; CMR, complete metabolic response; INRT, involved nodal radiotherapy; ISRT, involved site radiotherapy; OS, overall survival; PFS, progression free survival.

Table 4. Ongoing trials in early-stage HL incorporating novel agents

Clinical trial	Interventions	Phase	Anticipated enrollment
NCT04685616	ABVD vs Bv-AVD	III, randomized	1042
NCT05675410	Bv-nivo vs standard ABVD +/− radiotherapy	III, randomized	1875
NCT05627115	Tislelizumab +/− AVD and radiotherapy	II, single arm	80
NCT05900765	Zimberelimab	II, single arm	54
NCT05404945	Pembro plus Bv +/− AVD	II, multicohort	44
NCT04837859	Tiselizumab +/− AVD and radiotherapy	II, single arm	120
NCT04866654	ABVD +/− nivolumab	II, single arm	160
NCT03712202	Nivolumab plus Bv vs ABVD plus nivolumab vs Bv-AVD plus nivolumab	II, randomized	264

ABVD, doxorubicin hydrochloride, bleomycin, vinblastine sulfate, dacarbazine (DTIC); Bv, brentuximab vedotin.

Multiple trials investigating different combinations and strategies are currently ongoing, with details in Table 4. Of these, the North American study, which is a randomized phase 3 PET-adapted trial incorporating Bv, nivolumab, and/or RT in early-stage HL for children and adults, is particularly intriguing. It is planned to accrue 1875 patients from ages 5 to 60 with ABVD being the comparator arm to several experimental arms (NCT05675410).

Late toxicities and survivorship

While the majority of patients with early HL can be cured of their disease with the therapies discussed above, the long-term impact of these treatments can be significant. Late effects of cytotoxic therapies and radiation are well described and include cardiovascular disease, recurrent infections, second primary malignancies, endocrine dysfunction such as infertility, early menopause, and thyroid disorders. Cumulatively, this leads to HL patients having a 5.1-fold higher risk of death due to causes other than HL.[59-65] Dores et al conducted a large study that included 20 000 patients treated from 2000 to 2016 and determined that heart disease, infections, and interstitial lung disease were leading causes of noncancer-related mortality.[66]

In a large study over four decades with 2000 patients, Van Nimwegen et al noted the 40-year cumulative risk of cardiovascular disease was 50% higher in patients treated before age 25. Mediastinal RT increased the risk of coronary disease, valvular heart disease, and cardiomyopathy, while anthracyclines increased the risks of valvular heart disease and cardiomyopathy. Moreover, combining mediastinal RT with anthracycline and/or smoking had additive risk.[60] The study highlights the need for tools to predict late toxicities in HL survivors, and researchers have been developing models to answer these questions.[67,68] De Vries et al in the Netherlands established and validated a risk prediction model for heart disease in HL survivors that includes age at HL diagnosis, sex, smoking status, RT, and anthracycline treatment as predictors for coronary heart disease and heart failure, based on a multicenter cohort of 1433 patients with a median follow-up of 24 years. These models can assist in identifying HL survivors who may need closer follow-up and more intensive screening.[68] Recently, a double-blind randomized clinical trial conducted in lymphoma patients to receive atorvastatin vs placebo during anthracycline chemotherapy revealed that the odds of a 10% or greater decline in ejection fraction to a final value of less than 55% after anthracycline treatment was almost 3 times greater for participants randomized to placebo compared with those randomized to atorvastatin.[69] Further trials are needed to assess if early interventions or better patient selection may decrease the long-term risk of adverse cardiac events. Although optimal screening strategies are unclear for cardiovascular disease, monitoring and aggressive management of cardiovascular risk factors, including smoking, hypertension, diabetes, and hyperlipidemia, are recommended, with consideration of a baseline stress test or echocardiogram at 10-year intervals from treatment and, additionally, a carotid ultrasound at 10-year intervals if there was exposure to neck radiation.[70]

Second primary malignancies continue to rise, even up to 40 years from initial diagnosis, and constitute a leading cause of mortality for HL survivors.[71-73] Breast, lung, and gastrointestinal carcinomas as well as non-Hodgkin lymphoma and leukemia correlate with the use of radiation and alkylator chemotherapies, and studies demonstrate a dose dependent relationship between RT and risk of malignancy.[74-76] Travis et al reported that the risk of developing breast cancer in patients treated with chest radiation before age 25 was as high as 29% by age 55.[74] Increased risk of hematologic malignancies such as therapy-related acute myeloid leukemia and myelodysplastic syndrome are associated with regimen intensity as rates are lower with ABVD compared to the more intensive BEACOPP regimen.[61,77] Finally, endocrinopathies and thyroid disease can develop in up to 50% of patients, especially those who received radiation to the neck.[78] Infertility rates are significantly higher in patients exposed to BEACOPP compared to ABVD.[62,79,80] While fertility preservation options have expanded and improved over time, ongoing focus is necessary to decrease the infertility risk associated with therapy.

While there is no consensus on screening, in practice, we follow NCCN guidelines: initiate mammograms at age 40 or 8 years post-therapy, whichever comes first; if chest or axillary radiation therapy was administered for patients assigned female at birth who received RT to the chest between ages 10-30 years old, then breast MRI in addition to mammography is recommended.[70] HL survivors are often followed by PCPs are not aware of the resource of NCCN guidelines for surveillance.[81] Emphasis should be on developing a collaborative space

between patient care teams to convey the risk of late toxicities that may emerge 10 years or more from initial diagnosis and continue to persist for decades.

CLINICAL CASE (continued)

Our patient was started on treatment with ABVD and interim PET CT and after 2 cycles showed complete metabolic response (Deauville score of 2). She was offered two options: either 4 additional cycles of AVD without bleomycin (RATHL approach) or two more cycles of ABVD followed by consolidative RT up to 30 Gy. The patient decided to go with the additional 2 cycles and then RT and was counseled heavily on late toxicities from mediastinal RT.

Conclusions

Patients with early-stage HL continue to have excellent outcomes with time-limited chemotherapy or chemoradiotherapy. We recommend a risk-adapted approach to therapy using PET imaging and consider PET to be negative if the Deauville score is 1-3. If patient develops a score of 5 while on or after treatment, we advocate for a repeat biopsy to verify disease recurrence/progression. For patients with favorable disease and negative interim PET, we favor omitting radiotherapy in cases where the field includes breast or cardiac tissue. We recommend consultation with radiation oncology in patients with unfavorable or bulky disease to discuss the risk vs benefit of radiotherapy. Incorporating newer agents like Bv and CPI may avoid prolonged chemotherapy and/or radiotherapy and potentially the need for treatment intensification. Larger randomized studies are needed, however, before recommending practice change for early-stage disease. Finally, patient survivorship is crucial. A focus on late effects such as risk of cardiovascular disease, second primary malignancies, and endocrine dysfunction is essential, and all patients should have a fertility preservation consultation, if possible, before starting therapy.

Conflict-of-interest disclosure

Taha Al-Juhaishi: no competing financial interests to declare.
Sairah Ahmed: research support (to institution) for clinical trials: Seattle Genetics, Merck, Xencor, Chimagen, and Tessa Therapeutics; scientific advisory committee: Tessa Therapeutics, Chimagen; data safety monitoring board: Myeloid Therapeutics; consultancy: ADC therapeutics, KITE/Gilead.

Off-label drug use

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The optimal management of relapsed and refractory Hodgkin lymphoma: post–brentuximab and checkpoint inhibitor failure

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The treatment landscape of classical Hodgkin lymphoma has changed dramatically over the past decade. Relapsed and refractory mainstay therapeutics such as brentuximab vedotin (BV) and checkpoint inhibitors (CPIs) are being moved to earlier lines of therapy. However, the treatment of patients who progress after BV and CPI remains a challenge. Allogeneic stem cell transplantation still plays an important role in this patient population as the only current treatment approach with curative potential. Unfortunately, not all patients are transplant candidates, and many will still relapse afterward. Cytotoxic chemotherapy and radiation may be used for symptom palliation or as a bridge to transplant. Targeted therapies, including the antibody drug conjugate, camidanlumab tesirine, and transcriptional agents such mammalian target of rapamycin and histone deacetylase inhibitors have shown some potential in patients with refractory disease. In addition, combination therapies with CPIs and novel agents may help overcome resistance to therapy. Clinical trials with cellular therapies, including chimeric antigen receptor T cells targeting CD30 and allogeneic natural killer cells combined with AFM13, a CD30/CD16a-bispecific antibody, have shown promising results. The availability of more therapeutic options for this patient population is eagerly awaited.

LEARNING OBJECTIVES

- Describe the role of chemotherapy and radiation in relapsed/refractory Hodgkin lymphoma after brentuximab vedotin and PD-1 inhibitors
- Explain the role of allogeneic stem cell transplant in relapsed/refractory Hodgkin lymphoma and discuss outcomes after PD-1 inhibitors
- Discuss emerging therapeutic options, including novel agents and cellular therapies, in patients with relapsed/refractory Hodgkin lymphoma

CLINICAL CASE

A 56-year-old man presented with cough, night sweats, and palpable nodes. A lymph node biopsy led to a diagnosis of classical Hodgkin lymphoma (cHL). A positron emission tomography/computed tomography showed disease above and below the diaphragm consistent with stage IIIB disease. He received 6 cycles of doxorubicin, bleomycin, vinblastine, dacarbazine and achieved a complete response (CR). However, he relapsed within 6 months of completing treatment. He was treated with salvage ifosfamide, carboplatin, and etoposide, followed by carmustine, etoposide, cytarabine, and melphalan; autologous stem cell transplant (autoSCT); and maintenance brentuximab vedotin (BV). While on treatment with BV, he developed recurrent night sweats and relapsed disease was confirmed. He was treated with nivolumab but progressed after 3 months of therapy. What are his current treatment options?

Introduction

Although most patients with cHL are cured with frontline therapy, approximately 15% to 25% of patients will relapse.[1] The current standard of care for these patients is salvage therapy followed by autoSCT, with over 50% achieving a durable remission.[2,3] Over the past decade, BV and checkpoint inhibitors (CPIs) (ie, pembrolizumab and nivolumab) have transformed the care of patients with cHL, first in the relapsed setting and now in combination with frontline

regimens.[1,4-6] Therefore, in the modern era, most patients with multiply relapsed disease will have already been exposed to BV and CPI. The treatment of patients with cHL who have progressed after prior BV and CPI is an area of significant unmet need. In this review, we will discuss our treatment approach when caring for these patients.

BV and CPI rechallenge

One key question when evaluating these patients is to determine whether rechallenge with BV or CPI could be beneficial. If a patient previously achieved a CR with either of these agents, retreatment as a single agent or part of a multidrug chemotherapy regimen is a viable option.[7,8] In a long-term follow-up of the clinical trial of pembrolizumab in relapsed/refractory cHL, the overall response rate for the 19 patients who were rechallenged with pembrolizumab at time of progression after discontinuing therapy in CR was 71.4% with a median duration of response (DOR) of 16.6 months.[9] In addition, pseudo-progression can also be seen with CPI use, and some patients may derive benefit from being treated beyond progression.[10] There are limited data on the role of retreatment in truly refractory patients who progressed while on therapy, but it is likely that the efficacy of either drug as a single agent would be limited. However, there has been some success in combining targeted therapies with CPI with the goal of overcoming resistance, which will be further discussed below.

Chemotherapy

Traditional cytotoxic chemotherapy was the backbone of treatment for cHL for over half a century before immunotherapy and targeted agents came to the forefront of cHL management. While the role for chemotherapy in the relapsed setting has decreased in importance over the years, several situations still warrant this approach.

The decision to use traditional chemotherapy is based on patient factors, such as age, performance status, and comorbid conditions, as well as disease factors, including time to relapse from prior chemotherapy, aggressiveness of relapse, and whether there is any end-organ damage. The goal of treatment is also paramount; a palliative approach will warrant different treatment than a curative approach such as allogeneic stem cell transplant (alloSCT).

For young, otherwise healthy patients, alloSCT should be considered. These patients can be salvaged with combination chemotherapy with a goal of achieving a CR prior to transplant (Table 1). Traditional salvage regimens include gemcitabine, dexamethasone, and cisplatin[11]; gemcitabine, vinorelbine, and liposomal doxorubicin[12]; ifosfamide, carboplatin, and etoposide[13]; and dexamethasone, cytarabine, and cisplatin (oxaliplatin).[14] For patients who progress after an initial response to BV or CPI, a reasonable option would be using BV or a PD-1 inhibitor in combination with a standard salvage regimen.[15-17] While these combinations are highly effective at first relapse prior to autoSCT, their utility in the setting of prior exposure is less clear. In fact, CPI may actually sensitize patients to later lines of therapy, including chemotherapy.[18]

For patients who are not candidates for alloSCT, combination chemotherapy should be used sparingly as the toxicity of this approach usually outweighs the benefit. If chemotherapy is discussed, the focus should be on quality of life, and this is best achieved with a single-agent approach. Studies have shown some efficacy with gemcitabine,[19] vinblastine,[20] or bendamustine.[21,22] The decision of which to pursue will be based on prior treatments and patient factors.

Role of radiation therapy

cHL is generally radiosensitive, and even patients with refractory disease may obtain local disease control with radiation.[23] Radiation can be an option for patients with low burden disease or localized relapse and serve as a bridge to alloSCT.[24] Even for patients who are not alloSCT candidates, radiation is an effective tool for palliation. Although most patients with relapsed/refractory cHL who are treated with radiation alone will ultimately relapse, a small proportion of patients experience durable remissions.[23]

Table 1. Traditional chemotherapy for treatment of relapsed/refractory Hodgkin lymphoma

	N	ORR (%)	CR (%)	PFS	OS	Citation
GDP	23	69	17	NR	NR	Baetz et al.[11]
GVD*	41	62	20	4-y EFS: 52%	4-y OS: 70%	Bartlett et al.[12]
ICE	65	88	26	58% at 43 mo	NR	Moskowitz et al.[13]
DHAP	102	89	21	NR	NR	Josting et al.[14]
Nivolumab-ICE	35	100	88	1y: 90%	1y: 100%	Mei et al.[15]
Pembrolizumab-GVD	39	100	95	13.5 mo: 100%	13.5 mo: 100%	Moskowitz et al.[16]
BV-ICE	45	91	74	2y: 80.4%	2y: 97.8%	Lynch et al.[17]
Gemcitabine	23	39	9	6.7 mo	10.7 mo	Santoro et al.[19]
Vinblastine	17	59	12	8.3 mo	38.8 mo	Little et al.[20]
Bendamustine	35	53	33	5.2 mo	NR	Moskowitz et al.[21]

*Transplant-naive group only.

DHAP, dexamethasone, cytarabine, and cisplatin; EFS, event-free survival; GDP, gemcitabine, dexamethasone, and cisplatin; GVD, gemcitabine, vinorelbine, and liposomal doxorubicin; ICE, ifosfamide, carboplatin, and etoposide; NR, not reached; OS, overall survival.

Allogeneic stem cell transplant

AlloSCT remains a potentially curative option for patients with cHL who relapse after BV and CPI. A retrospective study of 209 patients who received alloSCT after PD-1 blockade with a median follow-up of 2 years demonstrated a 2-year progression-free survival (PFS) and overall survival of 69% and 82%, respectively.[25] Over the years, the nonrelapse mortality has declined from ~30% to 40% to about 10% to 20% in patients with cHL, largely because of adoption of nonmyeloablative and reduced intensity conditioning (RIC) regimens and improved supportive care.[26] In a large retrospective study, RIC alloSCT was reported to be superior to myeloablative alloSCT likely because of higher nonrelapse mortality associated with myeloablative alloSCT.[27] Additionally, use of alternative donors and posttransplant cyclophosphamide for graft-versus-host disease (GVHD) prophylaxis has been reported with good outcomes in cHL.[28] Disease status prior to alloSCT, especially achievement of CR, has been shown in multiple studies to be a predictor of favorable outcomes after alloSCT.[26,29] Given that most patients with relapsed cHL will be exposed to BV and CPI and these agents can stay in the system for weeks after exposure, the effect of these treatments has been explored in relation to outcomes following alloSCT. In a large multicenter retrospective analysis, it was demonstrated that treatment with a CPI prior to alloSCT (within 80 days) was associated with higher rate of acute GVHD and lower likelihood of relapse. Translational work from a subset of these patients showed that the alloreactive T cells were expanded and the ratio of T regulatory cells/CD4 conventional T cells was decreased in patients with prior exposure to CPI when compared with CPI-naive patients, suggesting this mechanism as a driver behind higher graft vs lymphoma and GVHD rates in these patients. However, the overall outcomes are similar to cohorts who underwent alloSCT without CPI exposure, indicating that treatment with CPI is not a contraindication for alloSCT.[25] A washout period of 6 weeks has been suggested in a review to reduce risk of severe GVHD[30]; however, prospective studies are needed to further elucidate the interplay of these therapies in this patient population.

CLINICAL CASE (continued)

With a goal of getting him to alloSCT, the patient was treated with gemcitabine, dexamethasone, and cisplatin and achieved a partial response (PR) with persistent fluorodeoxyglucose-avidity in a right subcarinal lymph node. After radiation to this region, he achieved a CR and subsequently underwent RIC matched unrelated donor alloSCT. Unfortunately, 6 months after transplant, his disease relapsed.

Targeted therapies

The best treatment option for patients with cHL whose disease has progressed after alloSCT is enrollment on a clinical trial. Several antibody-based therapies have garnered interest, including the antibody drug conjugate (ADC) camidanlumab tesirine (Cami) and lymphocyte activation gene 3 (LAG3) inhibitors such as favezilumab, both of which target the immune environment (Table 2).

Cami is an anti-CD25 antibody drug linked to a pyrrolobenzodiazepine dimer. CD25 is expressed in cHL, leading to direct cancer cell kill. In addition, anti-CD25 antibodies target CD25 expressing T regulatory cells, resulting in changes in the tumor microenvironment and increased antitumor immunity.[31] Cami has demonstrated activity in 117 patients with relapsed/refractory cHL after ≥3 lines of therapy, including BV and anti–PD-1 therapy with an overall response rate (ORR) of 70.1%, a CR rate of 33.3%, and a median DOR of 14.5 months for those patients who achieved a CR.[32] However, Cami did have concerning toxicities, and 27.4% of patients discontinued Cami due to treatment-emergent adverse events, including Guillain-Barre syndrome/polyradiculopathy,

Table 2. Novel agents for treatment of relapsed/refractory Hodgkin lymphoma

	Target	N	ORR (%)	CR (%)	PFS	OS	Toxicity	Citation
Camidanlumab tesirine	Anti-CD25 ADC	117	70.1	33.3	9.1 mo	~	Immune, GBS	Carlo-Stella et al.[32]
Favezilumab (with pembrolizumab)	LAG3 Inhibitor	34	29	9	10.7 mo	25.7 mo	Thyroid, GI	Timmerman et al.[33]
Lenalidomide	IMiD	36	19.4	2.8	4 mo	20 mo	Cytopenias, rash	Fehniger et al.[35]
Lenalidomide + temsirolimus	IMiD, mTOR-I	20	80	35	9.2 mo	39.6 mo	Cytopenias	Major et al.[37]
Everolimus	mTOR-I	57	47	5	7.2 mo	NR	Cytopenias, fatigue	Johnston et al.[36]
Ibrutinib (with nivolumab)	BTKi	17	51.9	29.5	17.3 mo	~	Cytopenias, rash	Hanel et al.[42]
Panobinostat	HDACi	129	27	4	6.1 mo	1-y OS: 78%	Cytopenias	Younes et al.[38]
Vorinostat (with pembrolizumab)	HDACi	32	72	34	8.9 mo	NR	Cytopenias, thyroiditis	Mei et al.[40]
Tinostamustine	Alkylating deacetylase inhibitor	20	40	10	3.8 mo	~	Cytopenias, CINV	Sureda et al.[61]

BTKi, Bruton tyrosine kinase inhibitor; CINV, chemotherapy-induced nausea and vomiting; GBS, Guillain-Barre syndrome; GI, gastrointestinal; HDACi, histone deacetylase inhibitor; IMiD, immunomodulatory imide drug; mTOR-I, mammalian target of rapamycin inhibitor.

which occurred in 6.8% of patients.[32] Therefore, although the CR rates reported in this trial were encouraging, it is not clear whether the confirmatory phase 3 trial that would be needed for possible US Food and Drug Administration approval will move forward.

PD-1 inhibitors are highly effective in the management of cHL; however, thus far, investigations into immunotherapy with alternative CPIs have had mixed results. Since LAG3 is expressed in the cHL microenvironment and regulatory T cells typically are in close proximity to PD-1, dual blockade with a LAG3 and PD-1 inhibitor is being investigated. The LAG3 inhibitor favezelimab, in combination with pembrolizumab, has shown some early efficacy in relapsed/refractory cHL after progression on prior anti–PD-1 therapy with an ORR of 29%.[33] Although these were mostly PRs, they were durable with a median DOR of 19.4 months. The drug combination was well tolerated, with hypothyroidism and gastrointestinal symptoms as the most common adverse events. There are minimal data investigating the role of the CTLA-4 inhibitor ipilimumab as part of a multidrug combination in patients with relapsed/refractory cHL.[34] This phase 1 trial of BV-ipilimumab, BV-nivolumab, and BV-nivolumab-ipilimumab reported complete response rates of 57% and 61% in the BV-ipilimumab and the BV-nivolumab arms, respectively, and a CR rate of 73% in the BV-nivolumab-ipilimumab arm, raising the possibility that the addition of ipilimumab could enhance responses. However, further research is needed to assess whether ipilimumab alone or as a drug combination could lead to responses in patients with relapsed/refractory cHL after prior BV or PD-1 inhibitors since patients previously exposed to immunotherapy were excluded, and only 13% had previously received BV. Research efforts into this and other alternative CPIs continue to determine their eventual role, if any, in relapsed/refractory cHL.

The immunomodulatory agent lenalidomide, transcriptional targets such as mammalian target of rapamycin (mTOR) inhibitors, and histone deacetylase inhibitors have also been used with varying success. Lenalidomide was studied in 36 evaluable patients with relapsed/refractory cHL, demonstrating an ORR of 19.4%, mostly PRs, and stable disease ≥6 months in an additional 13.9% of patients. Four patients continued lenalidomide for >1 year.[35] Everolimus is an oral mTOR inhibitor that led to an ORR of 47% in a small group of heavily pretreated patients with relapsed/refractory cHL, with a CR in 5%.[36] The median time to progression was 7.2 months, although 1 patient remained on treatment for >36 months. Early data hint that the combination of lenalidomide with the mTOR inhibitor temsirolimus may be an effective approach, with an ORR of 80% and a 35% CR rate in 20 patients with relapsed/refractory cHL.[37] The histone deacetylase inhibitor panobinostat led to an ORR of 27%, mostly PRs, in a phase 2 trial of patients with relapsed/refractory cHL after autologous stem cell transplant.[38] Patients had received a median of 4 prior therapies, but it was in the era before BV and CPIs, so its role in patients resistant to these therapies is unknown; any future role in relapsed/refractory cHL will likely be in a multidrug combination. The nuclear factor–κB pathway is an important pathway in cHL; however, a clinical trial of bortezomib in relapsed/refractory cHL was closed early due to inadequate response.[39] However, alternative agents or combinations targeting this pathway may show more promise. None of these options are expected to offer long-term disease control but may aid in symptom control and could bridge to other treatments or clinical trials.

The combination with CPI with epigenetic modulating agents may help overcome resistance to these immunomodulatory agents. A phase 1 trial of pembrolizumab plus vorinostat enrolled 32 patients with relapsed/refractory cHL.[40] Three-fourths had received prior PD-1 blockade, and 56% were refractory to PD-1 therapy. With a reported ORR of 72% and CR of 34% in the whole group, responses were even seen in the refractory subgroup with an ORR of 56% and a CR of 11%. Efficacy in the CPI refractory patient population has also been reported with the combination of the JAK2 inhibitor ruxolitinib with nivolumab.[41] A phase 1/2 trial in 19 evaluable patients with relapsed/refractory cHL after prior CPI therapy was well tolerated and demonstrated an ORR of 39% and a CR in 26% of patients. Ibrutinib was combined with nivolumab in 17 patients, resulting in an ORR of 51.9% and a CR of 29.5% without any unexpected toxicities.[42] Responses were similar in the 10 patients who had progressed on prior PD-1 inhibition with an ORR of 50.0% and a CR rate of 20.0%. Similarly interesting results have been seen with the combination of anti–PD-1 inhibitor camrelizumab plus decitabine with an ORR of 52% and a CR rate of 28% in patients previously exposed to CPIs.[43] Thus, there are increasing data suggesting that novel combinations with PD-1 inhibitors may augment the therapeutic immune response and even overcome resistance to CPI therapy.

Cellular therapies

Given the success of chimeric antigen receptor T (CAR-T) cells in other types of lymphomas as well as the fact that the malignant cells of cHL universally express CD30, which is ideal for targeted therapies, there has been interest in developing CAR-T cells in cHL. Patients with cHL who relapse or are refractory to BV generally retain CD30 expression,[44,45] so targeting of the antigen with subsequent therapies is still a feasible approach. CD30-directed CAR-T cells (CD30.CAR-Ts) were infused in heavily pretreated patients with relapsed/refractory cHL who received a median of 7 prior lines of therapy (Table 3).[46] Most patients had previously been treated with BV and CPI, with 90% of patients receiving prior BV and 81% of patients receiving prior CPI. CD30.CAR-Ts appear to have a better safety profile compared to CD19- or BCMA-directed CAR-T cells, with 24% of patients experiencing cytokine release syndrome (all grade 1) and no reports of immune effector cell-associated neurotoxicity syndrome. CD30.CAR-Ts demonstrated high response rates with an ORR of 72% and a CR rate of 59% in 32 patients who received lymphodepletion with fludarabine combined with cyclophosphamide or bendamustine. Although the rate of CR was high, responses were not always durable, with a 1-year PFS of 36% and median PFS for patients in CR at time of treatment of 444 days. In a multicenter phase 2 clinical trial of CD30.CAR-Ts, 15 patients with relapsed/refractory cHL with a median of 6 prior lines of therapy were treated with an ORR of 73.3% and a CR rate of 60%.[47] The median PFS was 6.5 months. Current studies of CD30.CAR-Ts are working to enhance the efficacy and DOR (Table 4).

One possible method of improving the efficacy of CD30.CAR-Ts is to enhance trafficking to the tumor site, which could give CAR-T cells increased opportunities to eliminate tumor cells before inhibitory mechanisms become more predominant. The malignant cells in cHL produce chemokines such as thymus and activation regulated chemokine (TARC) and macrophage-derived chemokine (MDC), which attract suppressive cells, including type 2 helper T cells and regulatory T cells that

Table 3. Cellular therapy clinical trial results in relapsed/refractory Hodgkin lymphoma

Therapy	Lymphodepletion	Patients	Efficacy	Toxicity	Reference
EBV-specific T cells	None	14 (EBV$^+$ HL)	Active disease (11 patients): 18% CR, 9% PR, 45% SD Some remissions up to 40 mo	Flu-like symptoms (14%)	Bollard et al.[62]
LMP 1/2-specific T cells	None	50 (EBV$^+$ lymphoma) 25 HL	Active disease (21 patients): 52% CR, 9.5% PR 2-y EFS: 50% Adjuvant therapy (29 patients): 28 patients in CR at median 3.1y of follow-up	No DLT	Bollard et al.[54]
LMP 1/2 specific T cells with DNRII	None	8 (EBV$^+$ HL)	Active disease (7 patients): 29% CR, 14% PR, 57% SD 2 patients with ongoing response >4y	No DLT	Bollard et al.[55]
Multiantigen targeted T Cells	None	32 (14 HL, 7 with active disease)	Active disease (7 patients): 29% durable CR (>3y)	No DLT	Vasileiou et al.[56]
Multiantigen targeted T cells with nivolumab	None	10 (6 received nivolumab)	Active disease (8 patients): 13% CR, 88% SD 38% in SD at 1y	No DLT	Dave et al.[57]
CD30 CAR-T cells	Flu/Cy; Gem/mustargen/Cy; nab-paclitaxel/Cy	18	39% PR, 33% SD Median PFS 6 mo	Grade 1–2 febrile syndrome within 24h (100%) Rash (11%)	Wang et al.[63]
CD30 CAR-T cells	None	9 (7 HL)	HL patients: 29% CR 1 durable CR >2.5y	No DLT	Ramos et al.[64]
CD30 CAR-T Cells	Benda; Flu/Benda; Flu/Cy	41	ORR 62%; for patients with flu: ORR 72% with 59% CR; 1-y PFS: 36%	Gr1 CRS (24%) Rash (48%)	Ramos et al.[46]
CD30 CAR-T cells with PD-1 inhibitor	Flu/Cy	12 (9 HL); 8 had prior CPI	ORR 92%; 50% CR Median follow-up 21.5 mo; PFS 45%	CRS (25%) Gr3 CRS (8%)	Sang et al.[51]
CD30 CAR-T cells	Flu/Benda	15	ORR 73.3%; 60% CR Median PFS 6.5 mo	Gr1 CRS (7%)	Ahmed et al.[47]
CD30 CAR-T cells (HSP-CAR30)	Flu/Benda	10 (8 HL)	ORR 100% with 50% CR Mean PFS: 235 d All CRs maintained	Gr1 CRS (60%) Rash (40%)	Caballero Gonzalez et al.[50]
CD30.CCR4 CAR-T cells	Flu/Benda	12 (10 HL)	HL: 70% CR, 30% PR Median follow-up 8.5 mo; mPFS for HL not reached	CRS (33%) Gr1 CRS (17%) Gr2 CRS (17%)	Grover et al.[49]
CD30.CAR—modified EBV-specific T cells (Allogeneic)	Flu/Cy	16	ORR 75% (38% CR, 38% PR)	Gr1 CRS (31%)	Ramos et al.[58]
AFM13 + NK cells	Flu/Cy	30 (28 HL)	ORR 97% with 67% CR EFS at 8 mo: 57%	Infusion reactions with AFM13 (37%) Gr3 infusion reaction (3%) Gr2 infusion reaction (33%)	Nieto et al.[60]

Benda, bendamustine; CRS, cytokine release syndrome; Cy, cyclophosphamide; DLT, dose-limiting toxicity; DNRII, dominant-negative TGF-β receptor type 2; Flu, fludarabine; Gem, gemcitabine; Gr, grade; HL, Hodgkin lymphoma; LMP, latency membrane protein; mPFS, median progression free survival; SD, stable disease.

express the TARC/MDC-specific chemokine receptor CCR4, producing an inhibitory barrier to cytotoxic T cells and leading to an immunosuppressed tumor microenvironment. Given promising results in preclinical studies,[48] a phase 1 clinical trial of CD30.CAR-Ts coexpressing CCR4 is currently ongoing (NCT03602157). In 10 patients with heavily pretreated cHL, with all patients having prior BV and CPI exposure, the ORR was 100% with a 70% CR rate.[49] Another clinical trial of CD30.CAR-Ts, in which the product is enriched in memory T cells with the goal of enhancing persistence, also has promising early results.[50] Other possibilities to enhance CD30.CAR-Ts in cHL include combination with CPIs.[51] Of interest, patients who progress after CD30.CAR-Ts, even if they had previously relapsed or been refractory to anti–PD-1 therapy, have had high response rates with some

Table 4. Clinical trials for relapsed/refractory Hodgkin lymphoma

Trial	Phase	Details	N (est)	Sponsor/location	Clinicaltrials.gov
Checkpoint inhibitor combinations (include PD-1 refractory)					
Magrolimab and pembrolizumab	2	Magrolimab: anti-CD47 mAb	24	Stanford/Merck	NCT04788043
Favezilumab/pembrolizumab vs physician's choice (bendamustine or gemcitabine)	3		360	Merck	NCT05508867
Nivolumab and axatilimab	2	Axatilimab: mAb inhibits CSF-1R	9	University of Utah	NCT05723055
Azacitidine and nivolumab	1		30	City of Hope	NCT05162976
Azacitidine and pembrolizumab	2		24	MDACC	NCT05355051
Chidamide/decitabine/camrelizumab vs decitabine/camrelizumab	2	Chidamide: HDAC inhibitor	200	Chinese PLA General Hospital	NCT04514081
PD-1 inhibitor after CD30 CAR-T cell therapy	1	Patients who progressed after CD30 CAR-T	20	University of North Carolina	NCT04134325
Novel agents					
AZD4573	2	AZD4573–CDK9 inhibitor	81	AstraZeneca	NCT05140382
SHR1701 alone or in combination with SHR2554	1/2	SHR-1701: bifunctional fusion protein targeting PDL1 and TGF-B SHR2554–EZH2 inhibitor	100	Chinese PLA General Hospital	NCT05896046
AZD7789	1/2	AZD7789: anti–PD-1/TIM3 bispecific antibody	180	AstraZeneca	NCT05216835
Adoptive cell therapies					
CD30 CAR-T cells (HSP-CAR30)	1/2A		30	Fundacio Institut de Recerca de L'Hospital de la Santa Creu I Sant Pau	NCT04653649
CD30 CAR-T cells	1		20	Immune Cell, Inc.	NCT03383965
CD30 CAR-T cells	1		60	Baylor	NCT02917083
CD30 CAR-T cells in R/R CD30$^+$ lymphoma	1		9	Zhejiang University	NCT05208853
CD30 CAR-T cells	1b/2	Pediatric patient cohort only open	40	UNC	NCT02690545
CD30 CAR-T cells coexpressing CCR4	1		59	UNC	NCT03602157
CD30biAb-AATC	1	Anti-CD30 bispecific antibody-armed, anti-CD3-activated, autologous T cells	42	MCW	NCT05544968
Allogeneic CD30.CAR-EBV specific T lymphocytes	1		18	Baylor	NCT04952584
AFM13 in combination with AB-101	2	AB-101–allogeneic NK cell therapy	154	Affimed	NCT05883449

HDAC, histone deacetylase; mAb, monoclonal antibody; TGF-B, transforming growth factor β.

durable remissions after rechallenge with CPI, raising the possibility that CD30.CAR-Ts could be rescued by CPIs.[52]

CD30.CAR-Ts are a promising therapeutic option for patients with relapsed/refractory disease. The future role of this therapy is still unknown, and larger studies with longer follow-up are needed to determine whether this treatment could be curative in a subset of patients.

Other cellular therapies have also been investigated in the treatment of cHL. Approximately 40% of cHL cases are Epstein-Barr virus (EBV) positive, so an EBV-directed approach could be a promising option for patients.[53] In a clinical trial of autologous EBV-specific cytotoxic T lymphocytes (CTLs) enriched for specificity against latency membrane proteins, 11 of 21 patients with active disease at the time of treatment achieved a CR, with a 2-year event-free survival of approximately 50%.[54] In a follow-up clinical trial, the autologous EBV-specific CTLs were engineered to express dominant-negative transforming growth factor β receptor, with the goal of decreasing the suppression caused by transforming growth factor β and enhancing efficacy.[55] Of 7 patients with cHL, 4 responded, including 2 patients

with continued response over 4 years from infusion. One challenge of EBV-specific CTLs is that over half of cHL cases are EBV negative. Trials of CTLs targeted against tumor-associated antigens have yielded promising early results in patients with refractory cHL.[56,57] There is also currently an ongoing clinical trial of allogeneic EBV-specific cytotoxic T cells engineered to express a CAR targeting CD30 in patients with $CD30^+$ lymphomas, therefore allowing for treatment of patients with EBV^- disease[58] (Table 4).

Another approach in targeting CD30 in cHL is via a bispecific antibody. AFM13 is a bispecific antibody that has specificity for CD30 as well as CD16a, which is expressed on natural killer (NK) cells, with the goal of activating NK cells so they can target Hodgkin Reed-Sternberg cells, which express CD30. In a phase 1 study of AFM13, the overall response rate was 23% in patients who received an optimal dose.[59] Given overall modest activity of this agent on its own but tolerable safety profile, there has been interest in combining AFM13 with other therapies. There is an ongoing clinical trial combining AFM13 with cord blood–derived allogeneic NK cells. Patients are treated with lymphodepletion, followed by AFM13-incubated NK cells and then AFM13 infusions.[60] There was no cytokine release syndrome or immune effector cell-associated neurotoxicity syndrome reported, and the ORR in 30 treated patients was 97% with a 63% CR rate. A phase 2 trial combining AFM13 with AB-101, cord blood allogeneic-derived NK cells, is planned.

Cell therapies have shown promise in patients with relapsed/refractory cHL. Future challenges include prolonging duration of response, as well accessibility and cost of therapy. Currently, these treatments are only available as part of a clinical trial with long wait lists for patients, but they remain promising as a major player in this patient population.

CLINICAL CASE (continued)

Our patient received lenalidomide for 6 months with progressive disease. He was then treated on a CD30 CAR-T cell clinical trial with complete remission. He remains in remission over 2 years from therapy.

Conclusions

There is a significant unmet need in the management of patients with cHL after BV and CPI progression. Patients are often young with frequently more indolent behaving disease compared to other lymphomas, making patients eligible for several lines of therapy. However, outside of alloSCT, there are limited curative therapeutic options for patients. More investigations are needed of novel agents and cellular therapeutic approaches for this patient population.

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Conflict-of-interest disclosure

Natalie S. Grover has served on an advisory board or consulted for Novartis, Kite, Seagen, ADC Therapeutics, Caribou Biosciences, Genentech, and Tessa Therapeutics.
Christopher Dittus has served on an advisory board for Beigene, Genentech, Seagen, and ADC Therapeutics.
Astha Thakkar: no competing financial interests to declare.
Anne W. Beaven: no competing financial interests to declare.

Off-label drug use

Natalie S. Grover: There is discussion of off label drug use in the management of relapsed/refractory Hodgkin lymphoma in the Targeted Therapies section, including lenalidomide, ibrutinib, everolimus, vorinostat, and panobinostat.
Christopher Dittus: There is discussion of off label drug use in the management of relapsed/refractory Hodgkin lymphoma in the Targeted Therapies section, including lenalidomide, ibrutinib, everolimus, vorinostat, and panobinostat.
Astha Thakkar: There is discussion of off label drug use in the management of relapsed/refractory Hodgkin lymphoma in the Targeted Therapies section, including lenalidomide, ibrutinib, everolimus, vorinostat, and panobinostat.
Anne W. Beaven: There is discussion of off label drug use in the management of relapsed/refractory Hodgkin lymphoma in the Targeted Therapies section, including lenalidomide, ibrutinib, everolimus, vorinostat, and panobinostat.

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Using disease-modifying therapies in sickle cell disease

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As curative therapy using allogeneic hematopoietic stem cell transplantation as well as gene therapy and gene editing remains inaccessible to most patients with sickle cell disease, the availability of drug therapies that are safe, efficacious, and affordable is highly desirable. Increasing progress is being made in developing drug therapies based on our understanding of disease pathophysiology. Four drugs, hydroxyurea, L-glutamine, crizanlizumab, and voxelotor, are currently approved by the US Food and Drug Administration, with multiple others at various stages of testing. With the limited efficacy of individual agents, combinations of agents will likely be required for optimal outcomes.

LEARNING OBJECTIVES

- Appreciate the general approaches to the management of SCD based on disease pathophysiology
- Describe the results of important drug trials in sickle cell disease, particularly those that resulted in drug approvals by regulatory agencies, while highlighting ongoing drug trials
- Describe our approach to using approved drug therapies for the management of patients with sickle cell disease

CLINICAL CASE

A 28-year-old man with hemoglobin SS (HbSS) sickle cell disease (SCD) complicated by retinopathy, acute chest syndrome (ACS), nephropathy, and frequent pain episodes requiring healthcare utilization was seen in clinic for follow up. He was on hydroxyurea (~20 mg/kg/d, his maximum tolerated dose) and losartan (25 mg/d) and was fairly adherent. Laboratory studies showed a white blood cell count (WBC) of 6.2×10^9/L; an Hb level of 9.7 g/dL; a mean corpuscular volume of 110 fL; a platelet count of 292×10^9/L; an absolute neutrophil count of 3.5×10^9/L; an absolute reticulocyte count of 114.8×10^9/L; a creatinine level of 0.8 mg/dL; and a cystatin C level of 0.8 mg/L. Hb electrophoresis showed a sickle Hb (HbS) level of 81.2%; fetal Hb (HbF), 15.6%; and HbA2, 3.2%. Given his frequent pain episodes, options for optimizing his care were discussed.

Introduction

SCD affects millions of individuals worldwide.[1] Although a rare disease in the United States, an estimated 230 000 children (representing the vast majority of worldwide births) were born with sickle cell anemia (referring to HbSS and HbSβ^0) in sub-Saharan Africa in 2010.[2] In addition to the presence of HbS, SCD is characterized by hemolytic anemia, vaso-occlusive complications, and progressive end-organ dysfunction. The mortality rate for children with sickle cell anemia remains high in sub-Saharan Africa, with estimated rates of 36.4% for those younger than 5 years and 43.3% for those younger than 10 years,[3] but most children in resource-rich countries live to adulthood.[4] Despite increased survival to adulthood, individuals with SCD in resource-rich nations have a reduced life expectancy compared to the general population.[5-8] SCD can be cured following allogeneic stem cell transplantation and possibly following gene therapy and gene editing. However, as most patients do not have access to these potentially curative treatments, the availability of safe, effective, and affordable drugs remains highly desirable.

This article reviews important historic and recent drug trials for SCD, highlighting regulatory agency–approved drug therapies and our approach to the use of these agents.

Pathophysiology

The development of effective drug therapies for SCD requires an adequate understanding of its pathophysiology. The primary event in the pathophysiology of SCD

is the polymerization of HbS following deoxygenation.[9] The polymerization of HbS depends on several factors, including the degree of HbS deoxygenation, the intracellular HbS concentration, and the amount of HbF.[9] HbS polymerization as well as its multiple consequences, including endothelial cell injury, endothelial dysfunction, increased oxidant stress, inflammation, coagulation and platelet activation, and complement activation, is a therapeutic target in SCD. The clinical manifestations of SCD appear to be driven by 2 major pathophysiological processes: vaso-occlusion with ischemia-reperfusion injury and hemolytic anemia.[1] SCD may also be divided into 2 overlapping subphenotypes: viscosity-vaso-occlusion (characterized by higher Hb levels, possibly increased blood viscosity, and clinical complications such as acute pain episodes, ACS, and avascular necrosis of bone) and hemolysis-endothelial dysfunction (characterized by lower Hb and higher levels of markers of hemolysis, including reticulocyte count, indirect bilirubin and lactate dehydrogenase, and clinical complications such as leg ulcers, priapism, stroke, and pulmonary hypertension).[10] This classification, while controversial, may facilitate an increased understanding of the pathobiology of SCD-related complications and the effects of therapeutic agents. The pathophysiology of SCD is beyond the scope of this article and is reviewed elsewhere.[11]

Drug trials for SCD

Although SCD affects multiple body organs, most trials of potentially disease-modifying drugs have focused on acute pain episodes (commonly referred to as vaso-occlusive crises, or VOCs) as their primary end point. The general approaches to the management of acute pain episodes are support, intervention, and prevention (Figure 1). No drugs have been approved for shortening the duration of acute vaso-occlusive complications (Table 1). As such, acute pain episodes are usually managed supportively. The majority of drug trials have focused on disease-modifying therapies to prevent acute pain episodes. For many years, hydroxyurea was the only drug approved by the US Food and Drug Administration (FDA) for sickle cell anemia. More recently, however, 3 other drugs, L-glutamine, crizanlizumab, and voxelotor, have been approved for SCD. The following sections focus on phase 3 and select multicenter phase 2 studies of disease-modifying agents.

Drugs that inhibit HbS polymerization

Multiple mechanisms can prevent HbS polymerization: 1) inhibition of sickle fiber intermolecular contacts; 2) increase in HbF; 3) decrease of intracellular HbS concentration; 4) increase of oxygen affinity; and 5) decrease in 2,3-diphosphoglycerate concentration.[12]

Hydroxyurea, an inhibitor of ribonucleotide reductase, is thought to exert its therapeutic effects largely by inducing HbF, although the mechanisms of HbF induction remain unclear. Hydroxyurea was approved for adults based on the results of the double-blind, placebo-controlled, multicenter trial of hydroxyurea in 299 patients with sickle cell anemia, which showed significant reductions of VOCs (median, 2.5 vs 4.5 crises per year; $P<.0001$), hospitalizations due to crises (median annual rates, 1.0 vs 2.4; $P<.001$), ACS (25 vs 51 patients; $P<.0001$), and blood transfusions (48 vs 73 patients; $P=.001$; and 336 vs 586 units of blood; $P=.004$) following treatment with hydroxyurea vs placebo.[13] Similar findings were observed in the multicenter BABY HUG trial in which treatment with hydroxyurea significantly reduced disease-related acute complications in young children with sickle cell anemia.[14] Based on this, a National Heart, Lung, and Blood Institute (NHLBI) expert panel strongly recommended offering hydroxyurea to children as young as 9 months with sickle cell anemia.[15] Hydroxyurea was approved in the United States for children aged 2 years or older in 2017 based on findings from an open-label, single-arm trial that showed significant decreases in acute vaso-occlusive events and transfusion requirements.[16] Although

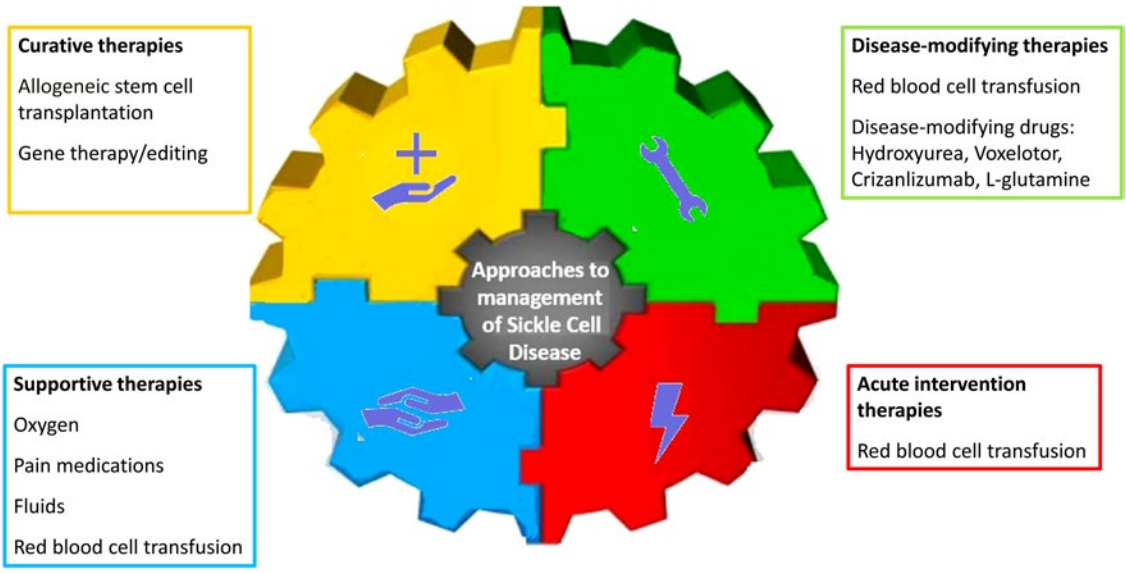

Figure 1. Approaches to the management of SCD. Of the disease-modifying therapies, 4 drugs, hydroxyurea, L-glutamine, crizanlizumab, and voxelotor, are currently approved by the FDA. In the absence of long-term data, gene therapy and gene editing are referred to as "potentially curative therapies."

Table 1. **Major historical acute intervention and prevention trials for VOCs in SCD**

Acute intervention trials for VOCs				Prevention trials for VOCs			
Drug	**Primary efficacy end point**	**Results**	**Reference**	**Drug**	**Primary efficacy end point**	**Results**	**Reference**
Cetiedil	Effect on course of pain crisis	Cetiedil (0.4 mg/kg) significantly reduced the number of painful sites on all treatment days and shortened the total time in crisis.	Benjamin et al[57]	Sodium cyanate	Effect on hemolytic rate and frequency of crisis	Decrease in the hemolytic rate (evidenced by increased Hb) and mean frequency crisis was seen in patients receiving cyanate. Increase in Hb was directly proportional to the amount of carbamylation achieved. Frequency of crisis was decreased in patients with higher carbamylation.	Gillette et al[58]
Methylprednisolone	Effect on duration and severity of pain crisis	Reduced duration of inpatient analgesic therapy, but majority of patients were readmitted for recurrent pain.	Griffin et al[59]	Ticlopidine	Effect on pain crisis	Treatment with ticlopidine decreased the number of VOCs, mean duration of VOCs, and severity of VOCs.	Cabannes et al[50]
Purified poloxamer-188	Effect on duration of pain crisis	In an earlier study, poloxamer-188 significantly reduced the duration of VOC, especially in children (≤15 years) and patients receiving concurrent hydroxyurea. Subsequent study showed no significant difference in the mean time to the last dose of parenteral opioids between poloxamer-188 and placebo.	Orringer et al[34]; Casella et al[35]	Hydroxyurea[a]	Effect on frequency of painful crises in adults and children	Treatment with hydroxyurea significantly reduced VOCs, hospitalizations due to VOCs, ACS, and blood transfusion compared to placebo.	Charache eta al[13]; Wang et al[14]
Inhaled NO	Effect on time to resolution of VOC	An earlier study showed significantly less morphine use over 6 hours but no difference between inhaled NO and placebo on duration of hospitalization. A subsequent larger study showed no significant difference in median time to resolution of VOC, length of hospitalization, or median opioid usage between inhaled NO and placebo.	Gladwin et al[41]; Weiner et al[60]	Senicapoc	Effect on frequency of painful acute sickle cell–related crises	Phase 2 study showed a dose-dependent increase in Hb with senicapoc vs placebo. Phase 3 study showed increase in Hb but no significant reduction in VOC compared to placebo.	Ataga et al[25]
Tinzaparin	Effect on painful crisis	Tinzaparin significantly reduced the severity and duration of VOC and duration of hospitalization.	Qari et al[53]	Prasugrel	Rate of VOC (composite of painful crisis or ACS)	No significant difference in the rate of VOC between prasugrel and placebo.	Heeney et al[51]
Arginine	Efficacy in children requiring hospitalization for severe pain necessitating parenteral narcotics	Arginine significantly reduced total analgesic usage, pain scores, time to crisis resolution, and total length of hospital stay.	Morris[61]	L-glutamine[a]	Number of pain crises	Treatment with L-glutamine resulted in reduced VOC, hospitalizations, and ACS compared to placebo.	Niihara et al[27]

Table 1. Major historical acute intervention and prevention trials for VOCs in SCD (*Continued*)

Acute intervention trials for VOCs				Prevention trials for VOCs			
Drug	**Primary efficacy end point**	**Results**	**Reference**	**Drug**	**Primary efficacy end point**	**Results**	**Reference**
Sevuparin	Effect on duration of VOC in hospitalized patients	No difference in time to VOC resolution or discontinuation of IV opioids between sevuparin and placebo.	Biemond[33]	Crizanlizumab[a]	Annual rate of sickle cell–related pain crises	In the phase 2 SUSTAIN trial, crizanlizumab significantly decreased median rate of VOC and increased median times to first and second VOC. In the phase 3 STAND trial, no significant difference in the annualized rates of VOC was seen with crizanlizumab compared to placebo.	Ataga et al[29]; Novartis AG[30]
Rivipansel	Effect on time to resolution of VOC	In phase 2 trial, treatment with rivipansel significantly reduced cumulative IV opioid dose and resulted in clinically meaningful reduction in time to crisis resolution. In the phase 3 trial, no significant benefit was seen in shortening the time to readiness for discharge, time to discharge, or discontinuation of IV opioids.	Telen et al[31]; Dampier et al[32]	NAC	Effect on frequency of SCD pain days	No reduction in the rate of SCD-related pain days per patient-year, hospital admission days, number of admissions, or days with home analgesic use with NAC+ compared to placebo.	Sins et al[28]
Regadenoson	Effect on reduction in invariant natural killer T-cell activation in patients admitted for acute pain crisis	Regadenoson infusion did not decrease the hospitalization duration, total opioid use, or pain scores compared with placebo.	Field et al[37]	Canakinumab	Effect on change in average daily pain scores	No decrease in daily SCA-related pain, but canakinumab significantly reduced markers of inflammation compared with placebo.	Rees et al[39]
Magnesium	Effect on length of stay in patients hospitalized for VOC	IV magnesium use did not shorten the length of hospitalization, reduce opioid use, or improve quality of life compared to placebo.	Brousseau et al[26]	Ticagrelor	Effect on the rate of VOCs (composite of painful crises and/or ACS)	No reduction in VOC with ticagrelor compared to placebo.	Heeney et al[52]

ACS, acute chest syndrome; Hb, hemoglobin; IV, intravenous; NAC, N-acetyl cysteine; SCA, sickle cell anemia; VOC, vaso-occlusive crisis.

[a]Clinical trials resulted in approval of these drugs by the FDA.

no significant differences were observed in the intention-to-treat analysis, hydroxyurea reduced the risk of conversion from conditional to abnormal transcranial Doppler (TCD) velocity compared with observation (0 vs 50%; $P = .02$) in post–host analysis of a multicenter trial.[17] In individuals with abnormal TCD on chronic blood transfusion and no severe vasculopathy, hydroxyurea was also noninferior to chronic red blood cell (RBC) transfusion in preventing stroke.[18] In this trial the final model-based TCD velocities for standard transfusions vs hydroxyurea were 143 cm/s (95% CI, 140-146) vs 138cm/s (95% CI, 135-142), with a difference of 4.54 (95% CI, 0.10-8.98; $P = 8.82 \times 10^{-16}$ for noninferiority, and $P = .023$ for superiority post hoc). In the REACH study, the treatment of 606 children from 4 sub-Saharan countries with hydroxyurea significantly increased total Hb (an increase of 1.0 g/dL; 95% CI, 0.8-1.0) and HbF levels (an increase of 12.5%; 95% CI, 11.8-13.1) and decreased the rates of vaso-occlusive pain (98.3 vs 44.6 events per 100 patient-years; incidence rate ratio [IRR], 0.45; 95% CI, 0.37-0.56), RBC transfusions (43.3 vs 14.2 events per 100 patient-years; IRR, 0.33; 95% CI, 0.23-0.47), and mortality (3.6 vs 1.1 deaths per 100 patient-years; IRR, 0.30; 95% CI, 0.10-0.88) when compared with the pretreatment period.[19] In a double-blind, parallel-group, phase 3 trial of 220 children with sickle cell anemia and abnormal TCD velocities conducted in Nigeria, no significant difference was seen in the stroke incidence rate with low-dose hydroxyurea (10 mg/kg) compared with moderate-dose hydroxyurea (20 mg/kg), although the incidence rate for all-cause hospitalization was lower with moderate-dose hydroxyurea.[20] Furthermore, no significant difference in the incidence rates of the primary outcome measures (stroke, transient ischemic attack, and death) was seen with low-dose vs moderate-dose hydroxyurea for secondary prevention of stroke.[21]

Voxelotor is an HbS polymerization inhibitor that reversibly binds to Hb and stabilizes it in the oxygenated (relaxed) state.[22] In the multicenter phase 3 HOPE study, 274 patients (12 years and older) with SCD were randomized to receive a daily dose of 1500 mg of voxelotor, 900 mg of voxelotor, or placebo for 72 weeks. In the intention-to-treat analysis, 51% (95% CI, 41-61) of participants who received 1500 mg/d achieved a Hb increase of greater than 1 g/dL from baseline after 24 weeks of therapy compared to 7% (95% CI, 1-12) in the placebo group. Treatment with voxelotor at 1500 mg/d also resulted in a significant increase in Hb (mean change, 1.14 g/dL vs −0.1 g/dL; $P < .001$) and significant decreases in indirect bilirubin (mean change, −29.1% vs −3.2%; $P < .001$) and percent reticulocyte count (mean change, −19.9% vs 4.5%; $P < .001$) compared to placebo.[23] Similarly, a Hb increase of more than 1 g/dL from baseline after 24 weeks of voxelotor was observed in 47% of children with HbSS or HbSβ^0 in the HOPE KIDS-1 trial.[24]

Senicapoc, a potent blocker of the Gardos channel, a calcium-activated potassium channel of intermediate conductance in RBCs, improves RBC hydration by reducing the loss of solute and water. However, an increase in Hb (mean change, 0.59 g/dL vs −0.1 g/dL; $P < .001$) and decreases in percent reticulocyte count (mean change, −2.46% vs −0.79%; $P < .001$) and indirect bilirubin (mean change −16.6 µmol/L vs −0.3 µmol/L; $P < .001$), both markers of hemolysis, following senicapoc administration were not accompanied by a significant reduction in acute pain episodes.[25] Magnesium inhibits K^+ efflux through the potassium chloride cotransport channel in RBCs and consequently prevents RBC dehydration. Treatment with intravenous (IV) magnesium when compared with placebo did not shorten the length of hospitalization (median, 56 hours; interquartile range [IQR], 27.0-109.0 vs 47 hours [IQR, 24.0-99.0]; $P = .24$), reduce opioid use (median, 1.46 mg/kg vs 1.28 mg/kg morphine equivalents; $P = .12$), or improve quality of life in children and young adults who were hospitalized for acute pain episodes.[26] Table 2 lists actively recruiting studies of antisickling agents.

Antioxidant, antiadhesive, and anti-inflammatory agents

Oxidative stress is a major contributor to the pathophysiology of SCD. L-glutamine, a conditionally essential amino acid, is a precursor for nicotinamide adenine dinucleotide (NAD), increases the NAD redox ratio within sickle RBCs, and may improve RBC health by reducing oxidative stress. In a multicenter, placebo-controlled trial of 230 patients with HbSS or HbSβ^0, L-glutamine, administrated twice daily, significantly reduced acute pain episodes (3.0 vs 4.0; $P = .005$) and hospitalizations (2.0 vs 3.0; $P = .005$) compared to placebo.[27] Treatment with L-glutamine also resulted in a significantly lower cumulative number of hospital days and fewer occurrences of ACS compared with placebo. Treatment with another antioxidant, N-acetylcysteine (NAC), at a dose of 600 mg twice a day for 6 months did not significantly decrease the rate of SCD-related pain days per patient-year, days of hospital admission, number of admissions, or days with home analgesic use compared with placebo.[28]

Antiadhesion agents may improve flow in the microvasculature by reducing abnormal cell-cell (RBC, leukocyte, platelet, endothelial cell) interactions. Crizanlizumab, a humanized monoclonal anti–P-selectin antibody, blocks the interaction between P-selectin (expressed on activated endothelial cells and platelets) and P-selectin glycoprotein ligand 1. In the SUSTAIN trial, a multicenter, randomized, placebo-controlled phase 2 trial, crizanlizumab administered at a dose of 2.5 mg/kg or 5 mg/kg via IV every 4 weeks (following an initial loading dose) in a 52-week period was compared with placebo in individuals with SCD.[29] Treatment with high-dose crizanlizumab (5 mg/kg) resulted in a significantly lower median rate of painful crisis (1.63 vs 2.98; $P = .01$), a lower median rate of uncomplicated crisis (1.08 vs 2.91; $P = .02$), and longer median times to occurrence of the first (4.07 months vs 1.38 months; $P = .001$) and second pain crises (10.32 vs 5.09 months; $P = .02$). The benefit in reducing pain crisis was observed regardless of the ongoing use of hydroxyurea, pain crisis frequency in the previous 12 months, or SCD genotype. However, recently reported results from the phase 3 STAND trial showed no significant difference between either crizanlizumab at 5 mg/kg or 7.5 mg/kg and placebo on the annualized rates of VOC leading to a healthcare visit over the first year postrandomization, although there were no new safety concerns.[30] Based on these results, the European Medicines Agency's Committee for Medicinal Products for Human Use concluded that the benefits of crizanlizumab do not outweigh its risks and recommended the revocation of its marketing authorization in the European Union.

Rivipansel (previously called GMI-1070) is a small-molecule panselectin inhibitor with highest affinity to E-selectin. In a multicenter phase 2 trial, rivipansel reduced the cumulative IV opioid dose during acute pain episodes by 83% compared to placebo.[31] However, in a multicenter, placebo-controlled phase 3 trial (RESET), no benefit was seen with rivipansel in shortening the times to readiness for discharge, hospital discharge, or discontinuation of IV opioids.[32] In a post hoc analysis, the early initiation

Table 2. Actively recruiting clinical trials of antisickling agents in SCD

Mechanism	Drug	Sponsor	NCT number (study acronym)	Clinical phase	Study design/Intervention	Number/age	Outcomes
HbF induction	Hydroxyurea	ADDMEDICA SASA	NCT03806452 (SIKAMIC)	Phase 2	Oral 15 mg/kg/d for 6 mo vs placebo	120/ ≥ 18y	Proportion of patients with at least a 30% decrease in ACR, mean change in GFR, change in ACR, systolic blood pressure, adverse events.
		Children's Hospital Medical Center, Cincinnati	NCT03789591 (HOPS)	Phase 3	Starting hydroxyurea dose 20mg/kg/d vs PK-guided initial hydroxyurea dose	116/6 mo-21y	Evaluate HbF, F cells, gene-expression patterns
			NCT02286154 (TREAT)	N/A	Open-label, single-arm study Old cohort: includes participants already on hydroxyurea upon study entry New cohort: includes participants starting hydroxyurea with starting dose predicted using PK/PD data	150/6 mo-21y	Evaluate time to reach maximum tolerated dose, hydroxyurea adherence, neurological function (TCD), splenic (pit count), kidney (BUN/creatinine, urinalysis, cystatin-c) and cardiac function (echo/ECG)
	Nicotinamide vs THU and decitabine	EpiDestiny Inc; National Institutes of Health; NHLBI	NCT04055818	Phase 1	Oral nicotinamide vs THU plus decitabine for 12 wk followed by combination for a further 12 wk	20/ ≥18y	Compare effect of oral nicotinamide vs THU-decitabine and in combination on Hb level at week 12
Allosteric modifier (to the R-state)	Voxelotor (formerly GBT440)	Global Blood Therapeutics	NCT02850406 (HOPE Kids)	Phase 2a	Oral, open-label, single- and multiple-doses study Part A: single dose Part B: 24 wk Part C: 48 wk	125/4–17y	Pharmacokinetics, change in Hb, effect on hemolysis, TCD velocity, safety
			NCT04188509	Phase 3	Oral, open-label	50/4–18y	Evaluate safety and tolerability, SCD-related complications
			NCT04335721	Phase 1/2	Open label, voxelotor vs standard of care (observation)	12/ ≥ 18y	Evaluate change in albuminuria and other kidney function measures (24-h urine protein, eGFR, serum creatinine, serum cystatin C)
			NCT05561140 (RESOLVE)	Phase 3	Oral daily voxelotor vs placebo for 12 wk	80/ ≥ 12y	Evaluate effect on healing of leg ulcers, time to resolution of target ulcer, change in total surface area of target ulcer, and incidence of new ulcers
			NCT05228834	Phase 3	Oral daily voxelotor vs placebo for 12 wk	80/8–17y	Evaluate change in executive abilities composite score, processing speed, nonexecutive cognitive abilities, change in hematological parameters, HRQOL score
		Robert Clark Brown	NCT05018728	Phase 2	Oral daily voxelotor×12 wk	50/4–17y	Change in cerebral blood flow, oxygen extraction fraction, cerebral metabolic rate of oxygen, Hb, voxelotor-modified Hb
		Emory University	NCT05018728 (VoxSCAN)	Phase 2	Open label, once daily for 12 wk	12/4–17y	Evaluate effect on cerebral hemodynamics including cerebral blood flow, oxygen extraction fraction.
	GBT021601–012	Global Blood Therapeutics	NCT05431088 (GBT021601–021)	Phase 2/3	Initial 1:1 randomization to 100mg and 150mg, after review of safety data of 150mg, then randomization 1:1:1 to 100mg, 150mg, and 200mg	480/6 mo-65y	Safety, pharmacokinetics, proportion of participants with increase in Hb >1 gm/dL at week 48

Table 2. Actively recruiting clinical trials of antisickling agents in SCD (*Continued*)

Mechanism	Drug	Sponsor	NCT number (study acronym)	Clinical phase	Study design/Intervention	Number/age	Outcomes
Allosteric activator of RBC pyruvate kinase-R	Mitapivat sulfate (AG-348)	Agios Pharmaceuticals	NCT05031780 (RISE UP)	Phase 2/3	Phase 2: oral, twice daily 50-mg dose vs 100-mg dose vs placebo×12 wk followed by open-label extension period for 216 wk Phase 3: based on phase 2 results either 50mg or 100mg twice daily vs placebo for 52 wk followed by open label extension period for 216 wk	267/ ≥ 16y	Safety, adverse events, change in Hb, effect on hemolysis, effect on annualized rate of pain crisis, pharmacokinetics, pharmacodynamics, QOL measures
	AG- 946	Agios Pharmaceuticals	NCT04536792	Phase 1	Single and multiple ascending dose study Part 1: single dose Part 2: once daily for 14 d Part 3: once daily for 28 d	64/18–70y	Safety, tolerability, pharmacokinetics, pharmacodynamics
	Etavopivat (FT 4202)	Forma Therapeutics	NCT04987489	Phase 2	Open label, 400mg once daily in transfusion-dependent sickle cell participants and in transfusion and non–transfusion dependent thalassemia participants	60/12–65y	Safety, evaluate proportion of participants with reduction in blood transfusion requirement, change in Hb, ferritin, and liver iron concentration
			NCT04624659 (HIBISCUS)	Phase 2/3	Phase 2: once daily, 200-mg dose vs 400-mg dose vs placebo for 24 wk Phase 3: based on phase 2 results either 200mg or 400mg once daily vs placebo for 28 wk, followed by 52-wk open-label extension period	344/12–65y	Safety, change in Hb, markers of hemolysis, effect on annualized pain crisis rate, patient reported outcome measures (PROMIS)

ACR, albumin creatinine ratio; BUN, serum urea nitrogen; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate;

HRQOL, health-related quality of life; N/A, not available; PK/PD, pharmacokinetics/pharmacodynamics; R-state, relaxed state; QOL, quality of life; THU, tetrahydrouridine.

of rivipansel within 26.4 hours of crisis onset resulted in clinically meaningful reductions in median time to readiness for discharge by 56.3 hours (from 122.0 to 65.7 hours; hazard ratio [HR], 0.58; $P = .033$), median time to discharge by 41.5 hours (from 112.8 to 71.3 hours; HR, 0.54; $P = .010$), and time to discontinuation of IV opioids by 50.5 hours (from 104.0 to 53.5 hours; HR, 0.58; $P = .026$), compared with placebo.[32] Sevuparin, a low-molecular-weight heparin-derived polysaccharide with antiadhesive properties but minimal anticoagulant activity, did not shorten the times to VOC resolution or discontinuation of IV opioids vs placebo when administered during acute pain episodes.[33]

Poloxamer-188, a nonionic block copolymer surfactant that improves microvascular blood flow and reduces hydrophobic cell-cell interactions, has been evaluated in patients hospitalized for acute pain episodes. In an earlier study, poloxamer-188 significantly shortened the duration of acute pain episodes when compared with placebo (mean, 133 hours [standard deviation, 41] vs 141 hours [standard deviation, 42]; $P = .04$).[34] However, with an absence of documentation of the study's crisis resolution criteria in approximately 24% of participants (30% in the placebo arm vs 18% in the poloxamer-188 arm), another trial was conducted. This subsequent study showed no significant difference in the time to discontinuation of IV opioids when poloxamer-188 was compared to placebo (81.8 hours vs 77.8 hours; $P = .09$).[35]

Multiple agents have been evaluated to mitigate the effects of inflammation in SCD.[36] Regadenoson, a partially selective adenosine A_{2A} receptor agonist that decreases the activation of invariant natural killer T cells, did not decrease the duration of hospitalization (3.96 days vs 3.99 days; $P = .80$), total opioid use (median morphine equivalent dose, 0.03 mg/kg/h vs 0.04 mg/kg/h; $P = .34$), or pain scores (−2.68 vs −2.80; $P = .91$) when compared with placebo.[37] Treatment with SC411, a docosahexaenoic acid ethyl ester formulation, for 8 weeks significantly reduced levels of D-dimer ($P = .025$) and soluble E-selectin ($P = .0219$) and increased Hb (mean change, 0.97 g/dL vs 0.33 g/dL; $P = .04$) when compared with placebo.[38] Canakinumab (ACZ885), a monoclonal anti–interleukin 1 beta antibody, was well tolerated in a 6-month study. Although the trial did not achieve its prespecified primary end point for diary-reported daily pain scores, treatment with canakinumab resulted in reductions in markers of inflammation (high-sensitivity C-reactive protein, absolute counts of leukocytes, monocytes) and number/duration of hospitalizations as well as trends for improvement in pain intensity, fatigue, and absences from school or work when compared with patients in the placebo arm.[39] Montelukast, a leukotriene inhibitor, did not significantly decrease levels of soluble vascular cell adhesion molecule 1 and reported pain when compared to placebo following 8 weeks of treatment.[40] Actively recruiting studies of antioxidants, antiadhesive and anti-inflammatory agents are shown in Table 3.

Nitric oxide and related agents, antiplatelet agents, and anticoagulants

Abnormalities of the nitric oxide (NO)-cyclic guanosine monophosphate (cGMP)-dependent signaling pathway may play a role in the inflammation and vascular dysfunction seen in SCD. As a result of ongoing hemolysis, scavenging of NO, and subsequent endothelial dysfunction, NO and related agents may provide benefit to patients with SCD. In a phase 2 multicenter study of SCD patients experiencing acute vaso-occlusive episodes, inhaled NO did not significantly shorten the median time to resolution of vaso-occlusive episodes (73 hours [95% CI, 46.0-91.0] vs 65.5 hours [95% CI, 48.1-84.0]; $P = .87$) or the median length of hospitalization (4.1 days [IQR, 2-6] vs 3.1 days [IQR, 1.7-6.4]; $P = .30$) or reduce the median opioid usage (2.8 mg/kg [1.4-6.1] vs 2.9 mg/kg [1.1-9.9]; $P = .73$) or the rate of ACS when compared to placebo.[41] In a separate study, inhaled NO did not reduce the rate of treatment failure in adult patients with mild to moderate ACS.[42] L-arginine is an obligate substrate for NO production. In a multicenter, double-blind, placebo-controlled study of children in Nigeria, oral L-arginine therapy administered within 6 hours of presentation for a pain crisis significantly reduced total analgesic usage, quantified using the mean analgesic medication quantification scale (73.4 [95% CI, 62.4-84.3] vs 120.0 [96.7-143.3]; $P < .001$), pain scores (1.50 [1.23-1.77] vs 1.09 [0.94-1.24]; $P = .009$), time to crisis resolution (75.8 ± 36 hours [95% CI, 63.4-88.2] vs 93.3 ± 32.7 hours [95% CI, 81.7-104.9]; $P = .02$), and the total length of hospital stay (105 hours [IQR, 72-144] vs 141 hours [IQR, 117-205]; $P = .002$).[43] However, patients treated with sildenafil, a phosphodiesterase-5 inhibitor that increases NO-mediated effects by inhibiting cGMP degradation, experienced more serious adverse events, predominantly hospitalization for pain, but no clinical benefits when compared to placebo.[44]

Platelet activation occurs in SCD at steady state, with further activation during acute pain episodes.[45-49] Although ticlopidine was previously shown to decrease the number, the mean duration, and the severity of acute pain episodes,[50] more recent phase 3 trials of the newer-generation $P2Y_{12}$ receptor blockers, prasugrel and ticagrelor, did not show a benefit in reducing the frequency of vaso-occlusive episodes compared to placebo.[51,52] Tinzaparin, a low-molecular-weight heparin, significantly reduced the durations of acute pain episodes (mean difference in duration of painful crises, −1.78 days; 95% CI, −1.94 to −1.62; $P < .0001$) and hospitalization (mean difference in duration of hospitalization, −4.98 days; 95% CI, 5.48 to −4.48; $P < .0001$) when compared to placebo.[53] However, it is uncertain whether the beneficial effects were a result of its anticoagulant or antiadhesive effects. Table 4 shows actively recruiting trials of NO and related agents, antiplatelet agents, and anticoagulants.

Our approach to the use of approved drugs

As most patients have limited access to curative therapies, pharmacotherapy may offer the best hope for improved patient outcomes at this time. In the absence of clinical trials comparing available drugs, the choice of initial therapy may be guided by a patient's clinical presentation as well as the availability and cost of drugs (Table 5). Patients with frequent vaso-occlusive complications (such as acute pain episodes or ACS) may obtain benefit from the use of hydroxyurea, L-glutamine, and crizanlizumab, while hemolytic anemia may be improved with the use of hydroxyurea and voxelotor. Despite the negative results of the STAND trial, we continue to use crizanlizumab on a case-by-case basis as several studies other than the SUSTAIN trial suggest a benefit to decreasing the frequency of painful episodes leading to health center visits.[54-56] As approved drug therapies have limited clinical efficacy, most complications related to SCD are unlikely to be ameliorated by a single drug. Consequently, patients are most likely to obtain maximum benefit using a combination of drugs with different mechanisms of action and nonoverlapping side effects. While more data are necessary to evaluate the effects of drug combinations, previous studies of

Table 3. Actively recruiting clinical trials of antioxidants, antiadhesive, and anti-inflammatory agents in SCD

Mechanism	Drug	Sponsor	NCT number (study acronym)	Clinical phase/status	Study design/intervention	Number/age	Outcome
P-selectin antagonist	Crizanlizumab	Novartis Pharmaceuticals	NCT03938454 (SPARTAN)	Phase 2	IV infusion every 2 wk for first month and then every 4 wk × 51 wk	56/ ≥16y	Evaluate efficacy in priapism, uncomplicated VOC events
			NCT04657822	Phase 4	Open-label extension study, IV infusion every 2 wk for first month and then every 4 wk	130/all ages	Evaluate the frequency of treatment-related adverse events
			NCT03474965	Phase 2	Open label extension study, IV infusion every 2 wk for first month and then every 4 wk	119/6 mo-17y	Pharmacokinetics, pharmacodynamics and safety, pain crisis rate
	Inclacumab	Global Blood Therapeutics	NCT04927247	Phase 3	Single IV dose (30 mg/kg) of inclacumab vs placebo after VOC event (that required hospitalization and IV pain medication)	280/ ≥12y	Evaluate proportion of participants with readmission for VOC within 90 d, time to readmission, pharmacodynamics
			NCT04935879	Phase 3	Inclacumab (30 mg/kg) vs placebo administered IV every 12 wk for 48 wk	240/ ≥12y	Safety and effect on VOC including frequency of VOC during treatment period, time to first and second VOC, hospitalization duration
			NCT05348915	Phase 3	Open-label extension study, inclacumab (30 mg/kg) IV every 12 wk	520/ ≥12y	Safety, evaluate annualized rate of VOC, hospitalizations, complicated VOCs, transfusions, pharmacokinetics
Blockade of FCγRIII receptors	IVIG	Albert Einstein College of Medicine	NCT01757418	Phase 1/2	Single dose of IVIG vs placebo given within 24 h of hospitalization	94/12–65y	Length of VOC, total opioid use, time to end of VOC, in vitro adhesion studies
Antioxidant	Glutamine	Ain Shams University	NCT05371184	Phase 4	0.3 gm/kg/dose twice daily orally (up to a maximum of 15 g/dose) for 24 wk	30/2–18y	Incidence of pain crisis, change in transcranial Doppler
Anti-inflammatory	Rifaximin (antibiotic, decrease aged neutrophils)	Bausch Health Americas Inc	NCT05098028	Phase 2a	Oral, extended release (high and low dose) vs delayed extended release (high and low dose) vs placebo	60/18–70y	Evaluate pharmacokinetics and pharmacodynamics
	Crovalimab (anticomplement C5 monoclonal antibody)	Hoffmann-La Roche	NCT04912869 (CROSSWALK-a)	Phase 1	Single IV infusion of crovalimab vs placebo for management of uncomplicated VOC	30/12–55y	Safety, adverse events, pharmacokinetics, pharmacodynamics, time to resolution of VOC, cumulative opioid dose, time to discontinuation of parenteral opioids, percentage of participants with VOC complication (ACS, ICU, blood transfusion)
			NCT05075824 (CROSSWALK-c)	Phase 2	IV loading dose of crovalimab on day 1, followed by SQ dose day 2 and then weekly for 3 wk. Monthly maintenance SQ dosing starting week 5 for 48 wk vs placebo.	90/12–55y	Evaluated effect on frequency of VOC events, ACS events, duration of hospitalization, change in urine albumin-creatinine ratio, tricuspid regurgitant jet velocity, and PROMIS score

Table 3. Actively recruiting clinical trials of antioxidants, antiadhesive, and anti-inflammatory agents in SCD (*Continued*)

Mechanism	Drug	Sponsor	NCT number (study acronym)	Clinical phase/status	Study design/intervention	Number/age	Outcome
	Tocilizumab (anti-interleukin 6)	University of Chicago	NCT05640271	Phase 2	Single 80-mg IV dose at time of ACS diagnosis followed by placebo (50mL normal saline) 48h later. Control arm receives placebo first followed by tocilizumab 48h later.	200/ ≥ 18y	Changes in peripheral oxygen saturation, changes in the route, rate, and FiO2 of supplemental oxygen delivery from day 0 to day 4. The time-weighted SaO2/FiO2 ratio will be calculated based on this.
	ALXN1820 (bispecific-antiproperdin antibody)	Alexion	NCT05565092 (PHOENIX)	Phase 2a	Open-label study to evaluate multiple dosing regimens (i) 300mg SQ weekly, (ii) 600mg SQ every 4 wk, (iii) 300mg SQ every 2 wk	30/18–65y	Safety, pharmacokinetics, changes from baseline in Hb, hemolysis markers, hemopexin, complement activity, and concentration of properdin and complement biomarkers

FiO2, fraction of inspired oxygen; ICU, intensive care unit; IVIG, intravenous immunoglobulin; PROMIS, patient-reported outcome measures; SaO2, oxygen saturation; SQ, subcutaneous.

Table 4. Actively recruiting clinical trials of NO and related agents, antiplatelet agents, and anticoagulants in SCD

Mechanism	Drug	Sponsor	NCT number (study acronym)	Clinical phase/status	Study design/intervention	Number/age	Outcome
Increased NO production	Arginine	Emory University	NCT02447874	Phase 1/2	IV infusion 3 times a day for maximum of 7 d	21/7–21y	Pharmacokinetics, NO metabolites
			NCT04839354 (STArT trial)	Phase 3	One-time L-arginine loading dose (200mg/kg IV) + standard dose (100mg/kg IV 3 times a day)	360/3–21y	Evaluate change in time to crisis resolution, pain scores, total parenteral opioid use, PROMIS pain interference, pain-behavior and fatigue score
	L-citrulline	Asklepion Pharmaceuticals	NCT04852172	Phase 1/2	IV infusion (bolus + continuous infusion for 7h) during VOC Part 1: identify optimum dose regime Part 2: doses selected from part 1 vs placebo	120/6–21y	Pharmacokinetics, adverse events, effect on VOC including amount of overall opioid use, time to resolution of VOC

Table 5. Summary characteristics of FDA-approved drugs for SCD

	Hydroxyurea	L-glutamine	Crizanlizumab	Voxelotor
Age (years)	≥2	≥5	≥16	≥4
Genotypes	HbSS, HbSβ^0 thalassemia	All genotypes (only studied in HbSS, HbSβ^0 thalassemia)	All genotypes	All genotypes (majority with HbSS, HbSβ^0 thalassemia)
Mechanism of action	Multiple, but primarily by increasing HbF production	Uncertain, but thought to increase NAD redox potential; may decrease cell adhesion	Anti–P-selectin inhibitor (decreases adhesion of WBC, RBC to endothelium and possibly of platelets to WBC)	Decreases HbS polymerization by increasing Hb-oxygen affinity
Route of administration	Oral (capsules/tablets)	Oral (powder)	IV	Oral (tablets)
Clinical effects of therapy	Decreased frequency of VOC, decreased frequency of ACS, decreased hospitalization, decreased RBC transfusion requirement, decreased stroke risk	Decreased frequency of VOC, decreased frequency of ACS, decreased hospitalization	Decreased frequency of VOC in phase 2 SUSTAIN trial. Results of recent phase 3 STAND trial showed no benefit.	Increased Hb
Effect size for primary end point (NNT)	44% decrease in VOC per year (median from 4.5 to 2.5); IRR, 0.56	25% decrease in VOC in 48 wk (median from 4 to 3); IRR, 0.75	45% decrease in crisis rate per year (median from 3 to 1.6); IRR, 0.55	7-fold increase in the Hb responders (7 to 51) at 24 wk, incidence proportion ratio=7.3[a]
Common toxicities	Myelosuppression, skin hyperpigmentation, nail discoloration, teratogenicity, decreased sperm counts, nausea and vomiting	Constipation, nausea, headaches, abdominal pain	Nausea, arthralgia	Headache, diarrhea, nausea
Pharmacokinetics	Excreted via kidneys. Adjust dose for eGFR <60 mL/min/1.73m^2	Use with caution with hepatic and renal impairment, but no recommended dose adjustment	No dosage adjustments in manufacturer labeling for renal and hepatic impairment (not tested in ESRD)	No dosage adjustment for renal impairment, but not yet studied in ESRD requiring dialysis. Dose reduction for severe liver disease (Child Pugh class C)
Cost	$	$$$	$$$$$	$$$$$

ACS, acute chest syndrome; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; HbF, fetal hemoglobin; HbS, sickle hemoglobin; IV, intravenous; NAD, nicotinamide adenine dinucleotide; NNT, number needed to treat; VOC, vaso-occlusive crisis.

[a]Patients treated with 1500 mg of voxelotor had 7.3 times the increased proportion of Hb responders (>1 g/dL increase from baseline at 24 weeks).

Data adapted from Rai and Ataga.[62]

L-glutamine, crizanlizumab, and voxelotor showed that these agents in combination with hydroxyurea were beneficial, without increased toxicity.

CLINICAL CASE (continued)

In the absence of identifiable precipitating factors and following confirmation of adherence with hydroxyurea, other treatment options for acute pain episodes were extensively discussed. While continuing hydroxyurea, the patient began on L-glutamine because he wished to avoid monthly infusion clinic visits. He was, however, switched to crizanlizumab due to poor tolerance of L-glutamine. He experienced a substantial reduction in the frequency of acute pain episodes over the next year.

Conclusion

Although SCD is an orphan disease in the United States, it is common worldwide. With advances in the understanding of disease pathophysiology, multiple drugs have been approved by regulatory agencies, with more in various stages of clinical testing. The development of new drugs for SCD offers opportunities to test drug combinations in the hope of improved clinical outcomes. Although the majority of drug trials in SCD have evaluated acute pain episodes as the primary clinical end point, other SCD-related complications and surrogate end points are increasingly being assessed. Demonstrating the benefit of drug therapies on end-organ dysfunction in SCD will provide further evidence for their role in improving patient outcomes.

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Parul Rai: consultancy: Global Blood Therapeutics.
Kenneth I. Ataga: research funding: Novartis, Novo Nordisk, Takeda Pharmaceuticals; advisory board member: Novartis,

Novo Nordisk, Fulcrum Therapeutics, Agios Pharmaceuticals, Pfizer; consultancy: Roche, Biomarin; data monitoring committee: Vertex.

Off-label drug use

Parul Rai: nothing to disclose.
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The range of haploidentical transplant protocols in sickle cell disease: all haplos are not created equally

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The ideal curative therapy for sickle cell disease (SCD) must be applicable across all ages and include individuals with strokes and preexisting heart, lung, and kidney disease. Myeloablative, matched sibling donor hematopoietic stem cell transplant (HCT) for children with SCD has shown excellent outcomes over the past 3 decades but has been restricted due to the limited availability of a human leukocyte antigen–matched sibling donor (10%-15%) and increased treatment-related death in adults with myeloablative conditioning. To overcome these 2 significant barriers to curative therapy in SCD, related haploidentical HCT has become an active area of research. The use of related haploidentical donors (first- and second-degree relatives) increases the donor pool to at least 90% of those eligible across the life span. Importantly, most adults, even with strokes or significant comorbidities, can tolerate the nonmyeloablative conditioning regimen without treatment-related death. Since 2013, at least 3 related haploidentical HCT strategies have emerged as potential curative therapies for SCD: (1) a nonmyeloablative, T-cell replete, bone marrow transplant with thiotepa and posttransplant cyclophosphamide with a goal of complete donor chimerism; (2) a nonmyeloablative, in vivo T-cell depletion, using peripheral blood stem cells (PBSCs) with a goal of stable mixed donor-recipient chimerism; and (3) a myeloablative, ex vivo T-cell depletion using PBSCs and advanced-technology graft manipulation, with a goal of complete donor chimerism. We review the similarities, differences, outcomes, and gaps in knowledge with these 3 haploidentical HCT approaches for SCD.

LEARNING OBJECTIVES

- Understand the evolution of current haploidentical stem cell transplant as a curative modality for sickle cell disease
- Determine the indication for haploidentical stem cell transplant for sickle cell disease
- Distinguish the different types of haploidentical stem cell transplants and evaluate their outcomes for sickle cell disease
- Describe pros and cons of the 2 prominent haploidentical stem cell transplant platforms to cure individuals with sickle cell disease

Introduction

Sickle cell disease (SCD) is no longer associated with early mortality for children living in high-income countries, with more than 98% living to 18 years of age.[1,2] Despite the dramatically increased survival in children with sickle cell anemia (SCA), overt strokes and silent cerebral infarcts remain the most common cause of progressive neurologic injury, even after starting regular blood transfusion therapy with a goal of keeping the hemoglobin >9.0 g/dL and HbS <30%.[3,4]

In contrast, adults with SCD have a significant risk of earlier death with no meaningful increase in median survival over 25 years. In a recent national cohort of adults with SCD receiving care based on Medicare and Medicaid claims data (2008-2016) in all 50 states, including 94,616 individuals, life expectancy at birth was only 52.6 years (95% CI, 51.9-53.4).[5] Further, a 2-center cohort of adults with SCD receiving medical care at tertiary care centers demonstrated median survival for HbSS/HbSβ^0/HbSD and HbSC/HbSβ$^+$, based on the pooled Kaplan-Meier estimates

and adjusted for entry age, was 48.0 years (95% CI, 44.4-58.4) and 54.7 years (95% CI, 38.6-62.9), respectively.[6]

Major causes of death in adults with SCD include progressive heart, lung, and kidney disease.[7] Pulmonary hypertension occurs in 20% to 40% of adults with SCD, with a 10-fold increase in the risk of premature mortality.[8] In a prospective cohort study of adults with SCA followed for a median of 5.5 years, low forced expiratory volume at 1 second was associated with earlier death.[9] Among adults with SCD, end-stage renal disease occurs in 4.2% with SCA and 2.4% with HbSC disease.[10] However, when present, end-stage renal disease when compared to a reference population is associated with higher mortality risk (hazard ratio, 1.66; 95% CI, 1.36-2.03) and higher hospitalization rates (incidence rate ratio, 2.12; 95% CI: 1.88-2.38), and it is associated with a 26% death risk within 1 year.[11,12] Unfortunately, no US Food and Drug Administration–approved therapy for adults with SCD has been developed to attenuate the progression of heart, lung, and kidney disease in this medically fragile population.

Related haploidentical, hematopoietic stem cell transplant (HCT), initially developed in adults with cancer,[13,14] has been adapted for SCD to overcome the barrier of a limited donor pool with matched sibling donor HCT for SCD. Earlier haploidentical approaches for SCD that used related donors were associated with high graft failure rates of greater than 30% and unacceptably high death rates.[15,16] These SCD haploidentical transplant strategies are evolving and have increased the curative therapy option to at least 90% of those eligible across the life span because of the corresponding increase in the donor pool.

Unfortunately, pooled analyses of haploidentical transplant outcomes in individuals with SCD have provided an outdated perception that this strategy is an unfavorable approach for curative therapy in SCD.[17] This pooled analysis included only 137 participants with SCD who underwent haploidentical-related donor transplantation between 2008 and 2017. During this period, most recipients received different conditioning intensities, graft sources, graft-versus-host disease (GvHD) prophylaxis, and supportive care regimens. While more haploidentical transplants were included in the pooled analysis from 2013 to 2017, 24% (33/137) were done from 2008 to 2012, when haploidentical transplant expertise was limited, and optimal supportive care measures were emerging and not standard care. Also, few participants were enrolled in multicenter trials with rigorous stopping rules and a data safety monitoring board. Importantly, the pooled analysis included early results of the related haploidentical bone marrow transplant (BMT) approaches with posttransplant cyclophosphamide (PTCy) at a stage when the approach was evolving,[16] nor did the results include the more recent alternate haploidentical transplant strategy of ex vivo T-cell depletion with $CD34^+$ selection, $CD3^+/CD19^+$, or T-cell receptor (TCR) $\alpha\beta^+/CD19^+$ depletion.[18-20] Taken together, these limitations attenuate any reasonable inference about haploidentical trial efficacy in curing SCD in the current era.

We review the similarities, differences, outcomes, and gaps in knowledge of recent haploidentical transplant approaches, namely, (1) T-cell replete haploidentical BMT with PTCy, with a goal of complete donor chimerism; (2) in vivo T-cell deplete haploidentical peripheral blood stem cell (PBSC) HCT approach with a goal of stable mixed donor-recipient chimerism; and (3) ex vivo T-cell deplete haploidentical HCT regimens with advanced-technology graft manipulation for SCD with a goal of complete donor chimerism.

CLINICAL CASE

A 34-year-old African American man with homozygous SCD (HbSS) presents with a history of overt stroke at 5 years of age and moyamoya vasculopathy. He was initially on regular blood transfusion therapy, but this was replaced with hydroxyurea therapy because of excessive iron and poor adherence to iron chelation. During hospitalization, he was found on the floor, combative and disoriented with seizure-like activity. The initial head computed tomography scan showed no acute abnormality. Follow-up magnetic resonance imaging of the brain 4 days later showed new acute infarcts in the left parietal, bilateral temporal, and occipital lobes. He was started on regular blood transfusion therapy with a goal HbS% of 15% and a target hematocrit of 28%. He is interested in curative treatment options but does not have a matched sibling or unrelated donor; he had a half-sibling who was a 6/10 haploidentical match.

Preclinical models of cyclophosphamide in HCT

Santos and Owens discovered in the 1960s the immunosuppressive properties of cyclophosphamide.[21] Subsequently, the team developed cyclophosphamide to replace total body irradiation in allogeneic HCT conditioning due to its beneficial effect on GvHD modulation.[22] Further studies on the immunobiology of cyclophosphamide showed that hematopoietic and other tissue stem cells, including memory T cells, express high levels of aldehyde dehydrogenase 1, the body's primary means of inactivating cyclophosphamide, conferring resistance to cyclophosphamide. In contrast, maturing lymphocytes generally express low levels.[23] Aldehyde dehydrogenase 1 inactivates cyclophosphamide by oxidizing the active metabolic aldehyde intermediate aldophosphamide to the inactive carboxylic acid carboxyphosphamide (Figure 1). More recently, investigators using animal models have tried to evaluate the immunologic underpinnings of PTCy and its effects on alloreactive conventional and regulatory T cells.[24-26]

Current haploidentical HCT approaches for SCD

The preclinical studies with PTCy led to phase 1 and 2 haploidentical clinical trials for adults with cancer with the addition of tacrolimus and mycophenolate mofetil for GvHD prophylaxis.[27-29] The beneficial effects of PTCy on GvHD appear to be independent of donor type, graft source, or conditioning regimen intensity,[30] with similar outcomes between non-first-degree and first-degree relatives, increasing the donor pool to an average of 2.7 donors per patient[31] and approximately >90% donor availability for children and adults with SCD. Over the past decade, improvements in transplant technology designed to overcome the human leukocyte antigen barrier have resulted in an alternative to T-cell–replete haploidentical transplant, namely, T-cell–deplete haploidentical HCT using PBSC grafts. This strategy uses advanced biomedical technology for graft

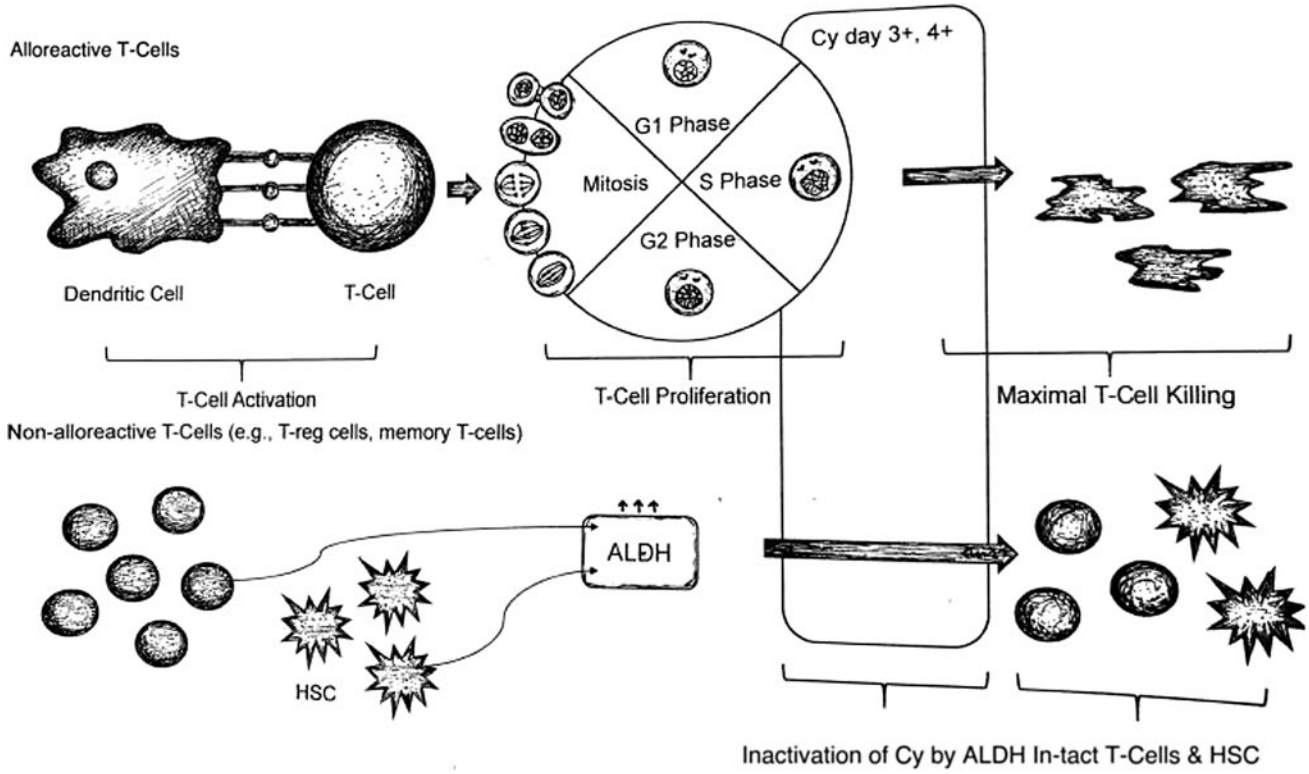

Figure 1. High-dose cyclophosphamide given early after transplant effectively prevents alloreactivity (GvHD and graft rejection) and to spare stem cells, allowing successful mismatched donor transplant. Hematopoietic and other tissue stem cells, including memory T cells, express high levels of aldehyde dehydrogenase 1 (ALDH1), the body's primary means of inactivating cyclophosphamide, whereas mature lymphocytes generally express low levels. Figure reproduced by Andrea Sikora, PharmD with permission; modification from *Expert Rev Hematol*. 2019;12(9):733-752.

manipulations for ex vivo T-cell depletion of GvHD mediating T cells ($CD3^+/CD19^+$ or $TCR\alpha\beta/CD19^+$).[18-20] The goal of therapy is complete donor chimerism.

T-cell replete haploidentical transplant approaches for SCD

Investigators at Johns Hopkins published an initial experience using a nonmyeloablative haploidentical BMT with PTCy in adults with severe SCD.[16] The initial cohort included 14 participants with a median age of 23.5 years, receiving a conditioning regimen with antithymocyte globulin, fludarabine, cyclophosphamide, and total body irradiation (TBI), using unmanipulated bone marrow grafts from related haploidentical donors, and mycophenolate mofetil, sirolimus, and PTCy for GvHD prophylaxis. At a median follow-up of 711 days, only 57% (8/14) of the recipients successfully engrafted, with a graft failure rate of 43% (6/14) and no deaths. All participants with graft failure had autologous reconstitution. Among engrafted participants, 14% (2/14) had mixed donor-recipient chimerism at the study conclusion with no incidence of acute or chronic GvHD and 100% survival. All engrafted participants had amelioration of SCD-related symptoms, which provided the momentum for further investigating this platform for SCD. To reduce the graft rejection rate, investigators at Johns Hopkins added granulocyte colony-stimulating factor bone marrow priming to improve T-cell content in the graft because T cells are essential for engraftment. However, T cells may also drive the pathogenesis of acute and chronic GvHD, resulting potentially in a net negative effect and no impact on engraftment. The Johns Hopkins team also avoided prohibitive donor-specific anti-human leukocyte antigen antibodies associated with high graft rejection rates in haploidentical HCT recipients.[32] To improve donor engraftment in participants, the Johns Hopkins team increased TBI from 200 cGy to 400 cGy in their related haploidentical BMT platform.[33] Among 17 participants with severe hemoglobinopathies transplanted with the modified protocol, full-donor myeloid engraftment was observed in 76% (13/17), 18% (3/17) had mixed donor-recipient chimerism, and 6% (1/17) had primary graft failure.

In 2013, the Vanderbilt international, multi-institutional learning collaborative with participants in both middle- and high-income countries sought to improve donor engraftment using the Johns Hopkins platform in a phase 2 trial of nonmyeloablative haploidentical BMT with PTCy for participants with SCD.[34] In the initial 3 participants with SCD, 2 participants experienced graft rejection. The investigators elected to add thiotepa to the conditioning regimen at 10 mg/kg (Figure 2). A near-final update of the trial results with thiotepa added was presented at the American Society of Hematology meeting in December 2022.[35] Among 80 evaluable participants who underwent

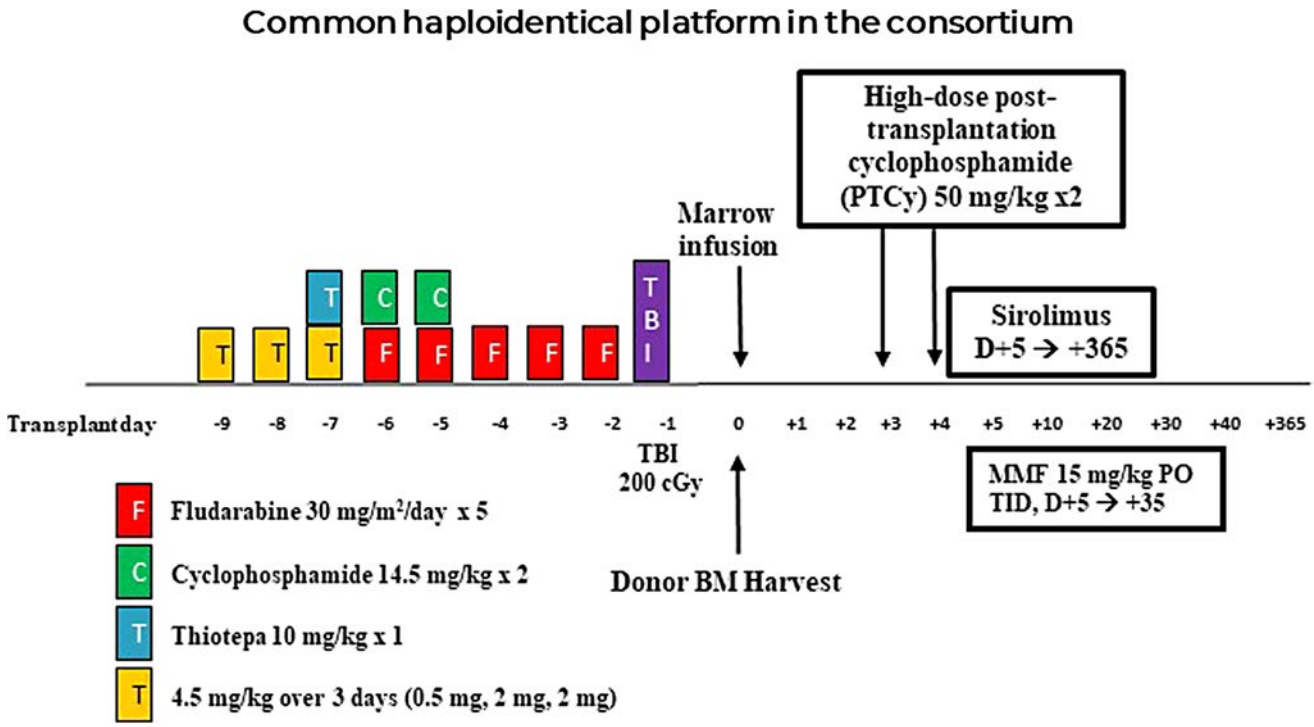

Figure 2. Conditioning schema for haploidentical BMT with thiotepa and PTCy used in the Vanderbilt Global Haploidentical Transplant Consortium.

haploidentical BMT with thiotepa and PTCy, the median age was 17.6 years, 97.5% had HbSS/HbSβ^0, and graft failure occurred in 12.5% (10/80) of the participants (4 primary and 6 secondary). Unexpectantly, all graft failures occurred in participants <18 years of age (P = .001), and all had autologous reconstitution. The Kaplan-Meier–based analysis for survival found no difference by age group <18 years or >18 years (P = .760). Kaplan-Meier–based overall survival probability was 96.7% (95% CI, 87.1%-99.2%) at 1 year and 94.3% (95% CI, 83.0%-98.2%) at 2 years. All engrafted participants had median whole-blood donor-recipient chimerism values at D + 180 and D + 365 post-transplant of 100.0%, respectively, and 97.7% (43/44) were off immunosuppression therapy at 1-year posttransplant. The prevalence of grades 3 to 4 acute and moderate to severe chronic GvHD was 8.8% (7/80) each. Mortality was 5.0% (4/80), attributable primarily to viral infections. The trial closed, and the final results are expected to be reported before the end of 2023.

In 2009, investigators at the National Institutes of Health designed a prospective phase 1/2 study, a non-chemotherapy-based, nonmyeloablative haploidentical protocol with PTCy, for participants with severe SCD, with the goal of stable mixed donor-recipient chimerism. The stem cell source was granulocyte colony-stimulating factor–mobilized PBSC; conditioning included low-dose TBI and in vivo T-cell depletion with alemtuzumab.[36] The trial was designed with 3 dosing cohorts for PTCy. Patients in cohort 1 (n = 3) did not receive PTCy, patients in cohort 2 received a single dose of 50 mg/kg (n = 8), and those in cohort 3 (n = 12) received 100 mg/kg in divided doses. The engraftment rate was 33% (1/3), 63% (5/8), and 83% (10/12) in cohorts 1, 2, and 3, respectively; 0% of patients in cohort 1 remained free of SCD, while 25% of patients in cohort 2 remained free of SCD compared to 50% in cohort 3, reinforcing the importance of the higher doses of PTCy. Three patients died, all of whom had graft rejection; 86% survived; 2 had grade 1 acute GvHD, and 1 had limited ocular GvHD, which resolved with systemic and topical steroids, respectively. However, 50% (6/12) of the participants in cohort 3 had graft rejection, an undesirable outcome. As anticipated, no participant achieved complete donor chimerism based on the primary trial's goal of stable mixed donor-recipient chimerism. All engrafted participants continued immunosuppression. The trial was closed early due to stopping rules related to graft rejection. Subsequently, 2 participants developed myeloid neoplasm after graft rejection, raising the investigator's concern about the association between stable mixed donor-recipient chimerism, graft failure, and an increased incidence rate of acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS). Recent evidence indicates AML/MDS rarely occurs after myeloablative matched-related donor transplants, where the goal is complete donor chimerism.[37,38] To replace the prior protocol with the goal of mixed donor-recipient chimerism, National Institutes of Health investigators have opened a new single-center haploidentical protocol with a goal of complete donor chimerism (NCT03077542).

Other investigators have attempted to improve the engraftment rate for SCD using the Johns Hopkins–based haploidentical BMT platform with PTCy using different conditioning regimens and stem cell sources with mixed results.[39-41] Most trials are small, single-institution studies that do not provide sufficient evidence to be considered standard therapy or replicated as a therapeutic option in a clinical trial. The absence of a SCD-specific BMT consortium that allows multiple phase 1, 2, and 3 trials to be open is a significant barrier to hastening curative therapy options for SCD. Table 1 reviews transplant outcomes from published studies using haploidentical HCT with PTCy for SCD.

Table 1. Transplant outcomes from published studies using haploidentical HCT with PTCy for SCD

Characteristic	Bolanos-Meade et al[16]	De la Fuente et al[33]	Fitzhugh et al[35]	Saraf et al[39]	Pawlowska et al[41]
Protocol	Phase 1/2, single center	Phase 2, multicenter	Phase 1/2, single center	Phase 2 single center	Phase 2 single center
Goal	Full donor chimerism	Full donor chimerism	Stable mixed donor-recipient chimerism	Full donor chimerism	Full donor chimerism (PTIS-HCT approach)
Stem cell source	Bone marrow*	Bone marrow*	Peripheral blood	Peripheral blood	Bone marrow (3) Peripheral blood (1)
Donor type	Haploidentical	Haploidentical	Haploidentical	Haploidentical	Haploidentical
Donor availability†	100%	>90%	100%	90%	100%
Number of patients	14	18	23 (21-SCD)	8	4
Age, median (range), y	30 (15–46)	20.9 (12.1–26)	36 (20–56)	29 (20–38)	18.5 (13 to 23)
Type of conditioning	Nonmyeloablative	Nonmyeloablative	Nonmyeloablative	Nonmyeloablative	Reduced toxicity
Conditioning	ATG, fludarabine, cyclophosphamide, TBI (200 cGy)	ATG, fludarabine, cyclophosphamide, thiotepa, TBI (200 cGy)	Alemtuzumab, TBI (400 cGy)	ATG, fludarabine, cyclophosphamide, TBI (300 cGy)	PTIS: fludarabine and dexamethasone ×2 courses; then ATG, busulfan, fludarabine
GvHD prophylaxis	PTCy, tacrolimus, sirolimus, MMF	PTCy, sirolimus, MMF	PTCy,‡ sirolimus	PTCy, sirolimus, MMF	PTCy, tacrolimus, MMF
Median follow-up, mo	23.4 (minimal 6.9)	13.3 (IQR, 3.8–23.1)	38 (range, 8–74)	17 (range, 12–30)	5–11
Engraftment rate	57% (8/14)	83% (15/18)	Cohort 1: 33% (1/3) Cohort 2: 63% (5/8) Cohort 3: 83% (10/12)	≥95% (7/8)	100%
Mixed chimerism	25% (2/8) at 6 months	None	100% (16/16)	13% (1/8)	None
EFS	NA	93%	NA	75% (6/8)	NA
OS	100%	100%	87% (11/12)	88% (7/8)	100%
Graft failure	43% (1 primary; 5 secondary)	6% (1/15)#	65% (7 primary; 8 secondary); 50% in Cohort 3	12.5% (1/8)	0%
TRM	0%	0%	9.5% (2/21)	13% (1/8)	0%
Acute GvHD >2	0%	13% (2/16)	0%	25% (2/8)	25% (1/4)
Chronic GvHD (moderate-severe)	0%	6% (1/16)	0%	13% (1/8)	75% (3/4)
Immunosuppression duration (IST)	14.2% (2/14) still on at publication	85% (6/7) off at 1 year	Continuing	3/6 on IST	1/4 on IST
Complications	PRES (3), viral reactivations	PRES (1), viral reactivations, VOD (1) with second transplant	Viral reactivation, CMV colitis, PTLD (1), MDS after graft failure (2)	SAH (2), viral reactivations,	Viral reactivations
Protocol status	Completed	Completed	Abandoned	unknown	unknown

ATG, antithymocyte globulin; CMV, cytomegalovirus; IQR, interquartile range; IST, immunosuppression therapy; MMF, mycophenolate mofetil; NA, not available; PRES, posterior reversible encephalopathy/neurological complications (means peripheral neuropathy, neuralgia); PTIS, pretransplant immunosuppressive therapy; PTLD, posttransplant lymphoproliferative disorder; SAH, sub-arachnoid hemorrhage; TRM, transplant-related mortality; VOD, veno-occlusive disease.

*Three patients received granulocyte colony-stimulating factor–primed bone marrow.

†Patient who had IST stopped due to severe reaction and PRES.

‡Escalating doses of PTCy: 0 mg/kg in cohort 1, 50 mg/kg in cohort 2, and 100 mg/kg in cohort 3.

Table 2. Transplant outcomes from competing strategies using T-cell deplete haploidentical HCT for SCD

Characteristic	Foell et al[19]	Gilman et al[42]	Gaziev et al[20]	Cairo et al[18]
Protocol	Phase 2 trial (single center)	Phase 2 trial (single center)	Phase 2 trial (single center)	Phase 2 trial (multicenter)
Goal	Full-donor chimerism	Full-donor chimerism	Full-donor chimerism	Full-donor chimerism
Stem cell source	Peripheral blood	Peripheral blood	Peripheral blood	Peripheral blood
Donor type	Haploidentical	Haploidentical	Haploidentical	Haploidentical
Donor availability	100%	100%	100%	100%
Number of patients	25	10	14 (3 SCD; 11 TDT)	19
Graft manipulation (T-cell depletion strategies)	CD3/CD19 T-cell depletion (19) or TCRαβ$^+$/CD19$^+$ depletion (6)	CD34$^+$ cell-selected, T-cell–depleted (8 Haplo; 2 MUD)	TCR αβ$^+$/CD19$^+$–depleted grafts	CD34$^+$ enrichment and mononuclear cell add-back
Age, median (range), mo	13 (3–31)	49 (14–60)	47 (6–62)	46 (1.9–76)
Type of conditioning	Myeloablative	Reduced intensity	Myeloablative	Myeloimmunoablative
Conditioning	ATG, fludarabine, thiotepa, treosulfan	Melphalan, thiotepa, fludarabine, and rabbit ATG + rituximab	PTIS: fludarabine, hydroxyurea, and azathioprine; then busulfan, thiotepa, cyclophosphamide, and ATG	PTIS: hydroxyurea and azathioprine; then fludarabine, busulfan, thiotepa, cyclophosphamide, total lymphocyte irradiation, and rabbit ATG
GvHD prophylaxis	Cyclosporine, MMF	DLI + methotrexate	Cyclosporine and methylprednisolone or MMF	None
Follow-up, median (range)	22 mo	49 (14–60) mo	3.9 (0.5–5.2) y	1409 (59–2330) d
Stable engraftment	84%	70%	93%	97.1%
Mixed chimerism	16% (4/25)	30%*	None	None
EFS	88% (22/28)	80%	69%	84%
OS	88% (22/25)	90%	84%	84%
Graft failure	0%	10%	14%	0%
TRM	12% (3/25)	10% (1/10)	14.2% (2/14)	15.7% (3/19)
Acute GvHD	Grades 1–2: 28% (7/25) Grades 3–4: 0%	Grades 2–4: 20% (2/10)	Grades 2–4: 28%	Grades 2–4: 6.2%
Chronic GvHD	Mild/moderate 14% Severe 0% (4/25)	Extensive skin (1/10)	Extensive 21%	Moderate-severe 6.7%
Immunosuppression duration	Off by 18 mo in patients with GvHD	Unknown	Unknown	Unknown
Complications	PRES (5), seizures (1), VOD (1), TAM/MAS (1). Viral reactivations 13 (52%)	PTLD (3), engraftment syndrome (2), PRES (2), viral reactivations	PTLD (3), DLBCL, viral reactivations (64%), AIHA (3), ITP (1); delayed immune reconstitution	Death from VOD (1), 1 each of acute and chronic GvHD
Protocol status	Ongoing	Ongoing	Ongoing	Completed

Viral reactivation indicates patients with reactivation of cytomegalovirus, herpes virus, Epstein-Barr virus, rotavirus, or polyomavirus.

AIHA, auto-immune hemolytic anemia; DLBCL, diffuse large B-cell lymphoma; DLI, donor lymphocyte infusion; ITP, immune thrombocytopenia; MAS, macrophage activation syndrome; MUD, matched unrelated donor; TAM, tumor-associated macrophages; TDT, transfusion dependent thalassemia.

*Received prophylactic DLI or second transplant.

T-cell–deplete haploidentical HCT approaches for SCD

Outcomes of initial attempts at graft manipulation before related haploidentical HCT for SCD were associated with high graft failure rate and significant GvHD.[13-15] Foell and colleagues[19] in Germany are conducting a phase 2 trial to assess α/β T-cell depleted haploidentical HCT in children and adults with SCD who lack sibling donors and have failed at least 1 year of hydroxyurea therapy (NCT04201210). In their pilot trial, investigators used a treosulfan (L-treitol-1,4-bis-methanesulfonate)–based myeloablative conditioning regimen with $CD3^+/CD19^+$ or $\alpha\beta/CD19^+$-depleted PBSC grafts and tacrolimus and mycophenolate mofetil for GvHD prophylaxis. A total of 17 participants were initially recruited, and the median age was 13 years. After a median follow-up of 22 months, event-free survival (EFS) and overall survival (OS) were 88% (22/25) each, transplant-related mortality was 12% (3/25), grade 1 to 2 GvHD was 28% (7/25), and mild to moderate GvHD was 16% (4/25). The main complications were early viral reactivation (52%), pain (72%), posterior reversible encephalopathy (20%), and 1 case each of veno-occlusive disease of the liver and tumor-associated macrophages/macrophage activation syndrome. An update, including trial data, was shared at the 49th annual European Society for Blood and Marrow Transplantation (EBMT) meeting in 2023 Paris, France. Of 37 participants with SCD enrolled, all were engrafted with OS 89% and EFS 85%, but 11% of participants maintained mixed donor-recipient chimerism. The incidence of grade 1 to 2 GvHD was 32% (12/37), and mild to moderate GvHD was 14% (5/37), all resolved 18 months posttransplant. The intensity of this conditioning may be too toxic for patients with SCD and significant organ dysfunction.

In August 2009, Gilman and colleagues[42] started a single-institution phase 2 study in children and young adults with severe SCD using a reduced-intensity conditioning regimen with $CD34^+$-selected, T-cell–depleted PBSC grafts, evaluating engraftment and GvHD. The median age was 14 years (range, 5-23), and 8 patients underwent haploidentical HCT, all engrafted, with an OS and EFS of 90% and 80%, respectively. The incidence of grade 2 to 4 acute GvHD was 20% and 1 chronic GvHD in a patient who received a donor lymphocyte infusion for a refractory posttransplant lymphoproliferative disorder.

In November 2017, Gaziev and colleagues[20] published a single-center retrospective study using myeloablative conditioning (busulfan, thiotepa, cyclophosphamide, and antithymocyte globulin preceded by fludarabine, hydroxyurea, and azathioprine), followed by $TCR\alpha\beta^+/CD19^+$-depleted grafts in 14 children with hemoglobinopathies. The median age was 7 years (range, 3-15.2), 3 had SCD, and 11 had thalassemia. The investigators showed improved outcomes in this cohort compared to 40 patients with hemoglobinopathies and similar baseline characteristics who received $CD34^+$-selected PBSC and bone marrow grafts. Table 2 reviews outcomes from published studies using T-cell–deplete haploidentical HCT approaches for SCD. These small single-center clinical trials, myeloablative conditioning platform, and requirements for ex vivo stem cell manipulations with complex technical equipment limit this approach for widespread use in middle- and high-income countries and are inaccessible for most adults with heart, lung, and kidney disease.[18-20]

CLINICAL CASE (continued)

The participant underwent a haploidentical BMT with thiotepa and PTCy from his half-sibling. His transplant was complicated by pure red cell aplasia that resolved after treatment with daratumumab. He remains 100% engrafted 2 years posttransplant, is on antiseizure therapy, and has had no recurrent strokes and improved cerebral hemodynamics (Figure 3).

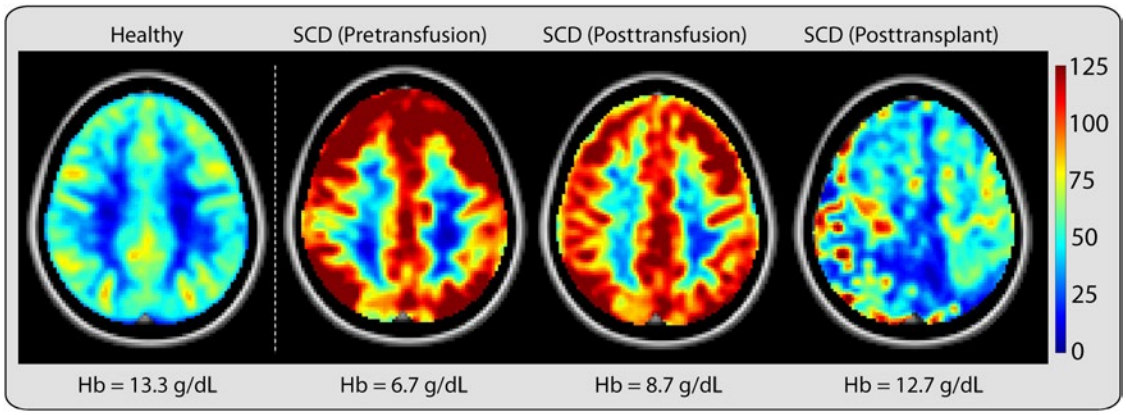

Figure 3. Changes in cerebral blood flow before and after blood transfusions and haplo-BMT with thiotepa and PTCy in a patient with SCD. Quantitative cerebral blood flow (CBF) maps show that CBF increases to maintain sufficient oxygen and glucose supply in people with anemia. The healthy image depicts the brain of an African American woman (aged 32 years, HbAA) with a hemoglobin concentration of 12.6 g/dL and a cortical CBF (assessed by arterial spin labeling magnetic resonance imaging) of 40 to 60 mL per 100 g per min. The SCD images are from an African American man with SCD (aged 34 years, hemoglobin SS); pretransfusion, his CBF was elevated to offset reduced oxygen content. Following blood transfusion, which increased the total hemoglobin and reduced the proportion of hemoglobin S, the CBF decreased but remained elevated relative to that in nonanemic individuals. After haploidentical BMT (haplo-BMT) with thiotepa and PTCy, both anemia and HbS were eliminated, and CBF approached that of a healthy control (HbAA). CBF heterogeneity was still present in this patient due to underlying moyamoya vasculopathy. Hb, hemoglobin concentration. (From *Lancet Neurol*. 2021 May;20(5):398-408.)

Table 3. Incidence of hematologic malignancies is highest in adults with mixed chimerism following HCT for SCD

	NHLBI HLA matched			NHLBI haploidentical		Gene therapy	French group	CIBMTR
Conditioning	**Alemtuzumab 300 cGy TBI**	**Pentostatin/Cy alemtuzumab 300 cGy TBI**	**(Chicago, Riyadh) alemtuzumab 300 cGy TBI**	**Alemtuzumab 400 cGy TBI ± PTCy**	**Pentostatin/Cy alemtuzumab 400 cGy TBI PTCy**	**Busulfan**	**Cy ± ATG busulfan**	**Cy ± ATG busulfan (mostly)**
Number enrolled in the study	57	24	64	21	19	44	234	908
At-risk time (person-years)	518.7	96	Chicago: 123 Riyadh: 87.5	176.4	49.4	111.9	1848.6	HLA-matched sibling: 1674
Hematologic malignancies/ 100 person-years*	0.57	**2.08**	Chicago: 0.8 Riyadh: 0	**1.70**	0	**1.79**	0.05	HLA-matched sibling: 0
Median follow-up, y	9.1	4.0	4	8.4	2.6	2.5	7.9	2.1–3.9

*Bolded values represent: Incidence of hematologic malignancies/100 person-years.

Personal communication from C. Fitzhugh and courtesy of C. Fitzhugh CD (*Blood*. 2022;140(23):2514-2518).

CIBMTR, Center for International Blood and Marrow Transplant Research; Cy, cyclophosphamide; HLA, human leukocyte antigen; NHLBI, National Heart, Lung, and Blood Institute.

Table 4. Pros and cons of the 2 promising different haploidentical HCT approaches

	Haploidentical nonmyeloablative, T-cell–replete, bone marrow transplant with thiotepa and PTCy[18,33,34]	Haploidentical myeloablative, ex vivo T-cell–deplete PBSC transplant using advanced-technology graft manipulation[19–21,42]
Pros	• Nonmyeloablative • >90% donor availability • Most adults can tolerate the conditioning regimen • Good rates of engraftment • Multicenter trial completed in middle- to high-income settings • Event-free survival: >90% in adults • Overall 2-year survival: >90% • Low rates of acute and chronic GvHD • Typically, discontinuation of immunosuppressive therapy in 1 year • Protocols easily replicable at different institutions and relatively inexpensive	• >90% donor availability • Event-free survival: >80% • Overall survival: 80%-90% • Low rates of acute and chronic GvHD • Prevents EBV-PTLD by removing $CD19^+$ cells ex vivo • Reduced need for posttransplant immune-suppressive medications
Cons	• Late health effects are not well established • Graft rejection (~12.5% in <18 years) • Increased risk of viral reactivations • May be prohibitive to patients with significant chronic kidney disease stage 4 or 5 • Limited insurance coverage, donor eligibility, and a high rate of DSAs may limit access • Late health effects on fertility are not well described, expected, or studied systematically	• Limited to single-center experiences • Expensive and labor-intensive • Variability in quality of cells depending on the source • Limited follow-up • Late health effects are not well established • May be prohibitive to patients with significant chronic kidney disease stage 4 or 5 • Specialized expertise required • Delayed immune reconstitution • Increased risk of viral reactivations • Graft rejection (0%-14%) • Fertility in women is likely to be low, not studied systematically

The third haploidentical transplant protocol is no longer open at the National Institutes of Health clinical center: nonmyeloablative, in vivo T-cell depletion with alemtuzumab using PBSC transplant.[35,37]

DSA, donor-specific antibody; EBV, Epstein-Barr virus.

Conclusion

For individuals with SCD, selecting the optimal nonmyeloablative haploidentical conditioning regimen that minimizes toxicity but ensures complete donor engraftment has become increasingly important in the past decade. The recent recognition that haploidentical trials with a goal of stable mixed donor-recipient chimerism are associated with increased risk of AML/MDS has resulted in at least 1 trial closure and careful consideration for future trials with a similar therapeutic goal of stable mixed chimerism (Table 3). Among the 3 haploidentical platforms, the most promising approach is the nonmyeloablative BMT with thiotepa and PTCy, an approach proven to be effective across all ages and transferable to almost all transplant programs in middle- and high-income countries.[43]

Preexisting comorbidities in the adult SCD population must be considered before haploidentical HCT, although only individuals with kidney failure are generally excluded. Further, the late health effects of all curative therapy results should be collected prospectively for better-informed decision-making for the full range of options. We predict the optimal curative therapy option among myeloablative matched related donor, nonmyeloablative haploidentical BMT with thiotepa and PTCy, myeloablative gene therapy, or myeloablative gene-editing treatment protocols will depend on each curative therapy's distinct late health effects and personal preferences.

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Conflict-of-interest disclosure

Adetola A. Kassim: no competing financial interests to declare.
Michael R. DeBaun and his institution are the sponsors of 2 externally funded research investigator-initiated projects. Global Blood Therapeutics will provide funding for these clinical studies but will not be a cosponsor of either study; he is not receiving any compensation for the conduct of these 2 investigator-initiated observational studies: he is a member of the Global Blood Therapeutics advisory board for a proposed randomized controlled trial, for which he receives compensation; he is on the steering committee for a Novartis-sponsored phase 2 trial to prevent priapism in men; he was a medical adviser for the development of the CTX001 Early Economic Model; he provided medical input on the economic model as part of an expert reference group for Vertex/CRISPR CTX001 Early Economic Model in 2020; and he provided consultation to the Forma Pharmaceutical company about sickle cell disease from 2021 to 2022.

Off-label drug use

Adetola A. Kassim: Nothing to disclose.
Michael R. DeBaun: Nothing to disclose.

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Gene therapy for sickle cell disease

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Sickle cell disease (SCD) is potentially curable after allogeneic hematopoietic stem cell transplantation (HSCT) or autologous HSCT after ex vivo genetic modification. Autologous HSCT with gene therapy has the potential to overcome many of the limitations of allogeneic HSCT that include the lack of suitable donors, graft-versus-host disease, the need for immune suppression, and the potential for graft rejection. Significant progress in gene therapy for SCD has been made over the past several decades, now with a growing number of clinical trials investigating various gene addition and gene editing strategies. Available results from a small number of patients, some with relatively short follow-up, are promising as a potentially curative strategy, with current efforts focused on continuing to improve the efficacy, durability, and safety of gene therapies for the cure of SCD.

LEARNING OBJECTIVES

- Describe the advances in gene therapy for patients with sickle cell disease
- Identify the risks and strategies to overcome these limitations associated with gene therapy for patients with sickle cell disease

CLINICAL CASE

A 27-year-old man with homozygous sickle cell disease on hydroxyurea is interested in transplantation as a one-time treatment strategy. His disease course has been complicated by frequent vaso-occlusive pain crises, recurrent severe acute chest syndrome, frequent transfusions with subsequent iron overload, and proteinuria. His pain crises have increased in frequency and intensity in his late adolescence and early adulthood, requiring more frequent and longer hospitalizations and chronic opioid use. He has been on hydroxyurea for more than 10 years, and despite good compliance and a maximally tolerated dose, his fetal hemoglobin remains <20%. His pain worsened with crizanlizumab, and he does not have a matched sibling.

Introduction

Sickle cell disease (SCD) is a life-limiting inherited hemoglobinopathy with significant complications that worsen over the life span of a patient. The currently available disease-modifying therapies are necessary but insufficient to address the growing burden of disease,[1] and therefore, treatment options that seek to fully eliminate disease complications, particularly for those with the more severe forms of SCD, are needed.

The current treatment paradigm for individuals with SCD centers on supportive care for acute complications, drug therapies to reduce disease severity, or potential cure through hematopoietic stem cell transplantation (HSCT).[2] Whereas the health and survival of children with SCD have improved considerably with the use of newborn screening, penicillin prophylaxis, and immunizations, mortality rates for adults have worsened in the same time frame.[3] Treatment has therefore shifted from a life-threatening disease of children to a chronic disease of adults, although irreversible and debilitating complications such as stroke can occur at any age. Disease-modifying therapies are not universally used, have variable clinical responses, must be continued indefinitely, require chronic monitoring, and do not fully eliminate disease complications. Allogeneic HSCT from a human leukocyte antigen–matched sibling is currently the only established curative intervention for SCD; however, broad use of this option is significantly limited by donor availability. While important strides have been made with the use of alternative donor HSCT, specifically haploidentical HSCT, to decrease graft rejection and graft-versus-host disease, higher rates of transplant-related mortality and morbidity limit the broad use of this therapy. Furthermore, specialized treatment centers that can manage the transplant and complex posttransplant care restricts access for many patients. Given the lack of universally beneficial

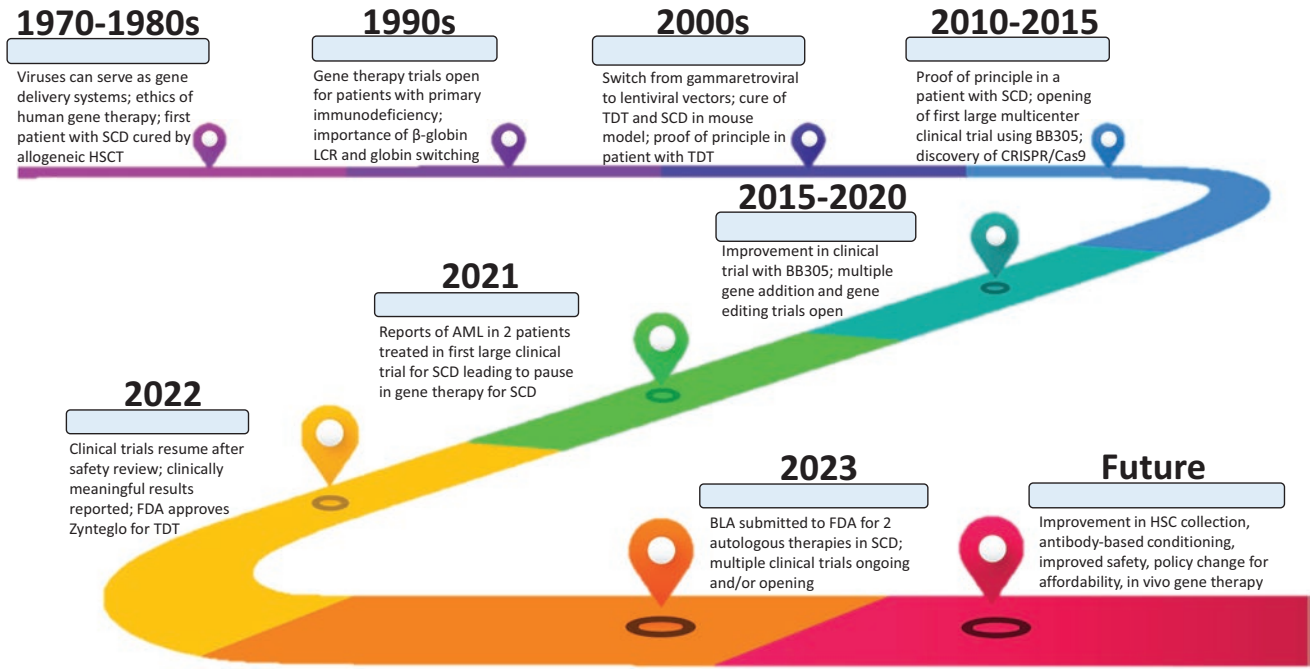

Figure 1. Historical timeline of gene therapy for sickle cell disease to present date. Highlights of decades of preclinical and clinical research leading to a possible FDA-approved autologous cell therapy product for sickle cell disease are presented. BLA, biologic license application; LCR, locus control region.

disease-modifying therapies, the lack of available human leukocyte antigen–identical sibling donors, the increased risks of complications with alternate donor HSCT, and the complex care following allogeneic HSCT, autologous HSCT after gene therapy is a theoretical universal cure for SCD that could eliminate major limitations of allogeneic transplantation. The historical perspective, current state, and future considerations of autologous gene therapy for SCD are reviewed herein.

Historical perspective

The concept that gene therapy may ameliorate human genetic diseases emerged in the 1970s after it was discovered that viruses could serve as a gene delivery system[4,5] (Figure 1). Proof of principle was first demonstrated in patients with advanced melanoma,[6] and the first clinical trials in gene therapy opened shortly thereafter. The first trials focused on ex vivo correction of hematopoietic stem cells (HSCs) from patients with primary immunodeficiencies (PIDs) using murine gammaretroviruses (specifically the murine leukemia virus), as this vector system permanently integrates into the target cell, which is a necessary feature for vector systems aimed at self-renewing stem cells and their progeny. The PIDs were an obvious early target given the relatively low-threshold gene transfer efficiency needed in the setting of a disorder where there existed a strong natural in vivo selection for gene-corrected cells. The long-awaited successful application of human gene therapy was indeed demonstrated first in X-linked severe combined immunodeficiency, but genotoxicity from insertional mutagenesis halted these initial studies after the retroviral vector inserted into protooncogenes driving a leukemia process in several patients.[7-9]

Initial challenges for gene transfer in hemoglobinopathies included an inability of gammaretroviral vectors to carry the large β-globin gene and the necessary regulatory elements, the potential for silencing, and the lack of high-level globin gene expression.[10] To overcome these challenges, the use of lentiviral vectors containing the β-globin locus control region enabled the first reversal of β-thalassemia in a mouse model by demonstrating viral transgene expression of nearly 20% of the total hemoglobin using a human β-globin lentiviral vector with crucial locus control region elements (TNS9 vector).[11,12] A similar lentiviral vector that substituted glutamine for threonine at amino acid 87 (β^{T87Q}) resolved anemia and reduced organ damage in 2 SCD transgenic mouse models.[13,14] The first clinical trials investigating gene therapy in patients with β-thalassemia and SCD therefore evaluated ex vivo delivery of β^{T87Q} using lentiviral transduction. Simultaneously, the discovery of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 for programmable editing was reported[15] ushering in a novel genome editing strategy for the cure of hemoglobin disorders.

A decade of lentiviral-based gene therapy for SCD

Proof of concept in a patient with β-thalassemia, and later in a patient with SCD, was reported in 2010[16] and 2017,[17] respectively, using ex vivo lentiviral delivery of β^{T87Q} into autologous HSCs. These first patients were among the group of initial patients treated with a lentiviral vector encoding β^{T87Q}, initially tested in phase 1/2 studies known as HGB-204 (NCT01745120), HGB-205 (NCT02151526), and HGB-206 (NCT02140554). HGB-204 focused on patients with transfusion-dependent thalassemia (TDT); HGB-205 treated 4 patients with TDT and 3 patients with SCD. Early reports demonstrated stable and durable

engraftment of gene-modified cells after myeloablative conditioning, leading to stable production of the therapeutic hemoglobin (HbA^{T87Q}).[17,18] Success in HGB-204 led to several phase 3 clinical trials in patients with TDT (NCT02906202 and NCT03207009), resulting in US Food and Drug Administration (FDA) approval for the product Zynteglo (LentiGlobin BB305, betibeglogene autotemcel) in 2022 as the first cell-based gene therapy to treat adult and pediatric patients with TDT.[19]

Following proof of concept in a single patient with SCD in HGB-205, HGB-206 was initiated as a large, multicenter study in patients with severe SCD. Data from the first cohort of patients (group A, n = 7) using bone marrow–derived HSCs demonstrated a relatively low peripheral blood vector copy number (VCN) and modest HbA^{T87Q} production, and therefore patients continued to have stress erythropoiesis and lacked a robust clinical benefit.[20] Improvements were subsequently made to the manufacturing process, resulting in higher VCN values in group B (n = 2) and later to the collection of a peripherally mobilized stem cell source with plerixafor, which was demonstrated to be safe and effective in patients with SCD and demonstrated a superior $CD34^+$ quality[21-24] in group C (n = 35).[20,25] Such improvements now demonstrate high and sustained near-pancellular expression of HbA^{T87Q} that was nearly 50% of total hemoglobin by 6 months posttreatment. Clinically, there was a marked reduction in sickle hemoglobin, reduction in the propensity of red blood cells to sickle, near normalization of hemolysis markers, and resolution of severe vaso-occlusive events. LentiGlobin BB305 (bb1111, lovotibeglogene autotemcel) for SCD is being evaluated in a phase 3 study (NCT04293185) in both children and adults with SCD and was submitted for FDA approval for adults with severe SCD in the spring of 2023 (Table 1).

Additional phase 1/2 studies have investigated lentiviral vector delivery of either wild-type β^A-globin (NCT01639690, NCT02453477, NCT03276455), β^{AS3} antisickling globin (NCT03964792), γ-globin (NCT02186418), or delivery of a transgene that results in erythroid-specific expression of a short hairpin RNA targeting *BCL11A*, leading to knockdown of this critical repressor of fetal hemoglobin (HbF) (NCT03282656) (Table 1). Of these, lentiviral delivery of a short hairpin RNA targeting BCL11A demonstrated stable HbF induction (percentage HbF/[F + S] at most recent follow-up, 20.4% to 41.3%), with HbF broadly distributed in red cells (F cells 58.9% to 93.6% of untransfused red cells) and HbF per F cell of 9.0 to 18.6 pg per cell,[26] and is now being investigated in a phase 3 study (NCT05353647).

Two cases of acute myeloid leukemia (AML) were reported following gene addition therapy for SCD; importantly, in both cases, it was determined the AML was unrelated to the lentiviral vector, as was previously documented in patients with early PID treated with gammaretroviral vectors.[27,28] The 2 patients who developed AML were among the first 7 patients treated in the phase 1/2 trial of LentiGlobin BB305 for SCD (NCT02140554) produced from bone marrow–harvested HSCs using an earlier version of the drug product manufacturing process. Current theories suggest the stress of switching from homeostatic to regenerative hematopoiesis by transplanted cells drives clonal expansion and leukemogenic transformation of preexisting premalignant clones, eventually resulting in AML/myelodysplastic syndrome.[29] Bone marrow harvests as the cell source resulted in lower cell doses with fewer $CD34^{hi/+}$ long-term HSCs, likely increasing proliferative stress on the repopulating cells. Low drug product VCNs for these patients resulted in modest transgene expression and an inadequate therapeutic response. These patients continued to experience hemolysis and persistent anemia, resulting in transplanted and endogenous bone marrow cells continuing to experience hematopoietic stress posttreatment, providing a further opportunity for accumulation of mutations. To date, no cases of insertional oncogenesis have been reported in gene addition trials using lentiviral vectors for TDT or SCD. There are no reports of leukemia in the 63 patients with β-thalassemia who received drug products manufactured with the identical BB305 LVV used for manufacture of LentiGlobin for SCD in separate clinical trials, suggesting a uniqueness to the pathophysiology of SCD. Constant erythropoietic stress with dysregulated hematopoiesis, chronic inflammation, repeat bony infarction, and the possibility of preexisting clonal hematopoiesis of indeterminant potential–related mutations compound the already known risks associated with genotoxic conditioning, autologous HSCT, and an increased relative but low absolute risk of AML/myelodysplastic syndrome in this patient population.[30-33]

In totality, the reported data from the completed or ongoing trials encompassing gene addition therapies using lentiviral vectors support this mode of treatment to be an efficacious option with an acceptable safety profile for patients with TDT and SCD. To date, no cases of graft-versus-host disease or immune rejection have been reported, there have been no cases of insertional oncogenesis, and the overall safety profiles of the ongoing and completed studies are generally consistent with that of HSCT requiring myeloablative conditioning and of the underlying

Table 1. Investigational lentiviral vector–based autologous cell therapy products for sickle cell disease

Product	Sponsor	Technology	Effect	Activation	Status
Lovotibeglogene autotemcel	Bluebird bio	Lentiviral gene addition	BB305 LVV/$b^{A\text{-}T87Q}$ (antisickling)	2014	Submitted BLA spring 2023
ARU-1801	Aruvant Sciences GmbH	Lentiviral gene addition	LVV/γ-globin (G16D) (antisickling)	2014	Active, not recruiting
DREPAGLOBE	Assistance Publique–Hopitaux de Paris	Lentiviral gene addition	GLOBE LVV/b^A (nonsickling)	2019	Active, not recruiting
BCH-BB694	Boston Children's Hospital	Lentiviral gene addition	shRNA targeting *BCL11A* (antisickling)	2018	Phase 3

BLA, biologic license application; LVV, lentiviral vector; shRNA, short hairpin RNA.

disease. While the 2 reports of AML after gene addition have excluded the genetic technology itself, the long-term risks of secondary malignancy after gene therapy are unknown. Given limited follow-up to date and potentially significant risks associated with genetic modifications, patients are required to be followed long term in accordance with the FDA's guidelines for participants who receive investigational genetically modified cellular products.

Advances in gene editing for SCD

Multiple gene editing therapies for SCD are currently in clinical development and include gene knockdown of regulators of fetal hemoglobin, gene editing of globin regulatory elements, and direct globin gene editing, with targeted nucleases such as zinc-finger nucleases, CRISPR/Cas9, and, more recently, with base or prime editors. The major advantage of these methods over gene addition strategies is the ability to significantly reduce or entirely avoid nonspecific integration that may lead to insertional oncogenesis, although risks of off-target editing, deleterious on-target editing, or consequences of double-strand breaks remain significant.[34-40]

Most gene editing clinical trials for SCD have sought to increase endogenous HbF expression through knockdown of *BCL11A*, either targeting the *BCL11A* erythroid enhancer on chromosome 2 or targeting the HbF repressor binding sites on chromosome 11. Two studies (NCT04819841 and NCT04774536) aimed to correct the underlying b^S glutamate to valine amino acid substitution at position 6 (E6V) in SCD by editing the β-globin gene and achieving repair via homology-directed repair by providing template DNA, but 1 study closed after the first patient developed pancytopenia,[41] and the other is not yet recruiting patients.

CTX001 (exagamglogene autotemcel, exa-cel) is an investigational autologous cell therapy that uses CRISPR/Cas9 to disrupt the *BCL11A* erythroid enhancer to increase endogenous HbF (NCT03745287). As of February 2022, 31 patients with SCD (age 22.5 [12-34] years) had been infused with exa-cel (follow-up 9.6 [2.0-32.3] months).[42] The mean proportion of HbF was >20%, and total hemoglobin levels were >11 g/dL 3 months postinfusion, with editing rates of 86.6% in the bone marrow $CD34^+$ HSCs 6 months postinfusion. All patients remained vaso-occlusive crisis (VOC)-free, and there were no serious adverse events, including deaths, discontinuations, or malignancies. Exagamglogene autotemcel (exa-cel) was submitted for FDA approval for SCD and β-thalassemia in the spring of 2023[43] (Table 2).

Two other therapies in development, SAR445136 (formerly BIVV003) and OTQ923, have presented early data but have been stalled in further development (Table 2). SAR445136 (formerly BIVV003) is an autologous cell therapy in development that uses zinc-finger nucleases to disrupt the *BCL11A* erythroid enhancer (NCT03653247, PRECIZN-1 study). Thus far, 5 patients have been treated with SAR445136 with up to 125 weeks of follow-up.[44,45] HbF fractions increased to 12.2% to 41.2%, and 3 patients sustained a protective level of ≥10 pg HbF/F cell at follow-up without any further VOCs. One patient who did not sustain a HbF/F level ≥10 pg per cell experienced 2 severe VOCs at 9 and 16 months postinfusion. OTQ923 is an autologous CRISPR/Cas9-edited $CD34^+$ cellular product with a targeted disruption of the *HBG1/HBG2* promoters on chromosome 11. As of July 8, 2022, 2 participants had received OTQ923, with follow-ups of 9 months and 3 months, respectively, and HbF levels of 22% and 15.9%, respectively.[46] Further development of OTQ923 has been suspended, and the focus has shifted on developing in vivo editing.[47] EDIT-301 and BEAM-101 are strategies using CRISPR/Cas12a to target the gamma-globin promoter and a base editor to target BCL11A expression, respectively (Table 2). Data from these studies are not currently available.

To date, cumulative data from the ongoing gene editing clinical trials for SCD suggest gene editing of the *BCL11A* erythroid enhancer could be an effective mechanism to induce HbF expression, whereas other studies have demonstrated that the need for sufficient HbF/F-cell induction, regardless of methodology, is important to achieve therapeutic benefit.

The future of gene therapy for SCD

Future iterations of gene therapies for SCD are currently in preclinical development and focus on overcoming the existing barriers of current ex vivo strategies. Critical areas of understanding, improvement, and exploration of gene therapy for

Table 2. Investigational gene-edited autologous cell therapy products for sickle cell disease

Product	Sponsor	Technology	Effect	Activation	Status
CTX001	Vertex Pharmaceuticals Incorporated	CRISPR/Cas9 Editing	CRISPR-Cas9/*BCL11A* erythroid enhancer (antisickling)	2018	Submitted BLA spring 2023
BIVV003 (now called SAR445136)	Sangamo Therapeutics	CRISPR/Cas9 editing	ZFN/*BCL11A* erythroid enhancer (antisickling)	2019	Further development discontinued
OTQ923/HIX763	Novartis Pharmaceuticals	CRISPR/Cas9 Editing	CRISPR-Cas9/*BCL11A* (antisickling)	2020	Further development discontinued
GPH101	Graphite Bio, Inc.	CRISPR/Cas9 HDR	CRISPR-Cas9/$b^S > b^A$ (nonsickling)	2021	Further development discontinued
EDIT-301	Editas Medicine, Inc.	CRISPR/Cas912a editing	CRISPR-Cas9/HGB1/2 (antisickling)	2021	Active, data not reported
CRISPR_SCD001	UCSF Benioff Children's Hospital Oakland	CRISPR/Cas9 HDR	CRISPR-Cas9/$b^S > b^A$ (nonsickling)	2021	Not yet recruiting
BEAM-101	Beam Therapeutics	Base Editing	Base editing *BCL11A* erythroid enhancer (antisickling)	2022	Not yet recruiting

BLA, biologic license application; HDR, homology-directed repair; ZFN, zinc finger nuclease.

hemoglobinopathies are 3-fold: safe collection of an adequate quantity of long-term HSCs, long-term expression with adequate engraftment of gene-modified cells with minimal toxicity to patients, and safe, efficient, and cost-effective manufacturing techniques enabling equitable access to therapies, including in vivo gene delivery.

Data suggest plerixafor mobilization is safe, efficient, and capable of yielding sufficient HSC quantities in most patients for clinical gene therapy applications,[21-24] although expanded options are urgently needed for those who do not mobilize sufficiently with plerixafor alone, particularly given the need for multiple mobilization cycles to collect sufficient quantities of HSCs for manufacturing.[48] Concerns regarding toxic conditioning, infertility, and secondary malignancy remain significant, leading to the development of multiple reduced toxicity conditioning strategies, largely antibody based.[49-51] Recently, a multiplex base-edited engineered HSC including a therapeutic edit at the gamma-globin promoter and a missense mutation in the extracellular domain of CD117 (cKIT), a receptor tyrosine kinase expressed by hematopoietic stem and progenitor cells (HSPCs) that regulates HSPC survival, proliferation, and differentiation, was reported.[52] Eighty percent of biallelic CD117 editing and near-complete editing of the HbF locus were achieved, and treatment of HSPCs with an anti-CD117 monoclonal antibody in vitro resulted in >85% reduction in viability of unedited HSPCs, while CD117-edited cells remained unaffected. A similar model is being trialed using base editing to produce HbG-Makassar along with a mutation in CD117.[53] Such strategies may enable less toxic pretransplant conditioning for autologous HSC-based SCD therapies and represent a promising potential alternative to busulfan-based myeloablative regimens that may preserve fertility.

Last, the overall projected costs of and equitable access to ex vivo gene therapies have fueled the ongoing preclinical development of in vivo gene therapies,[54] which could prove to be more portable and require less infrastructure, thus expanding access to these critical therapies. Whereas costs for gene therapy are high, gene therapy may be more cost-effective than a lifetime of emerging disease-modifying therapies[55] or more cost-effective than allogeneic transplantation as a result of significantly less costs in the posttransplant period.[56] Additional costs for gene therapy occur largely in the pretransplant and manufacturing stage; therefore, in vivo delivery methods that do not require stem cell mobilization or manufacturing to facilitate delivery are needed. Safe, in vivo delivery of CRISPR/Cas9 is possible for the treatment of human disease,[57] and targeting of CD117 lipid nanoparticles that can deliver RNA to HSCs in vivo is being investigated.[45,58] In vivo HSC prime editing was recently shown to rescue SCD in a mouse model[59] and may represent a simplified and portable strategy for autologous HSC-based SCD gene therapy.

Conclusion

Gene therapy for SCD has the potential to be curative, with preliminary data showing small successes that have improved over time, now with at least 2 cell therapy methods submitted for FDA approval. Early data in both gene addition and gene editing trials for SCD suggest the possibility of a future free from vaso-occlusive events, improvement in quality of life, and clinically meaningful improvements in patient outcomes. Data are limited by the small number of patients treated, a relatively short follow-up period, and concerns regarding long-term safety; however, hope remains for a large population of patients with SCD in need of curative therapeutic options. For the patient presented in this case, transplantation with autologous HSCs modified either by lentiviral vector gene addition or by gene editing to raise HbF is a viable strategy to offer this patient who does not have a matched sibling, whose disease is worsening with age, and who is interested in a curative option. The risks of these therapies should be well explained and well understood for this patient, who must weigh significant risks against the possible benefit of a clinically meaningful cure after autologous gene therapy.

Conflict-of-interest disclosure

Alexis Leonard: no competing financial interests to declare.
John F. Tisdale: no competing financial interests to declare.

Off-label drug use

Alexis Leonard: none to disclose.
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When to consider inherited marrow failure syndromes in adults

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The inherited bone marrow failure syndromes (IBMFS) are a heterogenous group of disorders caused by germline mutations in related genes and characterized by bone marrow failure (BMF), disease specific organ involvement, and, in most cases, predisposition to malignancy. Their distinction from immune marrow failure can often be challenging, particularly when presentations occur in adulthood or are atypical. A combination of functional (disease specific assays) and genetic testing is optimal in assessing all new BMF patients for an inherited etiology. However, genetic testing is costly and may not be available worldwide due to resource constraints; in such cases, clinical history, standard laboratory testing, and the use of algorithms can guide diagnosis. Interpretation of genetic results can be challenging and must reflect assessment of pathogenicity, inheritance pattern, clinical phenotype, and specimen type used. Due to the progressive use of genomics, new IBMFS continue to be identified, widening the spectrum of these disorders.

LEARNING OBJECTIVES

- Understand the clinical features and laboratory testing used to distinguish immune from inherited bone marrow failure (IBMFS) in adults
- Review when germline genetic testing for IBMFS is indicated and the common diagnostic challenges
- Learn why timely diagnosis of IBMFS is crucial for proper patient management

Introduction

Bone marrow failure (BMF), characterized by decreased production of 1 or more hematopoietic lineages, is classified as either inherited, due to germline variants, or acquired, most commonly immune mediated. Inherited bone marrow failure syndromes (IBMFSs) have been traditionally considered pediatric onset disorders; however, it is increasingly recognized that many present first in adulthood. Classical IBMFSs, including Fanconi anemia (FA), dyskeratosis congenita (DC), Shwachman Diamond syndrome (SDS), and Diamond Blackfan anemia (DBA) are mostly diagnosed in children but can present later in life, sometimes due to genetic somatic rescue or mosaicism. In adults, typical clinical findings may be missing, and diagnosis may be challenging without the use of specialized testing. Increased use of genetic testing has further characterized the broad spectrum of known IBMFSs and identified a wide range of new ones, the latter of which often present in adulthood and with predominant nonhematologic features.[1,2]

CASE 1

A 23-year-old male presented to the emergency room with pallor and bleeding and was pancytopenic: hemoglobin (Hb) 7.5 g/dL, absolute neutrophil count (ANC) 0.6×10^9/L, platelets 17×10^9/L, absolute monocyte count 0.08×10^9/L, and absolute reticulocyte count 50×10^9/L. No prior blood counts were available. No vitamin deficiencies or paroxysmal nocturnal hemoglobinuria clone were detected. Bone marrow examination showed hypocellularity of 30% with no dysplasia and a normal karyotype. Flow cytometry of the bone marrow showed a reduction in B-cells, B-cell precursors, and natural killer (NK) cells. Personal history was significant for recurrent pulmonary infections and warts. Family history was negative for malignancy or hematologic disorders. A diagnosis of acquired severe aplastic anemia (AA) was made.

Inherited versus immune marrow failure

Cytopenias and evidence of marrow hyperproliferation are the defining features of BMF but are present in both immune

and inherited forms.[3] Therefore, in evaluating BMF patients, careful consideration should first be given to the patient's disease history, concurrent medical comorbidities, and family history to assess for potential immune BMF versus IBMFS.[4] When positive, family history can aid in diagnosing IBMFSs, but varied clinical phenotype and disease heterogeneity even among family members make it less helpful when negative. Other causes of cytopenia, such as vitamin deficiency, viral infection, direct toxicity, autoimmune diseases, and medications, should be excluded. Patients with IBMFSs may have distinct clinical patterns of disease to guide diagnosis; for instance, limb and/or renal abnormalities in FA, lung and/or liver abnormalities in TBD, or recurrent atypical infections in GATA2 deficiency (Table 1).[5] More recently, disease-specific molecular profiles, including mechanisms of somatic genetic rescuing, have also been identified as potential markers of underlying IBMFS.[6]

Immune AA remains a diagnosis of exclusion; age of onset is bimodal with disease more common in younger and older patients. Clinical testing is focused toward excluding IBMFS; however, some features, such as the presence of glycosyl-phosphatidylinositol–negative paroxysmal nocturnal hemoglobinuria (PNH) clones and loss of heterozygosity in the chromosome 6 p arms (6pLOH), are reassuring markers of an immune etiology,[7,8] as is a clonal profile dominated by *PIGA*, *BCOR*, and *BCORL1*.[9,10] *BCOR* and *BCORL1* are the most common somatic mutations seen in AA and are often seen in isolation or co-occurring with *PIGA*.[10] While also present in myelodysplastic syndrome (MDS), they are not predominant and also tend to co-occur with other mutations.[11] T-cell large granulocytic leukemia clones are also more common in acquired than in inherited BMF but are less specific than PNH or 6pLOH (Figure 1).

Most specialized centers have routinely incorporated chromosome breakage studies and telomere length (TL) measurement by flow fluorescence *in situ* hybridization (FISH) in the clinical assessment of newly diagnosed AA. Other specialized testing, such as pancreatic dysfunction for SDS and erythroid adenosine deaminase activity for DBA, may be reserved for clinically suspected cases (Figure 2). Testing for primary immunodeficiency syndromes, using lymphocyte subsets and serum immunoglobulins, is pursued when there is a clinical history suggestive of recurrent and/or atypical infections, autoimmunity, or presence of severe lymphopenia.

CASE 1 (continued)

As the patient had no matched sibling donors, immunosuppressive therapy (IST) was promptly administered. Given his young age and history of warts and pulmonary infection, diagnostic genetic testing was performed. After 6 weeks, blood counts had not improved; ANC remained >0.5×10^9/L. Meanwhile the patient developed fever, progressive cough, and dyspnea. Computed tomography of the thorax showed patchy and nodular pulmonary infiltrates within the right middle, left, and lower lobes. Bronchoscopy samples grew mycobacterium avium complex (MAC). Immunosuppressive therapy was discontinued, and matched unrelated donor transplant was pursued. His germline genetic report returned and showed a pathogenic variant in the *GATA2* gene.

When to consider genetic testing

One of the most difficult considerations in work up of BMF is when to perform genetic testing. Most BMF is classified as immune (>90%), and genetic testing is costly and not always available. However, missing an IBMFS has significant clinical implications, and genetic testing is currently our best method of detection.[3,12,13] Increasingly, it is recognized that omission of genetic testing results in missed IBMFS diagnoses. A retrospective study including immune severe AA (SAA) patients using pre–hematopoietic stem cell transplant samples from the Center of International Blood and Marrow Transplant Research (CIBMTR) showed an undiagnosed IBMFS in ~7% of patients, one-third of whom were adults.[14] Most were nonclassical IBMFS (such as *RUNX1*, *MECOM*, *ANKRD26*, and *GATA2*) or TBD. Similarly in MDS, 7% of patients were found to have underlying germline predisposition, highest in the younger age group (33% for aged 11-20 years) but still significant in older patients (6%-8%).[15] One-quarter of germline genes mutated in MDS were implicated in IBMFSs.

Screening for IBMFSs should be also considered in young patients with atypical oncologic presentations or unexpectedly high toxicity to cytotoxic chemotherapy or hematopoietic stem cell transplant (HSCT).[16]

Recently, we developed a machine learning model to predict for immune versus inherited in adults with AA. By using patients' baseline clinical and laboratory characteristics, our model accuracy correctly predicted 88% of cases; TL, cutaneous findings, long-standing cytopenias, macrocytosis, and age/sex were top predictors.[17] Omission of TL dropped the model's accuracy, highlighting its importance. Adult patients with SAA without a positive family history or a clinical phenotype suggestive of inherited disease were rarely diagnosed with an IBMFS. Where genetic testing is not feasible, selection of patients for germline genetic screening should take into account age, clinical presentation, family history, and available laboratory and specialized test results.

Inheritance and penetrance as challenges for IBMFS diagnosis

IBMFSs can be linked to different inheritance patterns depending on the specific mutated gene being either autosomal recessive (AR) (2 mutated alleles required to cause disease), autosomal dominant (AD) (1 mutated allele required to cause disease), X-linked, or *de novo*. In general, AR disorders tend to have high penetrance and earlier disease onset while AD disorders have more variable penetrance and later onset, but there are exceptions to this.[18,19] *De novo* or AR variants represent a challenge for IBMFS diagnosis. *De novo* mutations first occur in the affected patient due to a mutation in the parental germ cell or during embryogenesis. In such cases, family history will be absent (as in case 1). Examples of IBMFSs that classically occur *de novo* are DBA (~50% of cases),[20] GATA2 deficiency, and the *TINF2* subset of TBD.[21] Family history may also be absent when consanguinity is present, family penetrance is incomplete, or due to AR inheritance. Therefore, one cannot omit specialized work up or genetic testing on the basis of family history alone.

Non-classical IBMFS

Newly described inherited monogenic diseases that may present as marrow failure have been defined, differing from the classical

Table 1. Inheritance and common clinical findings of inherited BMF syndromes

	Fanconi anemia[37]	Telomere biology disorders[1]	Shwachman Diamond syndrome[38]	Diamond Blackfan anemia[39]	GATA2	SAMD9/9L[28]	Platelet disorders[24]	MECOM-associated disorders[25]	ERCC6L2[2,40]	DADA2[41]
Affected genes	FANC genes, *BRCA2*	*DKC1, TERT, TERC, PARN, RTEL1, TINF2, CTC* + others	*SBDS, DNAJC21,* and others	RP genes, *TSR2, GATA1*	*GATA2*	*SAMD9/SAMD9L*	c-*MPL* (CAMT) *RBM8A* (TAR) *RUNX1, ETV6, ANKRD26,* and others	MECOM (MDS1 and EVI1 complex locus)	*ERCC6L2*	*ADA2*
Inheritance	AR except for FANCB (XLR) and FANCR (AD)	AD: *TERT, TERC, TINF2, RTEL1, PARN* AR: *CTC, RTEL1* XLR: *DKC1*	AR	AD, XLR or sporadic	AD	AD	AR: (CAMT, TAR) AD: *RUNX1, ETV6, ANKRD26*	AD	AR	AR
Common non hematologic clinic findings	- Limb abnormalities (absent radii/short thumbs) - Short stature - Renal anatomical defects - Café au lait spots - Microcephaly/ Microphthalmia	- DC triad: oral leukoplakia, dyskeratotic nails, reticulated skin - Pulmonary fibrosis - Liver disease (fibrosis, fatty liver) - AVM - Early grey hair - Immunodeficiency - Osteoporosis	- Failure to thrive/poor feeding - Steatorrhea - Recurrent infections - Skeletal abnormalities - Hepatomegaly - Intellectual disability - Congenital cardiac defects - Endocrinopathy	- Short stature/IUGR - Limb abnormalities (thumb) - Cardiac defects (VSD, ASD) - Cephalic malformation (microcephaly) - Developmental delay	- Immunodeficiency (atypical mycobacteria, recurrent warts from HPV) - Lymphedema - Thrombosis - Pulmonary alveolar proteinosis (dyspnea and cough)	- MIRAGE: myelodysplasia, infection, growth restriction, adrenal hypoplasia, genital problems, enteropathy - Ataxia Pancytopenia: cerebellar symptoms and pancytopenia - SAAD: nodular neutrophilic panniculitis, ILD, basal ganglia calcifications, cytopenia	- CAMT: some neurological associations, possibly related to ICH - TAR: skeletal defects (absent radii), cow's milk intolerance, renal tract abnormalities, cardiac defects) - RUNX1: platelet function defect	- Radioulnar stenosis - Clinodactyly - Hearing loss - Cardiac malformations - Renal malformations	- Microcephaly - Developmental delay	- Strokes - Vasculitis - Systemic inflammation - Hypogammaglobulinemia
Malignancy risk	- MDS/AML - SCC of skin, head/neck, anogenital	- MDS/AML - SCC skin, head/neck, anogenital - BCC skin	MDS/AML	- MDS/AML - Colon cancer - Osteogenic sarcoma	- MDS/AML - SCC skin, anogenital - BCC skin	MDS/AML	- RUNX1/ETV6/ ANKRD26: MDS/AML or ALL - ALL > ETV6, MDS/AML > RUNX1/ANKRD26	MDS/AML	MDS	None known

AD, autosomal dominant; AML, acute myeloid leukemia; AR, autosomal recessive; ASD, atrial septal defect; AVM, arteriovenous malformation; BCC, basal cell carcinoma; CAMT, congenital amegakaryocytic thrombocytopenia; DC, dyskeratosis congenita; HPV, human papilloma virus; ICH, intracranial hemorrhage; ILD, interstitial lung disease; MDS, myelodysplastic syndrome; RP, ribosomal protein; SAAD, SAMD9L-associated autoinflammatory disease; SCC, squamous cell carcinoma; TAR, thrombocytopenia absent radii; VSD, ventricular septal defect; XRL, X-linked recessive. References listed as superscript.

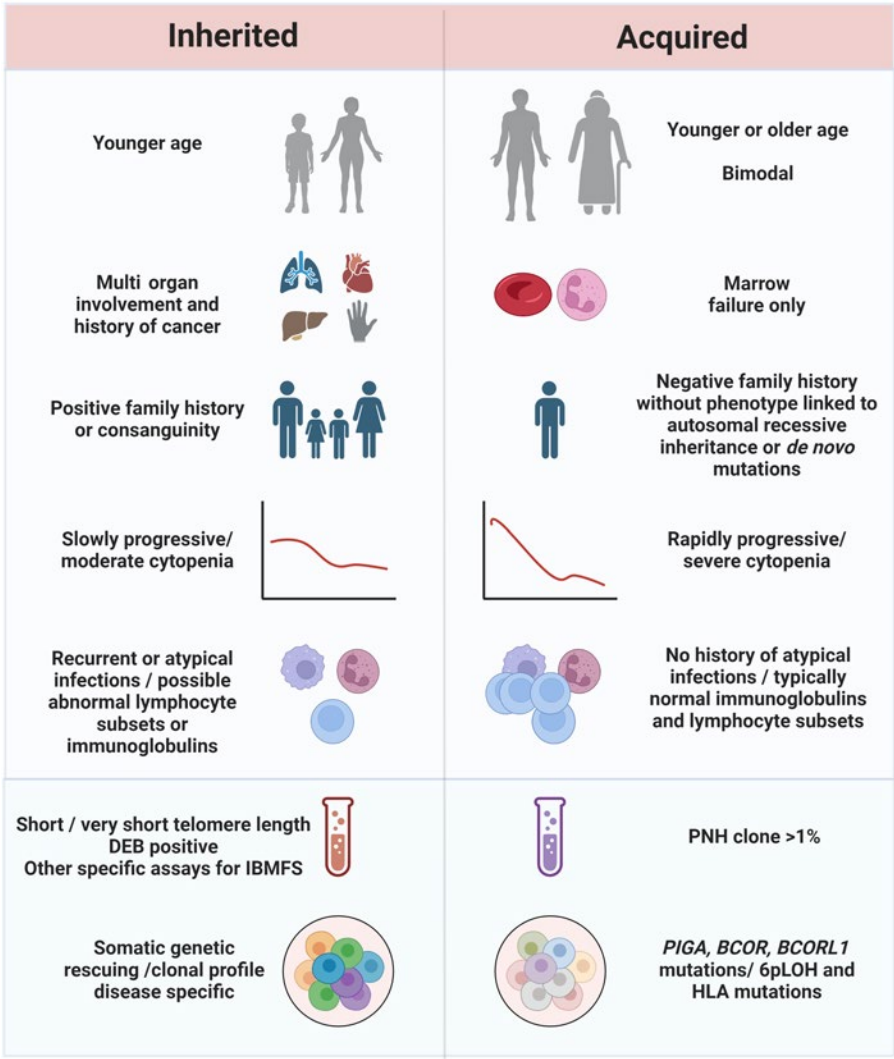

Figure 1. Considerations for inherited versus acquired bone marrow failure. Age, evidence of other organ involvement, and family history of cytopenia, hematologic malignancy, solid cancers, or other organ involvement (ie, familial pulmonary fibrosis in telomere biology disorders). Patients with IBMFSs may have had long-standing cytopenias for years; adult patients who present acutely with severe cytopenia more commonly have immune-mediated disease. Low immunoglobulins or lymphocyte subsets may point towards a primary immunodeficiency disorder; these are typically preserved in immune marrow failure. Paroxysmal nocturnal hemoglobinuria clones are commonly seen in immune bone marrow failure but very rare in IBMFS. Many IBMFS have disease specific clonal patterns and somatic genetic rescuing may occur. In immune BMF, ***PIGA*** (driver of PNH), ***BCOR/L1***, and human leukocyte antigen mutations predominate. DEB, diepoxybutane.

IBMFSs in terms of their typical age of onset, constellation of symptoms, and diagnostic testing. GATA2 deficiency was first described in 2010 as 4 different diseases based on the slightly different clinical observations. Patients can present in late adolescence or early adulthood, with a variable clinical phenotype, even among affected family members.[22] Patients with GATA2 deficiency may present with cytopenia and have a hypocellular marrow consistent with aplastic anemia. However, reduced numbers of B cells and precursors, NK cells, and monocytes are characteristic of GATA2.[23] Other manifestations include opportunistic infections, lymphedema, and predisposition to MDS and/or leukemia.[22]

Patients with isolated chronic thrombocytopenia, particularly with a pertinent family history, should be investigated for a familial platelet disorder. Congenital amegakaryocytic thrombocytopenia and thrombocytopenia with absent radii are usually apparent in early childhood. However, diseases related to RUNX1, ETV6, and ANKRD26 often present later in adolescence or adulthood; they are characterized by thrombocytopenia, variable bleeding phenotype, and predisposition to hematologic malignancy.[24] More recently identified, MECOM-associated syndromes (MDS1 and EVI1 complex locus) are typically pediatric and present with skeletal defects and amegakaryocytic thrombocytopenia.[25]

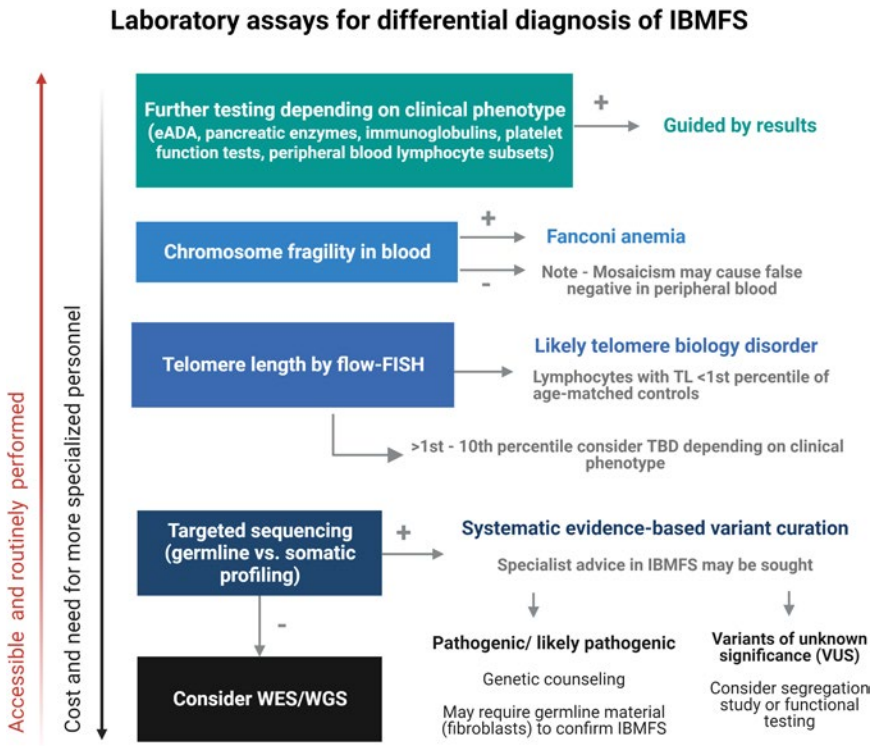

Figure 2. Approach to specialized work up of confirmed BMF. Specialized assays can be used for diagnosis of the IBMFS. Chromosome breakage studies for FA are performed by exposing cultured cells (usually peripheral blood lymphocytes) to diepoxybutane (DEB), a DNA cross-linking agents, and seeing how much chromosomal breakage is induced at a concentration of DEB that has little effect on normal cells.[42] Testing can be misleading or inconclusive for 2 reasons: 1) somatic reversion in the hematopoietic cells causes a false negative (this can be overcome by testing skin fibroblasts if you have a high clinical suspicion and negative peripheral blood DEB) or 2) recent chemotherapy administration (increase in baseline breakage). Telomere length is assessed in peripheral blood lymphocytes using flow-FISH and reported as a percentile for age. TL in lymphocytes <1st percentile is very sensitive and specific for TBD, ≥1st but <10th percentile is suggestive of possible TBD in the right clinical context, and ≥10th percentile is very unlikely to be TBD.[43] High erythroid adenosine deaminase (eADA) enzyme activity levels are found in cases of DBA. Targeted sequencing can identify both germline and somatic variants when peripheral blood is used; to confirm germline status, sequencing of a germline control tissue such as fibroblasts (skin biopsy) or testing of family members should be sought. Interpretation of genetic reports, particularly when VUS is reported, is challenging, and specialist input may be required. Testing for primary immunodeficiency syndromes is pursued when there is a clinical history suggestive of recurrent and/or atypical infections, autoimmunity, or presence of severe lymphopenia. Lymphocyte subsets and serum immunoglobulins are useful in this setting. FISH, fluorescence ***in situ*** hybridization; TBD, telomere biology disorder; WES, whole exome sequencing; WGS, whole genome sequencing.

Some inborn errors of immunity, characterized by immunodeficiency or other immune dysregulation, may also present as marrow failure, such as Toll-like receptor 8 gain of function mutations.[26] A careful history focused on infection and autoimmunity is required to identify such patients.

CASE 2

A 35-year-old female presented to the emergency department with dyspnea and pancytopenia: Hb 6.9 g/dL, ANC 1.2×10^9/L, platelets 6×10^9/L, and absolute reticulocyte count 55×10^9/L. Past medical history was significant for chronic immune thrombocytopenia and menorrhagia. Ferritin was 20 mcg/L with normal B12 and folate. Family history was significant for mother with chronic immune thrombocytopenia and iron deficiency and maternal aunt with leukemia. Bone marrow examination performed showed a mildly hypercellular marrow with megakaryocytic dysplasia (>10%), mild dysethryopoiesis, and dysgranulopoiesis. Blast count was 7% and karyotype was normal. Genetic testing identified variants in *RUNX1* (variant allele frequency [VAF] 55%) and *TET2* (VAF 35%). A diagnosis of MDS was made.

Clonal hematopoiesis vs germline predisposition in IBMFS

Most IBMFSs have an increased risk of myeloid malignancy, in particular MDS and acute myeloid leukemia.[16] Clonal hematopoiesis in myeloid-cancer genes may predict for clonal evolution in many IBMFSs, and distinct patterns of clonality can guide clinical suspicion for a particular disorder. In FA, malignancy has been linked to chromosome 1 q gain and cryptic *RUNX1/AML1* lesions;

in TBD, with *U2AF1*$^{S34/Q157}$ mutations; in SDS, with biallelic *TP53* mutations; and in *SAMD9/9L* disorders, with monosomy 7.[27-30] The genes and variants commonly found somatically mutated in typical MDS and acute myeloid leukemia are the same found in germline disorders, most commonly *RUNX1, ETV6, DDX41, TP53, GATA2, BRCA1, BRCA2,* and others.[31] Therefore, determining whether a variant is germline or somatic is crucial to distinguish *de novo* MDS from that secondary to IBMFS or another germline predisposition syndrome.

DNA sequencing assays covering genes related to hematologic disease often cannot distinguish whether variants are germline or somatic. In peripheral blood bulk DNA sequencing, germline variants are expected to be at allele frequencies of ~50% or ~100%, if heterozygous or homozygous, respectively. In ranges outside these limits (VAF <30% and >70%-85%), variants are often considered somatic.[32] However, revertant genetic rescuing can change the VAF of germline variants into somatic ranges, resulting in a false negative result; this should be considered when a suspicious variant outside the typical germline range is detected. When variants in germline predisposition genes are found at VAFs >30%, sequencing of germline tissues or affected relatives is important to distinguish between somatic and germline variants.[15,31,32] Cultured fibroblasts obtained by skin biopsies are considered optimal controls, but because their collection is difficult in many centers and extra time is required to culture the fibroblasts, results are delayed. Alternative sources of DNA are buccal swabs and hair follicles; buccal swabs are not recommended due to contamination with blood cells, and large numbers of hair follicles may be required to yield results. Co-occurrence of adaptive clonal hematopoiesis with germline variants related to IBMFS (mechanisms of somatic genetic rescuing) can be a natural proof-of-concept that a potential germline variant is pathogenic and disease causing. Examples of somatic markers of IBMFS include *PPM1D*, *POT1*, and the *TERT* promotor in TBD; *EIF6* mutations in SDS; transient monosomy 7 in SAMD9/9L disorders; and concurrent somatic *DDX41* mutations with germline *DDX41*.[33,34]

CASE 2 (continued)

The patient underwent a skin biopsy (with cultured fibroblasts) that identifies the same *RUNX1* variant but not the *TET2* in cultured tissue. The same *RUNX1* variant is confirmed in her mother. The patient is ultimately diagnosed with MDS with germline predisposition due to germline *RUNX1* and begins a work up for HSCT. The family history of thrombocytopenia, bleeding phenotype, and family history of leukemia are suspicious for a hereditary platelet disorder.

Difficulties in interpretation of genetic testing

A systematic evidence-based curation of variants and the incorporation of practical guides, often gene specific, for interpretation of genetic reports has been increasingly used in practice. The type of sample, timepoint of evaluation, and sequencing platform and depth are considered. Different methods with various sensitivities can detect types of genetic alterations: structural variations, small insertions and deletions (indels), single nucleotide variants, large copy-number variations (duplications and deletions), translocations, inversions, and aneuploidy. Large copy-number variations and small alterations in genes or intronic regions may not be covered by a targeted next generation sequencing panel.[6] Whole exome/genome sequencing as well as deletion/duplication analysis may identify uncharacterized genes linked to IBMFS.

Identification of variants of unknown significance (VUS) is a common challenge for interpretation of results, and negative reports may lead clinicians to often mistakenly rule out an IBMFS. Interpretation of a VUS should incorporate the patient's clinical phenotype, family history, frequency in the normal population, and prior reports in the literature of the variant's pathogenicity.[32] Segregation studies (assessment of affected and unaffected family members for the variant) may be useful. In cases with a suspicious family history and either negative germline testing or a VUS in a suspicious IBMFS gene, referral to specialist is recommended to guide further assessment.

Importance of detecting IBMFS for therapeutics

With high index of suspicion, early diagnosis of an IBMFS may improve outcomes. HSCT offers a cure for all marrow failure syndromes, inherited and acquired; however, IST is also standard for immune mediated marrow failure in adults and in older patients who lack a matched sibling donor.[3] Patients with IBMFSs do not respond to IST, leading to increased cytopenia-related complications, delay in appropriate care, and, potentially, suboptimal therapy if misdiagnosed. A recent study reported worse survival after HSCT in patients with unrecognized IBMFS compared to those with immune AA, most commonly due to organ failure.[14] When broken down by IBMFS subtype, patients with DNA damage response disorders (including FA) and TBD had significantly poorer overall survival while those with ribosome biology disorders (DBA and SDS) and hematopoiesis disorders (familial platelet disorders [RUNX1, MPL, ETV6, ANKRD26], MECOM, GATA2 deficiency and DADA2) had similar overall survival to those with immune AA.

HSCT is undertaken as a potentially curative option for the hematologic manifestations of a wide range of IBMFSs,[3,33] all with disease-specific HSCT considerations and outcomes. Differing genetic defects and associated clinical phenotypes make a uniform HSCT approach problematic. IBMFSs predispose patients to particular post-HSCT complications depending on underlying disease pathophysiology, including specific organ damage, increased graft-versus-host or graft-failure risk, or development of secondary malignancy. Choice of conditioning regimen is known to play an important role in improving outcomes in DNA damage response disorders and in TBD. In FA patients undergoing HSCT, secondary malignancies and endocrine complications predominate, which have been mitigated but not eliminated using radiation-free reduced-intensity conditioning.[35] TBD patients often have disease involving major organs, particularly the lung and liver, and vasculature, which may worsen with HSCT, resulting in increased morbidity and mortality; studies are ongoing to assess alkylator and radiation-free conditioning regimens (NCT01659606).[35,36] Therefore, at minimum, specialized testing for FA and TBD should be performed before HSCT in all BMF patients, even if a full genetic panel is not possible.

In cases in which HSCT is urgently indicated, the risks and benefits of waiting for genetic testing should be weighed against the likelihood of an IBMFS in an individual patient. Genetic

screening of familial donors in known IBMFS is also important and should occur prior to transplant, even in absence of clinical manifestations, to prevent unknowing use of an affected graft, although screening should also be balanced against the urgency of proceeding to HSCT.

Conclusions

Ideally, all new patients presenting with new onset BMF should undergo germline genetic screening regardless of age or clinical phenotype. Diagnostic work up of IBMFS can be challenging; in nonspecialist centers or poor-resource settings, specialized testing such as chromosome breakage studies, TL, or genetic testing may not be easily available. Interpretation of genetic results can be tricky and may require specialist input. Missing an IBMFS has important clinical consequences: such patients are at increased risk of other organ involvement and malignancy, and these patients require disease-specific monitoring. Identification of an IBMFS significantly impacts therapy; IST is typically ineffective, and HSCT may require modification from standard regimens or other special considerations.

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Conflict-of-interest disclosure

Fernanda Gutierrez-Rodrigues: no competing financial interests to declare.
Bhavisha A. Patel: no competing financial interests to declare.
Emma M. Groarke: no competing financial interests to declare.

Off-label drug use

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Management of Fanconi anemia beyond childhood

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Fanconi anemia (FA) has long been considered a severe inherited bone marrow failure (BMF) disorder of early childhood. Thus, management of this multisystem disorder has previously been unfamiliar to many hematologists specializing in the care of adolescents and young adults (AYA). The increased diagnosis of FA in AYA patients, facilitated by widely available germline genomic testing, improved long-term survival of children with FA following matched sibling and alternative donor hematopoietic stem cell transplantation (HSCT) performed for BMF, and expanding need in the near future for long-term monitoring in patients achieving hematologic stabilization following ex vivo gene therapy are all reasons why management of FA in AYA populations deserves specific consideration. In this review, we address the unique challenges and evidence-based practice recommendations for the management of AYA patients with FA. Specific topics addressed include hematologic monitoring in AYA patients yet to undergo HSCT, management of myeloid malignancies occurring in FA, diagnosis and management of nonhematologic malignances and organ dysfunction in AYA patients with FA, and evolving considerations for the long-term monitoring of patients with FA undergoing gene therapy.

LEARNING OBJECTIVES

- Delineate the unique medical challenges facing adolescents and young adults with Fanconi anemia
- Provide updated approaches for the management of hematologic disease in adolescents and young adults with Fanconi anemia

Introduction

Fanconi anemia (FA) is an inherited bone marrow failure (BMF) disorder caused by pathogenic variants in 1 of 23 genes[1] that result in defective repair of DNA interstrand crosslinks, genomic instability, cell cycle dysregulation, and cell death or transformation. In most affected individuals, including patients with biallelic mutations in *FANCA*, *FANCC*, and *FANCG* accounting for over 80% of cases,[2] FA is inherited in an autosomal recessive pattern, although distinct inheritance patterns and specific genotype–phenotype correlations are known (Table 1).

BMF in FA results from selective attrition of CD34$^+$ hematopoietic stem cells (HSCs) that significantly precedes the development of clinical cytopenias. This pathophysiology creates a unique challenge for autologous CD34$^+$ HSC collection for gene therapy applications.[3] HSC loss results from many factors, including excess DNA damage from endogenous reactive aldehydes, inflammatory cytokines released during typical childhood infections, and abnormal telomere shortening.[4,5] Recently, natural killer cell-mediated immune destruction through FA HSC upregulation of natural killer group 2 D ligand expression has been implicated as a key mechanism driving HSC attrition.[6]

Early studies suggested that 75% of patients with FA were diagnosed due to evolving BMF in the first decade of life, leading to initial impressions that FA was primarily an early pediatric disorder.[7] However, these studies mostly comprised patients with classic, severe *FANCA*, *FANCC*, and *FANCG* mutations. Recently expanded use of next-generation sequencing (NGS) to identify germline predisposition in adolescents and young adults (AYA) with BMF and myeloid malignancies (MMs) is now diagnosing FA in older patients with distinct phenotypes. Improvements in long-term survival following matched sibling and alternative donor HSC transplantation (HSCT) in children with FA and the advent of gene therapy that ameliorates but does not fully correct hematologic deficits have led to the need for increasing education and practice guidelines for hematologists specializing in the care of AYA patients with FA.

CLINICAL CASE

A 20-year-old man presents to the student health center at his university with increased fatigue and bruising. Medical history is significant for a ventricular septal

Table 1. Genes associated with Fanconi anemia

Gene	% of FA cases	Inheritance	Population distribution and unique phenotypes
FANCA	45–60	AR	Founder mutations: Middle Eastern, North African, Spanish Romani, Afrikaner, Sicilian Mutation-specific disease severity
FANCC	10–15	AR	Founder mutations: Ashkenazi, Saudi, northern Europe Exon 15 mutations: more severe phenotype c.67delG: milder phenotype
FANCG	5–10	AR	Founder mutations: Sub-Saharan Africa, Japan, Korea Severe hematologic disease
FANCB	1–2	XL	VACTERL-H common
FANCD1/BRCA2	1–4	AR	High leukemia risk: myeloid and lymphoid Early childhood solid tumors: brain, Wilms, neuroblastoma Aplastic BMF uncommon Carriers: risk of breast, ovarian, prostate, pancreatic cancer
FANCD2	3–5	AR	Sequencing challenging due to pseudogenes
FANCI	1–4	AR	VACTERL-H common
FANCJ/BRIP1	1–4	AR	Carriers: increased breast/ovarian cancer risk
FANCL	1–2	AR	Founder mutations: India, Pakistan VACTERL-H common
FANCM	<2	AR	Lower risk of congenital anomalies and BMF Early-onset cancer risk
FANCN/PALB2	<2	AR	Severe clinical presentation High leukemia risk Early childhood solid tumors: brain, Wilms, neuroblastoma Carriers: breast and pancreatic cancer risk
FANCQ/ERCC4	<2	AR	Overlap with xeroderma pigmentosum
FANCR/RAD51	<2	AD	Congenital anomalies common BMF and cancer not yet reported
FANCS/BRCA1	<2	AR	Severe solid tumor and leukemia risk Congenital anomalies BMF not yet reported Carriers: risk of breast, ovarian, prostate, pancreatic cancer
FANCE, *FANCF*, *FANCO*, *FANCP*, *FANCT*, *FANCU*, *FANCV*, *FANCW*, *FANCY*	<2	AR	Rare cases only

Data derived from Fanconi Anemia Clinical Care Guidelines.[2]

AD, autosomal dominant; AR, autosomal recessive; VACTERL-H, vertebral, anal, cardiac, tracheoesophageal fistula, esophageal atresia, renal, limb, hydrocephalus; XL, X-linked.

defect repair in early childhood and what he describes as slightly low blood counts detected on a sports clearance evaluation when he was 16 years old. Physical exam reveals only short stature (height 63 inches/160 cm) and a white patch on his left buccal mucosa. Laboratory evaluation reveals a normal white blood cell count and differential, macrocytic anemia (mean corpuscular volume [MCV], 105 fL; hemoglobin, 8.5 g/dL) and thrombocytopenia (platelets, 33 000/μL). He is admitted to the nearby university hospital, where hematology performs a bone marrow aspirate and biopsy, revealing hypocellularity (20%) multilineage dysplasia and no excess blasts by morphology. Metaphase cytogenetics and fluorescence in situ hybridization reveal a gain of chromosome 3q (20% by fluorescence in situ hybridization). Chromosome stress testing reveals increased breakage upon exposure to diepoxybutane. NGS revealed biallelic pathogenic mutations in *FANCA*.

Diagnosis of FA in adolescence and young adults

Any AYA patient (up to age 40 years) who presents with BMF or MM associated with 1q, 3q, or 7q copy number abnormalities/translocations or unusual solid tumors for age (oral, head/neck, genital) should undergo a diagnostic evaluation for FA. Screening should include at minimum a chromosome stress test performed on peripheral blood lymphocytes exposed to DNA crosslinking agents diepoxybutane and mitomycin C. This screening by chromosomal stress test remains the gold standard in diagnosing FA. A positive test should trigger NGS testing and

Table 2. Factors that should trigger high clinical suspicion for FA in adolescent and young adult patients presenting with bone marrow failure or myeloid malignancies

Clinical features
• Positive family history of bone marrow failure • Long-standing cytopenias • Characteristic congenital abnormalities including VACTERL-H, café au lait, short stature, microcephaly • Myeloid malignancy with +1q, +3q, or −7/del7q cytogenetics or FISH • Excessive toxicity with chemotherapy • Unusual solid tumor for young adults (oral, head/neck, liver, stomach, genital)

FISH, fluorescence in situ hybridization.

sensitive copy number analysis of the 23 genes associated with FA. Notably, 1 mechanism resulting in delayed onset of hematologic abnormalities in patients who present with FA at older ages is revertant hematopoietic clonal evolution resulting in elimination of 1 FA-associated mutation.[8] Such mosaicism can result in equivocal or even normal results on blood-based chromosome stress and NGS testing. Thus, in AYA patients with a high clinical suspicion for FA (Table 2) but in whom peripheral blood screening is normal or equivocal, screening results should be confirmed using skin fibroblasts. Once a mainstay of FA classification, complementation group testing is now used only in situations where NGS is equivocal in assigning subclassification due to variants of uncertain significance.

Hematologic status of AYA patients with FA

In a recent single-institution study of young adults with FA (age range 18-37 years at last follow-up), Wang et al.[9] demonstrate that AYA patients with FA have quite variable hematologic function. Of 52 adults, 8 (15%) had normal blood counts without prior HSCT, and 31% of the total cohort overall had not required HSCT. Patients with *FANCA* mutations had decreased likelihood of requiring HSCT compared to those with other genotypes (57% vs 83%),[9] consistent with known mild phenotypes and later onset of malignancies conveyed by some *FANCA* mutations.[10] Twenty-seven (52%) had undergone HSCT for BMF at a median age of 10.5 years, and 9 (13%) had undergone HSCT for MM at a median age of 15.4 years.[9] While the trend toward higher likelihood of presentation with MM vs BMF in AYA patients is consistent with earlier registry studies, the increased percentage of AYA patients who have maintained normal blood counts or exhibit only mild BMF in the Wang et al.[9] cohort without ever requiring HSCT may reflect improved diagnosis of patients with milder phenotypes.[9-11]

These findings emphasize that HSCT is not necessarily inevitable in FA and should not be performed in the absence of BMF or MM. This lack of inevitability also creates a challenge for ex vivo gene therapy, because while autologous collection for FA gene therapy is ideally performed at a young age prior to onset of HSC attrition and subsequent severe BMF,[12] proceeding to gene-modified autologous HSC infusion should not be done prior to BMF onset as some patients will not ultimately require stem cell therapy. This temporal disconnect between collection and infusion will make reimbursement models for commercialization of FA gene therapy challenging. For AYA patients with FA who have not undergone successful HSCT, adherence to hematologic malignancy screening is recommended as outlined in the Fanconi Anemia Clinical Guidelines[2] and in Table 3.

Therapy for severe BMF in AYA patients with FA

Several groups have published data demonstrating inferiority of HSCT outcomes for AYA vs young children with FA for BMF indications. In European Society for Blood and Marrow Transplantation (EBMT) published outcomes of adults with FA undergoing HSCT through 2014,[13] 4-year overall survival (OS) for the 64 patients receiving HSCT for BMF was only 48%, much inferior to outcomes in younger pediatric cohorts where OS now exceeds 80% to 90%.[1] These findings are comparable to prior Center for International Blood and Marrow Transplant Research and multicenter analyses of matched sibling donor and alternative donor outcomes,[14] as well as a prospective multicenter study of low-dose busulfan base conditioning for alternative donor HSCT,[15] which all demonstrate poorer outcomes for patients ≥10 vs those under 10 years old.

One practice-changing consequence of this age-based dichotomy in HSCT outcomes is a recommendation to avoid giving therapies long term that forestall onset of severe bone marrow failure but do not definitively fix hematopoiesis, as the consequence of delaying HSCT until the AYA period may result in poorer outcomes. Thus, strategies such as androgen therapy,[16] metformin,[17] and eltrombopag (NCT03206086) should be used in young children only as bridging therapy while completing diagnostics and identifying optimal HSCT donors, or in patients ineligible for HSCT due to lack of feasible donors or comorbidities. In contrast, use of these supportive strategies is reasonable for new-onset BMF occurring in AYA patients, as these individuals are already at high age-based risk for HSCT complications. While early data on use of metformin and eltrombopag hold promise, data supporting the use of androgen therapy are the most extensive. Indeed, AYA patients with FA may exhibit stable hematopoietic function on androgen therapy for many years.[18] For AYA patients with FA unresponsive to these approaches with good organ function, novel HSCT strategies testing risk-adapted alkylator dosing are currently in clinical trials (NCT02143830) to reduce rates of severe toxicities responsible for poor OS in AYA patients.

Hematologic malignancy management in patients with FA

Most myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) cases that arise in patients with FA occur in AYA patients.[19] Rarely, cases of lymphoma and T-cell acute lymphoblastic leukemia have also been seen,[20,21] although these are mostly limited to rare genetic subtypes. MDS/AML in FA has characteristic cytogenetic abnormalities, although not all abnormalities are clear indicators of malignant transformation.[22] Gain of chromosome 1q is the most common abnormality in FA and was once thought to not necessarily represent MDS transformation.[2] Recently, however, +1q has been shown to trigger a specific pathway driving leukemogenesis (Figure 1), starting with MDM4 triplication, which downregulates p53 pathways that in turn rescues BMF but also drives clonal dominance enabling AML development.[23] Prognostic significance of the similarly common chromosome 3q gain remains controversial, whereas chromosome 7q loss represents a late step, signaling imminent AML transformation.[24] In general, gene-specific somatic mutations are less commonly seen in FA compared to other BMF syndromes, although

Table 3. Screening recommendations for adolescent and young adult patients with Fanconi anemia

Specialty/type of screening	Frequency of follow-up screening
Hematology CBC monitoring Bone marrow biopsy/aspirate with cytogenetics	Pre-HSCT: Every 3–4 months Post-HSCT: At least annually Pre-HSCT: Yearly* Post-HSCT: only if clinically indicated
Endocrinology Endocrinology consultation Thyroid function Growth axis 25-OH vitamin D and calcium DXA scan Fasting glucose Oral glucose tolerance test Lipid profile Gonadal function monitoring	Yearly Yearly if normal Screen young adolescents with short stature Yearly Every 5 years Yearly If indicated based on fasting glucose Every 3 years if normal Yearly
Head/neck cancer screening Dental/oral surgery assessments Nasolaryngoscopy Audiology	Every 6 months Every 6 months Based on symptoms
Dermatologic cancer screening	Yearly. Initiate by age 18
Gynecologic screening External and cervical exams	Yearly. Initiate by age 13
Breast cancer screening Mammogram, ultrasound, or MRI	Yearly. Age of initiation depends on genotype
Gastroenterology screening Esophagogastroduodenoscopy Colonoscopy	Only if indicated based on symptoms
Hepatology Liver function tests Liver ultrasound MRI for liver iron concentration	Yearly Every 3–5 years if normal If history of chronic red cell transfusions
Pulmonary Spirometry, diffusing capacity	Every 1–2 years post-HSCT

Based on the Fanconi Anemia Clinical Care Guidelines.[2]

CBC, complete blood count; DXA, dual-energy X-ray absorptiometry; MRI, magnetic resonance imaging.

*More frequent bone marrow (BM) screening recommended in patients with high-risk acquired cytogenetic lesions.

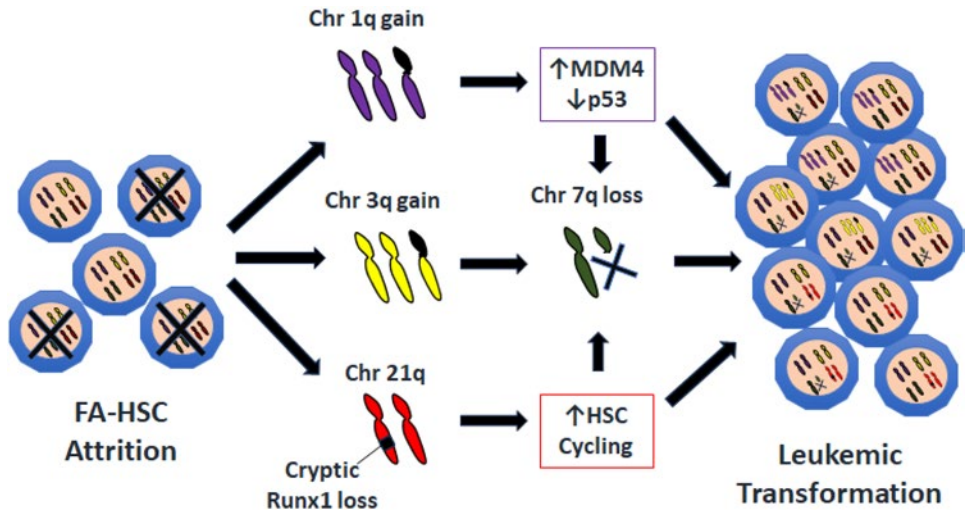

Figure 1. Schematic of leukemic transformation pathways in patients with Fanconi anemia.

Table 4. Recent studies reporting hematopoietic stem cell transplant outcomes for patients with Fanconi anemia and pre-HSCT evolution to myeloid malignancies

Reference	Patients (n)	Era of HSCT	Conditioning	Survival
Mitchell et al. (2014)[35]	MM: 21	1988–2011	Various	All MM: 5-year OS 33%
Bierings et al. (2018)[13]	Total: 199 MM: 54	1991–2014	Various	MDS: 4-year OS 48% AML: 4-year OS 17%
Giardino et al. (2020)[32]	MM: 74	1999–2016	Various	All MM: 5-year OS 42% 5-year EFS 39%
Bernard et al. (2021)[33]	Total: 82 MM: 11	1999–2018	Most fludarabine + cyclophosphamide	All MM: 5-year OS 40%
Mehta et al. (2017)[15]	Total: 45 MM: 11	2009–2014	Low-dose busulfan + cyclophosphamide, fludarabine, ATG	MDS: 3-year OS 63.6%
Chattopadhyay et al. (2023)[34]	Total: 60 MM: 10	1990–2021	Low-dose busulfan + fludarabine	All MM: 5-year OS 46%

ATG, antithymocyte globulin; EFS, event-free survival.

the exceptions are cryptic RUNX1 mutations, occurring in up to 20% of patients with FA, that reverse HSC quiescence through abrogating cell cycle checkpoints, resulting in restored hematopoietic output but promotion of malignant transformation.[25,26]

For patients with early-stage MDS without excess blasts such as the one in our case, most centers recommend proceeding directly to HSCT with best available donor.[27] Prior to the year 2000, even early-stage MDS conveyed dismal prognosis in FA, with 5-year OS <25%.[28] Subsequently, improved outcomes have been made possible by T-cell depletion strategies such as CD34 selection and TCRαβ depletion to limit graft-versus host disease (GvHD) and by use of low-intensity regimens to reduce organ toxicity.[29,30] Posttransplant cyclophosphamide has also proven to be an effective method of in vivo T-cell depletion for patients with FA undergoing HSCT with haploidentical donors.[31] Unfortunately, 5-year OS for patients with MDS/AML (30%-50%) remains considerably lower than for patients with BMF (Table 4) in recent studies owing notably not just to relapse but also to ongoing high rates of nonrelapse mortality.[13,32-34] Whether increased nonrelapse mortality is driven by distinct pathophysiology of MDS/AML in FA or is confounded because MDS/AML occurs in AYA patients, whereas BMF occurs in younger patients, remains uncertain.

Pretransplant cytoreduction in patients with FA and advanced MDS/AML remains controversial. Intensive AML therapy has been associated with prolonged aplasia and significant toxicity,[35] although 1 recent series suggests improved outcomes in patients exhibiting pre-HSCT complete remission.[32] Sequential strategies of fludarabine/cytarabine-based chemotherapy followed by HSCT several weeks later regardless of aplasia status may improve relapse-free survival.[27] Combination azacytidine/venetoclax as pretransplant cytoreduction is currently being tested in a combined safety/efficacy basket trial that includes patients with FA and MDS/AML (NCT05292664).

Hematologic monitoring in patients with FA who have received prior gene therapy

Ex vivo autologous gene therapy may soon be a commercially available option for patients with FA, although to date, trials have been limited to patients with a *FANCA* genotype. The tremendous potential of this approach is tied to the elimination of conditioning from this platform.[12] If efficacious, autologous HSC gene correction avoids not only GvHD but also long- and short-term chemotherapy radiation toxicities seen after allogeneic HSCT. However, while effects on restoring health of HSC based on in vitro assays have been promising,[36] hematopoietic restoration in clinical trials has been somewhat inconsistent. In the FANCOLEN-1 study,[12] despite impressive percentages of gene-corrected leukocytes, gene therapy did not halt progressive thrombocytopenia. In a recent presentation of 12 patients under age 6 at the time of treatment on the RP-L102 study, hematopoietic stabilization was achieved in 7 of 12 patients, but blood counts failed to normalize in any patient.[37] Thus, for AYA patients who receive this therapy on clinical trials or if made commercially available, we would continue to recommend annual bone marrow (BM) screening and routine complete blood count (CBC) monitoring, as patients may remain at risk for developing severe BMF or MM.

CLINICAL CASE (continued)

After undergoing unrelated donor stem cell transplant with TCRαβ depletion, our patient remains engrafted with 100% donor chimerism and no evidence of relapse 3 years post-HSCT. He is planning to transition care to an adult hematology center in another city. At his final visit, he asks about his malignancy risks and about other subspecialty care he needs to reestablish.

Solid tumors in AYA patients with FA

Most solid tumors in patients with FA will occur between age 20 and 40 years, although exceptions include liver tumors associated with androgen use that have been seen in younger patients and childhood cancers (Wilms, brain, neuroblastoma) associated with rare *FANCD1* and *FANCN* subtypes.[2] Notably, nearly one-third of AYA patients are diagnosed with FA because of a preceding malignancy diagnosis.[2]

AYA patients should thus undergo routine screening as recommended in the FA clinical care guidelines (Table 3). Recent

updates from the US National Cancer Institute FA cohort show that the cumulative incidence of solid tumors by late adulthood (age 60) is 18% to 24%, with head/neck squamous cell carcinoma (SCC) being the most common type, followed by basal cell and SCC skin cancers, and vulva/vaginal/cervical cancers in females.[38] Median age of onset for these malignancies was over 30 years of age, a full decade later than the median age of leukemia onset. Head/neck and skin cancers were the only solid tumors seen in adolescents. These findings parallel those seen in Wang et al.,[9] where 19% of patients developed solid tumors, 80% of which were SCC. In the National Cancer Institute cohort, earlier onset of solid malignancies was seen in patients with prior HSCT. Whether HSCT is the driver of earlier cancer occurrence or earlier tumor onset simply parallels earlier onset of BMF, necessitating HSCT in patients with more severe phenotypes, remains uncertain. Supporting the hypothesis that genotype may be the primary driver of age of tumor onset, 60% patients with severe *FANCA* variants impacting function of exons 27 through 30 developed a solid tumor by age 40 years.[38]

Prevention of solid tumors in FA is a critical focus of ongoing research. New brush biopsy techniques measuring aneuploidy have shown high sensitivity/specificity in diagnosing early oral dysplasia in FA.[39] A long-enrolling study (NCT03476330) with data yet to be released is assessing whether daily supplementation of the flavonoid quercetin, which possesses antioxidant, anti-inflammatory, and antineoplastic properties, can achieve the primary end point of reducing buccal micronuclei formation, a marker of malignant transformation risk. In vitro studies suggest combination therapy of quercetin and mammalian target of rapamycin (MTOR) inhibition may provide synergistic reduction in DNA damage.[40] Skin cancer prevention strategies include basic sun avoidance, avoidance of GvHD post-HSCT, and avoidance of medications such as voriconazole that may increase skin cancer risk. Whether human papillomavirus (HPV) drives development of head/neck and anogenital cancer in FA has long been debated. While we still recommend HPV vaccination to eliminate this risk factor, a recent comprehensive sequencing study of FA-associated SCC vs sporadically occurring SCC demonstrates that unlike in sporadic SCC, most FA-SCCs arise in the absence of HPV genome marking. Instead, FA-SCCs arise from TP53 loss and copy number alterations in other SCC driver mutations.[41]

Organ dysfunction monitoring in AYA patients with FA

In young children with FA, surgical intervention is often required for congenital anomalies, including the vertebral, anal, cardiac, tracheoesophageal fistula, esophageal atresia, renal, limb/digit, and hydrocephalus complex; hypospadias; and structural ear abnormalities. AYA patients with FA may need ongoing postsurgical follow-up for these congenital anomalies.

In addition, all AYA patients with FA need ongoing endocrinology care because of high rates of anatomic pituitary stalk abnormalities and other hormone changes. Short stature in FA may be driven in up to 25% of cases by growth hormone (GH) deficiency. GH replacement can help patients with FA achieve adequate adult height, and although early literature raised malignancy risk concerns, such risks for GH use in FA have not been proven. AYA patients with FA also have high rates of hypothyroidism (30%-40%) and osteoporosis (up to 50%), and diabetes (both type 1 and 2) occurs in 10% to 17% of patients.[9,38] In contrast, few patients have pulmonary complications unless induced by HSCT.

Finally, reproductive health remains an unmet challenge in FA. Both delayed and precocious puberty may occur and require hormonal intervention. Testicular failure and premature ovarian failure occur in over 40% of adults with FA.[2] Most males have a reduction in sperm counts, and women often reach menopause in their 20s or 30s, even without prior HSCT. A large retrospective study of gonadal function post-HSCT in FA demonstrates that longitudinal tracking of inhibin B levels in males and anti-Mullerian hormone in females may be better predictors of testicular and ovarian failure than traditional markers like follicle-stimulating hormone.[42] Biologic pathways that could be exploited to provide new approaches to improve fertility in patients with FA are only beginning to be explored.[43]

Conclusions

Improved outcomes for pediatric patients with FA and increased diagnosis of FA in older patients with late symptom onset are driving an increased need for multispecialty providers for AYA patients with FA. Increased recognition of patients with later-onset hematologic manifestations and the advent of gene therapy, which stabilizes but does not fully restore hematopoietic function, mean that many AYA patients with FA may not require allogeneic HSCT but will still need long-term hematologic monitoring. Furthermore, new techniques to prevent, diagnose, and treat solid malignancies will hopefully soon lead to decreased morbidity and mortality. The complex, long-term screening and treatment needed by AYA patients with FA require enhanced care models centered on a medical home with FA expertise, ensuring efficient and durable access to care.

Conflict-of-interest disclosure

Timothy S. Olson: no competing financial interests to declare.

Off-label drug use

Timothy S. Olson: All uses of medications discussed in this article are off-label, as there are no approved medications for use in Fanconi anemia.

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Clinical manifestations of telomere biology disorders in adults

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Telomere biology disorders (TBDs) are a spectrum of inherited bone marrow failure syndromes caused by impaired telomere function due to pathogenic germline variants in genes involved in telomere maintenance. TBDs can affect many organ systems and are often thought of as diseases of childhood. However, TBDs may present in mid- or even late adulthood with features similar to but not always the same as the childhood-onset TBDs. Adult-onset TBDs are often cryptic with isolated pulmonary, liver, or hematologic disease, or cancer, and may lack the classic disease-defining triad of abnormal skin pigmentation, nail dysplasia, and oral leukoplakia. Diagnostics include detection of very short leukocyte telomeres and germline genetic testing. Notably, adult-onset TBDs may show telomeres in the 1st to 10th percentile for age, and some cases may not have an identifiable genetic cause. TBD genetic etiology includes all modes of inheritance, with autosomal dominant the most frequent in adult-onset disease. Variable symptom onset due to incomplete penetrance, variable expressivity, and genetic anticipation add to the diagnostic challenges. Adult-onset TBDs are likely underrecognized, but their correct identification is of utmost importance, since affected patients are faced with numerous clinical complications, including but not limited to an increased risk of malignancies requiring close surveillance for early detection. Currently lung, liver, or hematopoietic cell transplants are the only curative therapeutic approaches but can be complicated by comorbidities, despite improved medical care. This review highlights the challenges of identifying adult-onset TBDs and addresses currently recommended clinical screening measures and therapy options.

LEARNING OBJECTIVES

- Identify clinical clues consistent with an adult-onset TBD and determine the correct tools for diagnostic work-up
- Understand the correlations between mode of inheritance, gene, and phenotype of TBDs in adults
- Determine appropriate clinical surveillance modalities and discuss therapeutic approaches for adults with TBDs

What is a telomere biology disorder?

Telomeres consist of $(TTAGGG)_n$ nucleotide repeats and a protein complex at chromosome ends that protect against loss of protein-encoding DNA during cell replication and are thus essential for genome stability.[1] Telomeres shorten with each cell division, and over time critically short telomeres trigger cellular senescence or apoptosis.[1,2] Telomere biology disorders (TBDs) represent a heterogeneous group of multi-organ diseases caused by pathogenic germline variants in genes encoding key telomere biology proteins. The best known subtype is classic dyskeratosis congenita (DC), which typically manifests during childhood with the mucocutaneous triad of nail dysplasia, abnormal skin pigmentation, and oral leukoplakia, but can also present in adults.[3] The TBD designation encompasses phenotypes also called cryptic DC, which may manifest in adulthood with one or two isolated features such as bone marrow failure (BMF), interstitial lung disease (ILD), head and neck squamous cell carcinoma (HNSCC), or liver fibrosis. Underdiagnosis of TBDs impacts clinical outcome, since affected patients need specific surveillance measures and treatment considerations.

The biology connecting the spectrum of TBDs is impaired telomere maintenance resulting in short telomeres for age.[2,4] Currently, pathogenic variants in at least 17 genes involved in telomere biology are associated with TBDs (Table 1).[2,5] All modes of inheritance have been reported in TBDs, including X-linked recessive (XLR), autosomal recessive (AR), and autosomal dominant (AD). Patients with de novo germline variants have

Table 1. Genotypes associated with adult-onset telomere biology disorders

Protein complex	Function in telomere biology	*Gene*/ Protein*	Functional effect of pathogenic variants	Main adult-onset phenotypes†	Inheritance*
Telomerase core components	Telomere elongation	*TERT*/TERT	Reduced telomerase activity, processivity, and/or recruitment	BMF/MDS, PF, LD	AD
				BMF, HNSCC	AR¶
	RNA template	*TERC*/hTR	Reduced telomerase activity	BMF/MDS, PF, LD	AD
				PF	AR¶
Telomerase enzyme complex	Telomerase assembly, hTR stability	*DKC1*/dyskerin‡	Reduced hTR stability and telomerase activity	BMF, PF	XLR‖
		NHP2/NHP2	Reduced hTR stability and telomerase activity	BMF, PF	AD
				BMF, LD	AR¶
		NOP10/NOP10	Reduced hTR stability and telomerase activity	PF, LD	AD
				BMF	AR¶
	Telomerase maturation/ activation/ trafficking	*WRAP53*/ TCAB1	Impaired telomerase trafficking and recruitment to telomeres	BMF, LD	AR¶
Shelterin complex	Telomerase recruitment/ activity/processivity	*ACD*/TPP1	Impaired telomerase recruitment	PF	AD
				BMF	AR¶
	Telomerase regulation/ recruitment, telomere protection	*TINF2*/TIN2‡	Multifactorial interruption of telomere maintenance	BMF, PF#	AD
	Telomerase regulation, telomere stability, 3′ G-overhang regulation	*POT1**/POT1	Impaired telomerase regulation and telomere replication	PF, familial melanoma§	AD
hTR biogenesis/ stability factors	hTR stability	*NAF1*/NAF1	Reduced hTR stability and telomerase activity	MDS, PF, LD	AD
	hTR maturation/stabilization	*PARN*/PARN	Reduced telomerase activity and hTR stability	PF, kidney disease	AD
				BMF, PF	AR¶
	hTR maturation and stability	*ZCCHC8*/ ZCCHC8	Impaired telomerase function	BMF, PF	AD
Telomeric accessory factors	DNA replication/repair, prevention of telomere loss during cell division	*RTEL1*/RTEL1	Impaired telomere replication and stability	BMF/MDS, PF, LD	AD
				BMF	AR¶
	DNA replication/repair	*RPA1*/RPA1	Impaired telomere maintenance	PF, [BMF]**	AD
Other (proposed TBD associated) ††	Ribosomal RNA maturation	*NPM1*/NPM1	Impaired ribosomal RNA maturation (altering hTR stability)	BMF	AD
	Inhibition of p53 activity	*MDM4*/MDM4	Hyperactivation of p53	BMF/MDS, HNSCC	AD
	De novo nucleotide synthesis (thymidine nucleotide metabolism)	*TYMS-ENOSF1*/ TYMS	Impaired telomerase regulation	Classic DC	AR (digenic)

*TBD-related genes/proteins pathogenic changes (associated inheritance patterns) not listed since to date solely reported in childhood-onset disease: Shelterin complex: *POT1*/POT1 (AR), telomeric accessory factors: *CTC1*/CTC1 (AR), *STN1*/STN1 (AR), and *DCLRE1B*/Apollo (AR).

†Phenotypes listed are not comprehensive but meant to highlight the primary clinical manifestations in adult-onset TBDs.

‡Pathogenic germline variants in all listed genes can occur de novo but are more common in *TINF2* and *DKC1*.

§Monoallelic, pathogenic germline *POT1* variants resulting in longer telomeres have been associated with cancer predisposition to a range of malignant and benign tumors, particularly familial melanoma.

‖Skewed X chromosome inactivation may in some cases result in phenotypically affected females heterozygous for pathogenic variants in *DKC1*.

¶The first manifestations of AR TBDs are typically seen in childhood but may also occur in young adults.

#*TINF2* AD occurs frequently de novo and is primarily associated with severe disease in childhood. However, families with TBDs due to inheritance of heterozygous *TINF2* pathogenic variants have been reported. BMF in young adults (<40 years) has been reported as well as rare adult *TINF2* cases with PF as the primary clinical complication.

***RPA1* was recently identified to belong to realm of TBD genes and was reported in 3 pediatric cases with BMF/MDS, immunodeficiency, and post-hematopoietic cell transplant PF, as well as 1 adult case with PF.[33]

††*NPMI*, *MDM4*, and *TYMS* have all recently been proposed to be TBD associated, but data are limited. *NPMI*: Germline monoallelic variants were reported in 2 individuals with symptoms indicative of a TBD.[53] *MDM4*: A germline missense variant was reported in a family with TBD features and showed in vitro decreased telomere length.[54] *TYMS*: heterozygous germline variants in *TYMS* and *ENOSF1* leading to TYMS deficiency were reported in children and young adults (<40 years) with classic mucocutaneous features of DC.[50]

hTR, human telomerase RNA.

also been reported. TBD-causing variants show both variable expressivity and incomplete penetrance as the result of several factors, including but not limited to somatic genetic rescue mechanisms such as acquisition of variants in the *TERT* promoter region.[2,6] Genetic anticipation with shorter telomeres and earlier onset clinical manifestations has been reported in successive generations.[2] All these phenomena add to TBD pleiotropy, making them frequently challenging to recognize in adults.

CLINICAL CASES

The presented individuals participated in the National Cancer Institute IRB-approved Inherited Bone Marrow Failure Syndromes study (NCT00027274) or the Aachen TBD registry (EK206/09, Aachen, Germany). The patients or their legal guardians signed informed consent.

Case 1: A 16-year-old male presented with portal hypertension without alcohol use history or infectious causes. Banding of esophageal varices and a transjugular intrahepatic portosystemic shunt was performed for progressive portal hypertension (Figure 1A). At age 26 years, mild pancytopenia was noted without evidence of myelodysplastic syndrome (MDS) on bone marrow exam, and was interpreted in the context of liver disease. Hepatic diffuse large B-cell lymphoma was diagnosed at 35 years of age. Prolonged pancytopenia with several severe infections occurred during chemotherapy, leading to dose reductions and discontinuation after 4 cycles. The patient later became transfusion dependent for platelets at 36 years of age. He had no mucocutaneous manifestations consistent with DC. He also had a history of a malabsorption syndrome with growth retardation and severe periodontitis with loss of several teeth. There was no family history of TBD-related features. Bone marrow biopsy after chemotherapy showed persistent hypocellularity (Figure 1B), and liver biopsy revealed signs of cirrhosis.

Case 2: A 42-year-old woman presented with persistent cytopenias. Mild thrombocytopenia was first noted at age 18 years, followed by leukocytopenia and macrocytic anemia. Light ridging of fingernails and lacy skin pigmentation were found on physical exam. Additional features included early graying at age 14 and two skin squamous cell carcinomas in her thirties. Family history was notable for transfusion-dependent cytopenias and pulmonary fibrosis (PF) in the patient's mother and mild thrombocytopenia and dysplastic fingernails in the patient's child. The patient's bone marrow biopsy revealed hypocellularity without significant dysplasia.

When to suspect an adult-onset telomere biology disorder?

The phenotypic spectrum associated with aberrant telomere function is broad, and consensus diagnostic criteria for TBDs have not been established. However, there are clinical findings in adults that are suspicious for an underlying TBD (Figure 2).

Skin findings: The classic TBD feature is the mucocutaneous triad of nail dysplasia, reticulated skin pigmentation, and mucosal leukoplakia.[5,7] However, triad features may not be present or may be subtle; the mucocutaneous triad often progresses with age.[8] For adult patients another nonspecific, yet suspicious, clue is premature graying (<30 years).[8]

Pulmonary disease: Individuals with familial PF (FPF), or PF with findings suggestive of TBDs in their personal or family history, should be evaluated for TBDs.[9] TBDs can manifest with different forms of ILD, with idiopathic PF being the most frequent. There also appears to be a predisposition to severe smoking-related emphysema.[10] PF is the leading manifestation of adult-onset TBDs, and pathogenic variants in TBD-related genes are found in 30% to 35% of FPF and 10% of sporadic PF cases.[10] PF in the context of TBDs is rapidly progressive and associated with high morbidity and mortality.[7,11,12] PF patients present with mainly a restrictive pattern on spirometry, decreased diffusion capacity for carbon monoxide (D_{LCO}), and commonly a pattern consistent with usual interstitial pneumonia on high-resolution computed tomography (HRCT).[12] Important additional pulmonary manifestations include hepatopulmonary syndrome (HPS) and/or pulmonary arteriovenous malformations (PAVMs), both of which may co-occur with PF, and are associated with early-onset telomere disease, including young adults.[13,14] PAVMs have been reported in the absence of overt HPS. However, it is not clear if PAVMs develop independently or if their diagnosis is connected with

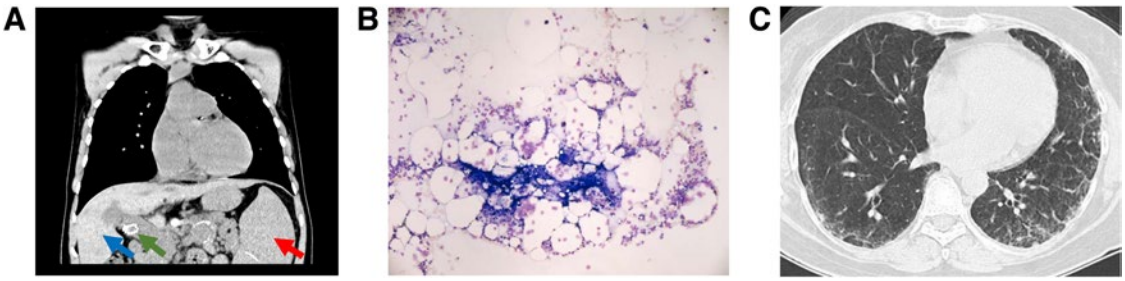

Figure 1. Clinical manifestations of telomere biology disorders in two adults. (A) Computed tomography scan (coronal plane) of a 35-year-old male with cryptogenic hepatic disease (case 1). Transjugular intrahepatic portosystemic shunt was set in place at the age of 21 years. Blue arrow indicates heterogenous liver parenchyma, green arrow transjugular intrahepatic portosystemic shunt, and red arrow splenomegaly. (B) Image of the hypocellular bone marrow biopsy of a 35-year-old TBD patient with severe cytopenia (case 1). (C) Pulmonary high-resolution computed tomography scan (axial plane) of a 54-year-old female with a TBD due to a heterozygous *TERC* mutation (case 2). Pulmonary bases bilaterally show peripheral interstitial and ground-glass opacities with early honeycombing. Findings are consistent with usual interstitial pneumonia pattern of pulmonary fibrosis. For all images written permission from patients was obtained. Figure 1C was previously published in Giri et al. 2019.[12]

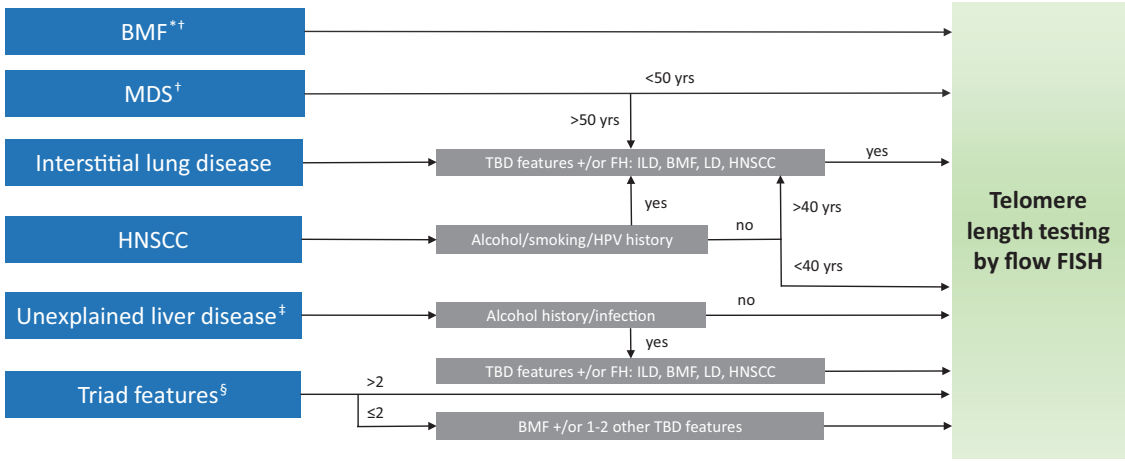

Figure 2. When to consider telomere length measurements in adults. *Includes unexplained, persistent cytopenia, aplastic anemia. †Consider chromosome breakage testing to exclude Fanconi anemia. ‡Includes liver cirrhosis, fibrosis, hepatopulmonary syndrome, idiopathic portal hypertension. §Mucocutaneous triad consisting of leukoplakia, reticular skin pigmentation, and nail dysplasia. FH, family history; HPV, human papilloma virus.

existing or developing HPS/liver disease and therefore are the component of one symptom complex.[14]

Hematopoietic manifestations: Screening for TBDs is recommended for individuals of all ages with BMF and for those younger than 50 years of age with MDS.[3,15] A recent study of a reportedly acquired aplastic anemia cohort found that approximately 2% of individuals had an unrecognized TBD by germline genetic testing.[16] Additionally, MDS is a frequent manifestation of TBDs with an increased risk of 145-fold to 500-fold compared with the general population.[15,17]

Liver disease: Cryptogenic liver fibrosis and/or cirrhosis, unexplained through lifestyle or infectious causes, or idiopathic portal hypertension can be a clue for a TBD. TBD-related hepatic involvement is variable and may first present as an asymptomatic liver enzyme elevation, nonspecific ultrasound abnormalities, and/or nodular regenerative hyperplasia on biopsy.[18,19] TBD liver disease is progressive and should be considered part of the differential diagnosis for patients with nonalcoholic/noninfectious liver fibrosis/cirrhosis, idiopathic portal hypertension, and HPS.[13,18-20]

Cancer susceptibility: Cancer presenting at a younger than expected age, especially HNSCC, may indicate an underlying TBD. Patients with TBDs have a 4-fold increased risk for development of malignancies compared with the general population.[15] Telomeres may play a role in cancer development due to chromosomal instability (short telomeres) or accumulation of somatic DNA changes (long telomeres).[21,22] T-cell exhaustion was recently proposed as an additional contributor to solid tumor development in TBDs.[23] Acute myeloid leukemia (AML) and HNSCC are the predominant cancer entities associated with TBDs, with each having an estimated 70-fold (observed/expected) increased risk in TBDs compared with the general population.[15] Other frequent malignancies include anal and cutaneous squamous cell carcinomas.[15,17] The median age at MDS/acute myeloid leukemia diagnosis is usually younger than the general population.[17] HNSCCs also show an unusually early age at onset in TBDs (<40 years of age) and predominantly affect the tongue.[15,17]

Family history: Pedigree construction often yields important clues for an adult-onset TBD since each of above-mentioned organ manifestations may present in isolation or precede development of other features.[7,20,24] Consideration should be given to the fact that the complex pathophysiological background of TBDs leads to both a variety of affected organs and variable time of disease onset, even within members of the same family. However, de novo occurrence of variants is possible, reported most frequently in *TINF2* and *DKC1*.

Additional phenotypes: With more study, the clinical spectrum associated with telomere dysfunction is growing. Vascular disease, including PAVMs and gastrointestinal telangiectasias, were recently recognized as an important cause of morbidity in TBDs, with gastrointestinal hemorrhage being noted as an initial manifestation of TBDs in children or young adults even in the absence of overt portal hypertension.[25] While abnormal immune status is predominantly associated with childhood-onset TBDs, T-cell immunodeficiency has been reported in adult TBDs even in the absence of BMF.[26] Signs of impaired immune response may therefore be an additional clue for an underlying TBD.

CLINICAL CASES (continued)

The presentation with one feature of a TBD (liver disease), as highlighted in case 1, illustrates the complex diagnostic journey of such patients. It wasn't until his disease progressed and other evaluations were not diagnostic that a TBD was suspected (Table 2). Adult patients may initially not present to the hematologist but to other subspecialties, illustrating the importance of interdisciplinary clinical care. The patient in case 2 presented with BMF as a classic TBD feature, but her cutaneous phenotype was subtle. The early graying was an additional hint, yet the most important clue in her case was the family history (Table 2).

What are the diagnostic tools to identify telomere disease?

Traditionally, classic DC was diagnosed by the presence of mucocutaneous triad or a combination of 1 triad feature plus

Table 2. Summary of clinical features and diagnostic results leading to the diagnosis of TBD in 2 adult cases

	Clinical case 1	Clinical case 2
Clinical features	Cryptogenic liver disease Bone marrow failure	Pulmonary fibrosis Bone marrow failure
Mucocutaneous features	None	Nail dysplasia Lacy skin rash
Patient history	Severe periodontitis as a teenager with loss of several teeth Suspected malabsorption syndrome with growth retardation Prolonged pancytopenia with several severe infections during chemotherapy	Gray hair, 14 years SCC, 30 years SCC, 36 years
Family history	No disease reported	Mother: PF, cytopenia Child: nail dysplasia, thrombocytopenia, LTL <1st percentile for age
Telomere length by flow FISH (lymphocytes)	<1st percentile	<1st percentile
Genetics	Heterozygous, pathogenic variant in *TERC*	Heterozygous, pathogenic variant in *TERC*

SCC, skin squamous cell carcinoma.

BMF. The addition of telomere length testing as a diagnostic tool and germline genetic testing have greatly improved diagnostics and expanded the phenotypic spectrum of TBDs.[3,5]

Telomere testing: Lymphocyte telomere length (LTL) testing by fluorescent in situ hybridization and flow cytometry (flow FISH) measures mean telomere length and is a powerful tool in diagnosing individuals with TBDs.[27-29] In the clinical setting it is currently considered the primary diagnostic test for TBDs yet is labor intensive and only established in specialized laboratories.[3,28,29]

The recently established high-throughput single telomere length analysis (HT-STELA), which determines the distribution of telomere length, has been implemented as a diagnostic tool for TBD in the United Kingdom and may identify asymptomatic individuals with TBD not readily detected with other methods.[4,29] Other measurement approaches, including quantitative PCR or telomere shortest length assay (TeSLA), are useful in research but not validated in the clinical setting.[29,30]

Interpreting TL results in adults can be challenging and complicated by a few factors: First, due to normal age-associated telomere attrition, LTL must be interpreted as age adjusted.[3,27,28] Telomeres shorten more slowly in middle age than in childhood, making the diagnostic window between normal for age and critically short telomeres sometimes challenging to interpret in adults.[28] Second, some genotypes are associated with short but not very short telomeres. Childhood-onset TBDs typically exhibit TL below the 1st percentile for age whereas adult-onset disease may exhibit telomeres between the 1st and 10th percentile.[24,28] Therefore in a clinically suspected adult TBD case, the detection of LTL <10th percentile for age should trigger genetic workup.[3] Of note, variants in *DCLRE1B/Apollo*, which are to date solely reported in childhood-onset TBD, are not associated with a global TL reduction.[31] Third, granulocyte TL is often routinely measured with LTL. While LTL less than the first percentile is sensitive and highly specific for TBDs, very short granulocyte TL lack specificity for TBDs and may also be observed in acquired aplastic anemia or clonal myeloid disorders.[27,32]

Genetic testing: Genetic testing of the patient and their family members is helpful for both clinical guidance and family counseling. In cases of LTL <10th percentile, detecting a pathogenic germline variant can confirm a TBD diagnosis.[3] The TBD-associated genetic spectrum continues to expand, with pathogenic variants in at least 17 different genes associated with disease reported to date (Table 1). However, in approximately 20% of TBD cases, an underlying genetic cause cannot be identified, and newly discovered or rare TBD genes may not yet be included in clinical gene panels.[2,5,7,29,33] While potentially uncovering unrecognized TBDs, the implementation of panel or exome sequencing in routine diagnostics has also led to increased detection of variants of unknown significance, which are difficult to interpret in the absence of an obvious TBD phenotype. Additionally, in rare cases relatives from yet unidentified TBD patients may inherit short telomeres and exhibit TBD symptoms despite their wild-type genotype, a phenomenon called phenocopy.[2] These patients would be missed by exclusive genetic testing. LTL can sometimes be helpful in assessing the functional impact of the identified variant, but additional basic science studies are often required.

Genotype-phenotype correlations: Typically, AR TBDs become evident in childhood or young adulthood, whereas AD pathogenic variants, except for those in *TINF2*, are primarily associated with symptom onset in adults (Figure 3).[7] In general, genes found in adulthood include predominantly heterozygous pathogenic variants in *TERT*, *TERC*, *RTEL1*, or *PARN*.[3,24] In FPF cases AD *TERT* pathogenic changes are most frequent, followed by AD *RTEL1* and AD *PARN* variants, while AD *TERC* changes are identified less often but present at a younger age.[11,34] In adult BMF, autosomal dominant (AD) *TERC* and *TERT* changes are predominant.[3,35] Of note, *TINF2* and *DKC1* TBDs commonly manifest in childhood, but *DKC1*-associated TBDs have been reported to present in late adulthood, and monoallelic *TINF2* pathogenic germline variants have been observed in rare cases of adults with PF.[7,36]

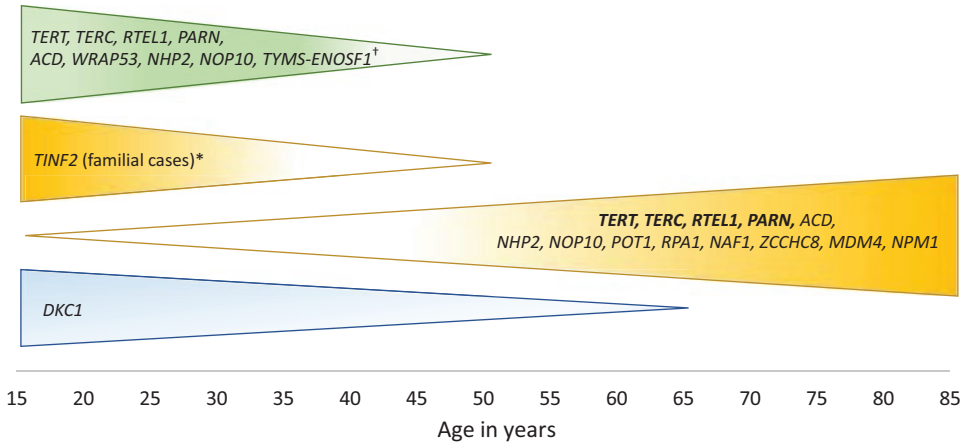

Figure 3. Genetic etiology of telomere biology disorders: genes and associated inheritance patterns primarily to consider in adults. Depicted are typical age groups for clinical manifestations of each gene and associated inheritance pattern. Yellow shade indicates AD, green AR, and blue X-linked disease. Genes in bold are more frequently reported. For all genes, de novo occurrence is possible but most frequently reported for *TINF2* and *DKC1*. *De novo *TINF2* is associated with a severe phenotype and onset in childhood. †The combination of germline variants in *TYMS* and *ENOSF1* appear to follow an AR inheritance but are the result of digenic inheritance.[50] There are some pathogenic gene variations in combination with specific inheritance patterns that are to date solely reported in children and therefore not depicted. These include the following genes with the associated inheritance pattern in brackets: *POT1* (AR), *STN1* (AR), *CTC1* (AR), *DCLRE1B* (AR).

CLINICAL CASES (continued)

In both clinical cases, very short LTL were detected (Figure 4) and monoallelic pathogenic *TERC* variants identified, fitting the common genotype spectrum in adult-onset TBDs.

How does a TBD diagnosis change clinical care?

Outcome analyses have found a better overall survival in AD compared with AR/XLR TBDs, possibly related to the older age at onset and fewer clinical features seen in non-*TINF2* monoallelic TBDs.[7] However, it is important to recognize that adult-onset TBDs are often accompanied by high morbidity and mortality due to progression of BMF, PF, liver disease, HNSCC, or other complications.[5,7]

Surveillance: Once a TBD diagnosis is established, regular surveillance is recommended (Table 3). This includes regular blood counts to monitor progression of cytopenias, and there should be a low threshold for a bone marrow aspirate and biopsy when blood counts change. Some providers recommend a bone

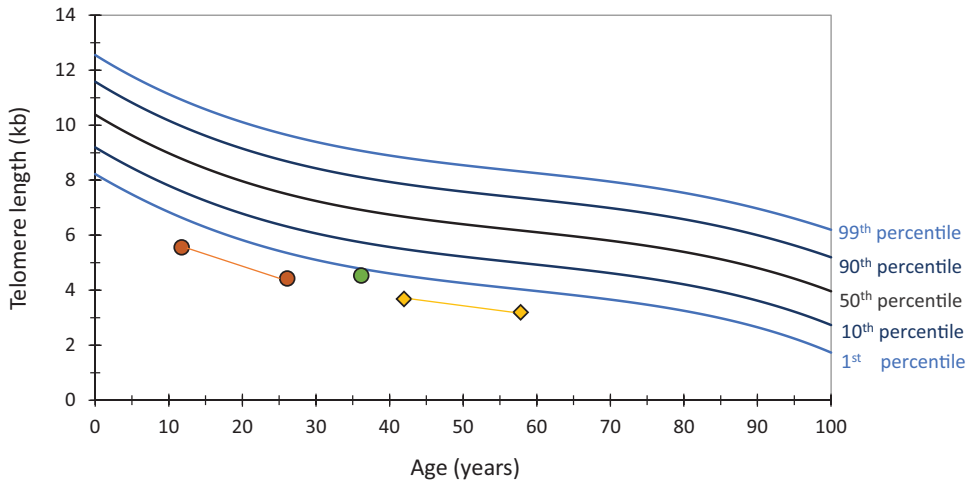

Figure 4. Telomere lengths in cases 1 and 2. Depicted are lymphocyte telomere length by fluorescent in situ hybridization and flow cytometry (flow FISH) of 3 patients with *TERC*-associated TBD. The green circle shows lymphocyte telomere length of a 36-year-old male with liver disease and bone marrow failure (case 1). Yellow diamonds indicate 2 lymphocyte TL taken over time in a female patient with pulmonary fibrosis and bone marrow failure (case 2). Orange circles show lymphocyte TL of the child of patient 2 who presented with thrombocytopenia and dysplastic fingernails and was found to carry the same *TERC* variant as their mother.

Table 3. Recommended surveillance in adults with telomere biology disorders

General recommendations	
	• Regular use of sunscreen, avoid excessive sun exposure • Avoid exposure to cigarette smoke • Patient should be taught how to perform a monthly self-examination for oral, head and neck cancer • Maintain good oral hygiene • Vitamin D and calcium as needed to optimize bone health
Basic surveillance	
Hematology	Baseline • CBC with differential and reticulocyte count • BM aspiration and biopsy • Conventional and molecular cytogenetics • Consider NGS myeloid panel to assess for somatic variants/clones. Monitoring • CBC normal/no cytogenetic abnormality: CBC every 6–12 months. BM evaluation if cytopenia develops. • Mild cytopenias/no cytogenetic abnormality: CBC every 3–4 months. BM evaluation based on clinical development, consider regular intervals (eg, every 1–3 years). • Abnormal cytogenetics: clonal cytogenetic abnormalities require more frequent CBC/BM evaluation to evaluate potential leukemic or MDS progression; intervals depend on development of CBC counts. High-risk abnormalities such as chromosome 7 change need immediate referral to HCT center. • Progressive decline or rise in blood counts require CBC and BM evaluation based on clinical situation. • On androgen therapy: CBCs prior to therapy; repeat CBCs every 4–6 weeks to assess response, when counts are stable every 2–3 months
Dermatology	Baseline and monitoring • Perform regular skin self-examination for new or changing skin growth • Annual dermatologist evaluation*
ENT	Baseline and monitoring • Annual cancer screening by an otolaryngologist
Dentist	Baseline and monitoring • Dental hygiene and screening every 6 months
Pulmonology	Baseline • Pulmonary function test • Consider HRCT based on results and risk factors Monitoring • Annual pulmonary function test • HRCT as clinically indicated Bubble echocardiogram for pulmonary symptoms in the absence of pulmonary fibrosis
Gastroenterology/ hepatology	Baseline • Evaluate for risk factors for hepatic disease (alcohol, drug use) • Liver function tests • Liver ultrasound and/or fibroscan • Evaluate for clinical signs of esophageal stenosis Monitoring • Liver function tests annually • Consider imaging (fibroscan/ultrasound) every 2 years • On androgen therapy: Check liver function tests prior to starting and every 1–2 weeks for first month, then every 6–12 weeks. Check lipid profile prior to starting and every 6–12 months. Perform liver ultrasound examination prior to starting androgens and semiannually to evaluate for adenomas, carcinomas, or fibrosis
Gynecology/ obstetrics	Baseline and monitoring: • Annual gynecologic evaluation with HPV testing starting at 18 years of age or at start of sexual activity • HPV vaccination in females and males <27 years of age if not adequately vaccinated in childhood Pregnancy: referral to maternal-fetal medicine specialist for high-risk pregnancy
Orthopedics	Bone density scan at baseline†
Oncology	• Follow surveillance guidelines for breast cancer, cervical cancer, colon/rectal cancer, lung cancer, prostate cancer • In case of cancer diagnosis: increased sensitivity to therapeutic radiation and chemotherapy may require dose reductions
Additional surveillance based on clinical presentation per case	
Cardiology	Regular assessment for hypertension Baseline lipid level
Urology	Baseline assessment for genitourinary anomalies, including symptoms of urethral stenosis, penile leukoplakia

Table 3. Recommended surveillance in adults with telomere biology disorders (*Continued*)

Immunology‡	In case of suspected immunodeficiency such as increased sinus/lung infections: • Serum immunoglobulin levels (total and fractions) • Flow cytometry for peripheral blood leukocytes including lymphocyte subsets • Consider evaluating childhood vaccine antibody titers
Neurology‡	MRI assessment for cerebellar hypoplasia in individuals with developmental delay or learning problems
Ophthalmology‡	Annual examination to detect/correct vision problems, abnormally growing eyelashes, lacrimal duct stenosis, retinal changes, bleeding, cataracts, and glaucoma

Surveillance recommendations are modified from Niewisch and Savage[55] and based on expert opinion in Agarwal et al. (published on www.teamtelomere.org) and Walsh et al.[56] These recommendations are tailored toward individuals above 18 years of age without previous HCT. Following HCT, surveillance intervals may need to be adjusted.

*Cutaneous squamous cell carcinomas have frequently been described in young adults with TBDs.[15] Regular dermatologic exams may therefore be advisable starting before the age of 30 years.

†TBDs in younger patients have an increased risk of avascular osteonecrosis and unexplained fractures. Therefore, a baseline bone density scan is advisable even in young adults.

‡Immunodeficiency (commonly with lymphopenia) and developmental delay (often cerebellar hypoplasia) is predominantly observed in TBD cases with onset in early childhood, specifically Hoyeraal-Hreidarsson syndrome. Ophthalmologic manifestations are frequent in childhood-onset TBDs, including classic dyskeratosis congenita, Hoyeraal-Hreidarsson syndrome, Revesz syndrome, or Coats plus.

BM, bone marrow; CBC, complete blood count; HPV, human papilloma virus; NGS, next generation sequencing.

marrow evaluation at diagnosis and annually. Abnormal pulmonary function tests are common in TBD patients and associated with development of significant pulmonary disease.[12] Baseline pulmonary function tests are recommended at diagnosis and annually thereafter. Annual screening for oral cancer is recommended since it may detect HNSCC early, when still amenable to surgical resection. Additional specific screening recommendations are available in the TBD Diagnosis and Management Guidelines (https://teamtelomere.org).[37] Family members should be offered genetic counseling and testing based on the patient's genetic status. Related individuals with pathogenic variants causative of TBDs should be offered a clinical and diagnostic evaluation, even in the absence of symptoms, to establish a baseline given the considerable variation in respect to time of disease onset. Genetic counseling is essential for either the patient or family members and should address the possibility of genetic anticipation.

Therapy options: Research advances in telomere biology have led to a better understanding of the etiology of TBDs and their associated clinical manifestations. However, there are few therapeutic options. The only curative option for TBD-related lung, bone marrow, or hepatic disease is organ transplant. Each of these modalities can be complicated by the concomitant involvement of other organs and concerns for increased treatment-related toxicity.[38-40] This warrants close interdisciplinary care both pre- and posttransplant and highlights the importance of multicenter prospective trials in this setting. Implementation of reduced-intensity hematopoietic cell transplant (HCT) regimens and advancement in HCT donor matching have significantly improved its outcome in patients with TBDs.[40,41] After HCT, patients remain at high risk of PF, PAVM, liver disease, HNSCC, and/or gastrointestinal bleeding complications.[12,25,40,41] Lung transplant in TBD patients with PF has successfully been performed, yet patients are prone to complications and show inferior outcome compared with non-TBD lung transplant recipients.[10,38] A current collaborative effort by the Clinical Care Consortium of Telomere Associated Ailments (CCCTAA) is evaluating liver transplant outcomes in TBD patients. "Tandem" transplants of either lung/bone marrow or lung/liver have been considered as a possible therapeutic approach yet are restricted to a few specialized centers, and outcome data are sparse.[42]

If lung transplant is not an option, antifibrotic agents such as nintedanib and pirfenidone could be considered. There are very few data on their use in TBDs, but overall reports suggest they are likely safe in TBD-related PF and may slow lung function decline.[43] Immunosuppressive therapy is not effective in TBD-related BMF. In lieu of HCT, androgen treatment can result in a hematologic response and transfusion independence for several years. Danazol is often used due to its somewhat more favorable side effect profile compared with nandrolone or oxymetholone. Their efficacy appears similar, but they have not been systematically studied in this setting.[44] In some studies androgens have been proposed to lengthen telomeres, but the effect has been inconsistent and the long-term risks or benefits of lengthening telomeres in TBDs is not known.[45-48] One study reported a potential positive effect of nandrolone on pulmonary function in TBD patients with ILD.[47] Solid tumors should be treated according to entity-specific recommendations with careful following for increased chemotherapy-related complications, especially for cytopenias. Patients with TBDs also have higher rates of complications from therapeutic radiation, including severe tissue reactions and avascular osteonecrosis.[7,49]

CLINICAL CASES (continued)

For both cases, screening measures as outlined in Table 3 were initiated. The progressive BMF in case 1 required treatment, and the patient was started on low-dose danazol because of the severe hepatopathy. His blood counts improved such that he no longer requires transfusions, and his liver disease has not worsened.The patient in case 2 was started on androgen treatment for BMF and remained hematologically stable for more than 10 years. The first restrictive changes on pulmonary function tests and D_{LCO} reduction were noted at 52 years of age. PF

was diagnosed 2 years later (Figure 1C) and was progressive, leading to oxygen dependency. Unfortunately, a suitable lung transplant donor could not be identified, and pirfenidone treatment did not lead to a significant improvement. She died due to respiratory failure at 59 years of age while awaiting a combined lung and bone marrow transplant. This clinical course sadly highlights 2 key problems of TBD patients: (1) the unavailability of suitable organs for transplants while experiencing rapid progression of disease and (2) the severe disease that can occur in several organs and limit therapeutic options.

Outlook

Discovery of new genotypes: A growing understanding of telomere biology and the growth of next-generation sequencing has led to numerous discoveries of pathogenic variants in patients with TBDs. Most recently, germline variants involving both *TYMS* and *ENOSF1* were reported to result in classic DC features in children and young adults and introduced the possibility of digenic inheritance of TBDs.[50] Consideration of variants in noncoding sequences, which may affect regulatory regions, or synonymous variants altering splicing may elucidate the genetic etiology in patients with TBDs and no currently known cause.

Emergence of new therapeutics: The development of therapeutic agents targeting disease-specific defects are required to expand treatment options and improve patient outcomes.[5] Future targets may include pathways connected to telomere maintenance, telomerase-directed gene therapies, and/or CRISPR-Cas9 editing to elongate telomeres.[2,5,10,51,52] Major challenges in developing TBD-related therapeutics include their broad clinical spectrum and the multiple and variable genetic etiologies. Given the role telomeres play in genome integrity and potentially in carcinogenesis, long-term follow-up will also be required.

Until new therapies are discovered, early diagnosis of TBDs and careful surveillance for disease progression, including cancer, are essential to improve the health and well-being of all affected with TBDs.

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Conflict-of-interest disclosure

Marena R. Niewisch: no competing financial interests to declare.
Fabian Beier: no competing financial interests to declare.
Sharon A. Savage: no competing financial interests to declare.

Off-label drug use

Marena R. Niewisch: Androgen-use in the context of telomere biology disorder related bone marrow failure.
Fabian Beier: Androgen-use in the context of telomere biology disorder related bone marrow failure.
Sharon A. Savage: Androgen-use in the context of telomere biology disorder related bone marrow failure.

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Leveraging health care technology to improve health outcomes and reduce outcome disparities in AYA leukemia

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Significant improvements have occurred for adolescent and young adult (AYA) B-cell acute lymphoblastic leukemia (B-ALL) patients following the widespread adoption of "pediatric-inspired" treatment regimens for AYA patients cared for in adult oncology settings. However, for AYA patients, aged 15 to 39, an outcomes gap remains in B-ALL, necessitating the incorporation of novel therapies into up-front treatment regimens. As a result, clinical trial enrollment remains the current standard of care for AYA B-ALL across disease subtypes when available and accessible. Currently, several up-front trials are looking to incorporate the use of inotuzumab, blinatumomab, and chimeric antigen receptor T-cell therapy into existing chemotherapy backbones for AYA patients, as well as tyrosine kinase inhibitors for both Philadelphia-positive (Ph+) and Ph-like B-ALL. In addition to ongoing attempts to improve up-front treatments by incorporating immunotherapy and targeted approaches, the increased use of next generation sequencing for measurable residual disease evaluation has led to superior risk-stratification and a decreased need to pursue consolidative hematopoietic stem cell transplantation during the first complete remission for many patients.

LEARNING OBJECTIVES

- Evaluate pediatric and "pediatric-inspired" regimens for the treatment of AYA B-ALL
- Compare current clinical trial approaches for AYA B-ALL subtypes in the up-front setting
- Discuss changing standards for MRD assessment and the impact on consolidative HSCT in first CR

CLINICAL CASE

A 20-year-old man presents to an urgent care clinic for bruising and profound fatigue. Blood work shows anemia, thrombocytopenia, and a white blood cell count (WBC) of 32×10^9/L. Since he is over 18 years old, the patient is referred to the adult emergency room, where a repeat complete blood count shows 68% lymphoblasts on manual differential with morphology suggestive of ALL (acute lymphoblastic leukemia). The patient is admitted to the Adult Leukemia Service for a further work-up and initiation of treatment.

"Pediatric-inspired" regimens for adolescent and young adult B-cell ALL

Up-front treatment for adolescent and young adult (AYA) patients with B-cell ALL (B-ALL) at pediatric centers consists of a 4-drug induction, augmented Berlin-Frankfurt-Münster (BFM) consolidation, interim maintenance with high-dose methotrexate, delayed intensification, a second interim maintenance with Capizzi methotrexate, and, finally, an extended maintenance therapy (Figure 1). This treatment backbone has experienced only minor changes over the last few decades, including the shortening of maintenance to 2 years for males and a decrease in frequency of steroid/vincristine pulses to every 12 weeks.[1] These modifications were adapted for the Children's Oncology Group (COG) current up-front high-risk trial AALL1732 (NCT03959085). While variations on the COG backbone exist, like the Associazone Italiana Ematologia Oncologia Pediatrica-BFM (eg, AIEOP-BFM ALL 2017, NCT03643276) or St Jude Total Therapy (eg, St Jude Total XVII, NCT03117751), many of the same general treatment principles persist. Compared to adult ALL regimens, pediatric approaches are less myelosuppressive and include more asparaginase with greater total doses of steroids, vincristine, and intrathecal chemotherapy.[2] Despite the ability of many pediatric hospitals to treat

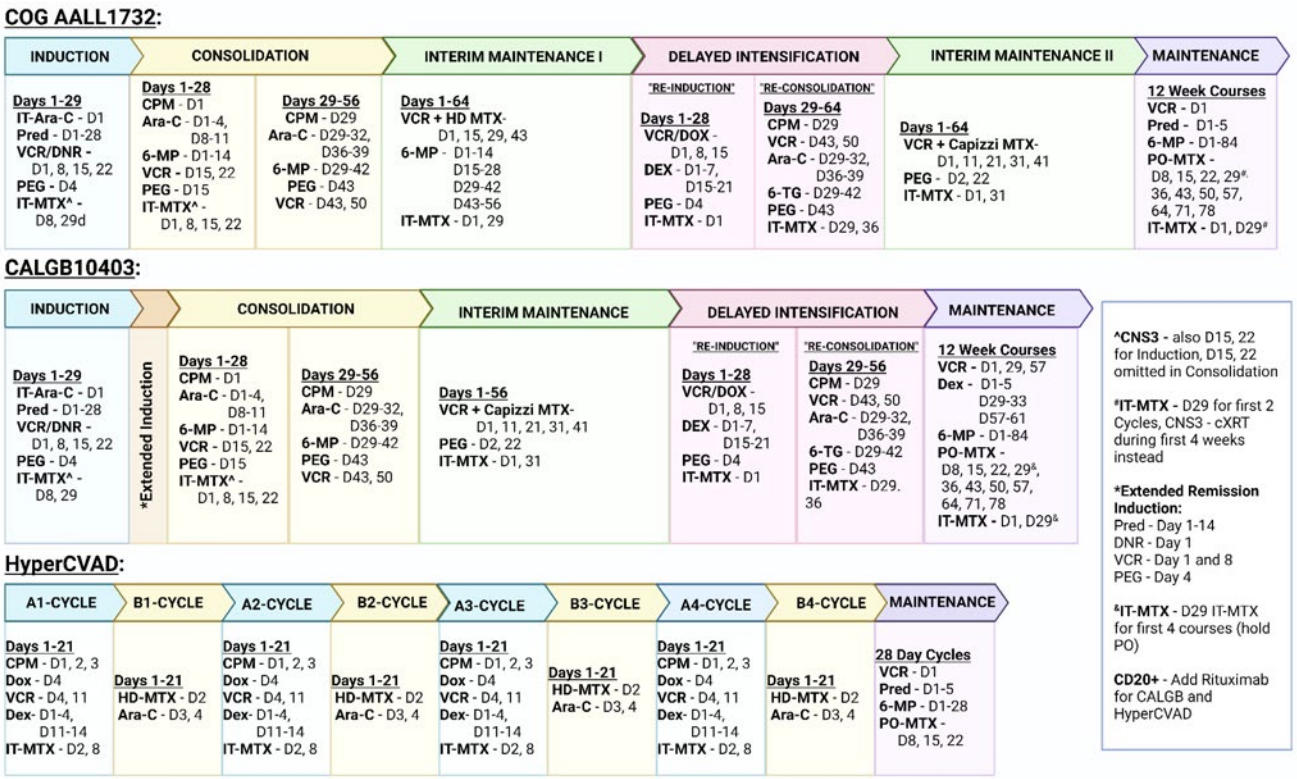

Figure 1. Up-front AYA B-ALL treatment schemas. Ara-C, cytarabine; CNS3, central nervous system; CPM, cyclophosphamide; cXRT, cranial radiation; DEX, dexamethasone; DNR, daunorubicin; DOX, doxorubicin; HD-MTX, high-dose methotrexate; IT-MTX, intrathecal methotrexate; IT-Arac, intrathecal cytarabine; PEG, pegaspargase; PO-MTX, oral methotrexate; Pred, prednisone; 6-MP, mercaptopurine; 6-TG, thioguanine; VCR, vincristine.

older adolescent B-ALL patients, even up to the age of 21 to 24 years, the majority of AYA patients over 18 (87.1%) are managed by adult oncologists, often in community settings, which has traditionally limited exposure to pediatric regimens.[3]

Following a large retrospective study showing a doubling of survival for AYA patients treated on US pediatric cooperative group trials compared to US adult cooperative group trials (7-year event-free survival [EFS], 63% vs 34%; $P<.001$),[4] a prospective phase 2 trial evaluated the use of a "pediatric-inspired" regimen by adult oncology providers. Based on the pediatric AALL0232 backbone, CALGB10403 showed both an improved EFS of 59% and overall survival (OS) of 73% when compared to historical controls. The regimen also had acceptable toxicity rates in the treatment population, and delivery was feasible in the adult oncology setting.[5] Superior outcomes using pediatric-inspired regimens have also been demonstrated across multiple prospective trials by other cooperative groups (Table 1),[2] resulting in the pediatric-inspired approach becoming the new standard of care for AYA B-ALL patients treated in adult centers.[6]

Alternative AYA treatment approaches do exist, including MD Anderson Cancer Center's hyperCVAD regimen (Figure 1), as well as its augmented hyperCVAD that adds blocks of pegylated asparaginase. This regimen was shown in its single-center retrospective analysis to produce similar results to the BFM-based approach in AYA patients.[7] However, in other retrospective analyses it was found to be inferior compared to pediatric-inspired regimens in regard to 3-year OS (48.5% vs 72.6%; $P=.4$), mean OS (41.5±6.4 months vs 53.9±5.4 months; $P=.012$), mean relapse-free survival (RFS; 39.1±6.8 months vs 53.9±5.4 months; $P=.009$), and 3-year disease-free survival (DFS; 54.7% vs 76.4%; $P=.44$).[8] An additional comparison of 2 pediatric-inspired regimens with hyperCVAD found superior complete remission (CR) rates (79.5% vs 64.2%; $P=.02$), lower relapse rates (44.1% vs 60.0%; $P=.04$), and improved 24-month OS (41.5% vs 28.1%; $P=.012$) with the pediatric-inspired regimens. Treatment per CALGB10403 was also the only independent prognostic factor for OS in patients older than 20 years (hazard ratio, 0.44; 95% CI, 0.20-0.97; $P=.04$).[9]

CLINICAL CASE (continued)

Following admission to the Adult Leukemia Service, the patient is confirmed on multiparameter flow cytometry (MFC) to have B-ALL that is CD20+. He is initiated on treatment per CALGB10403 with fluorescence in situ hybridization, mutational profile, and next generation sequencing (NGS) testing pending.

Philadephia-negative B-ALL

Regardless of the up-front treatment utilized in the AYA population (Figure 1), a survival gap exists in AYA B-ALL when compared to pediatric outcomes. Following up-front therapy, children aged 1 to 14 years now achieve a 5-year OS of greater than 93%, whereas the OS for AYA patients ranges from 60% to

Table 1. "Pediatric-inspired" B-ALL trials

Trial	No. of patients	Age (years), median (range)	CR (%)	OS (%)	EFS (%)	DFS (%)	Duration of follow-up	AlloHSCT
JALSG 202-U	139	19 (16–24)	97	74	-	71	4 year	t(4;11)
DFC1 01–1756	92	28 (18–50)	86	70	-	71	4 year	t(4;11), +8, Ph+
DFCI 06–254	110	32 (18–50)	89	75	-	73	3 year	t(4;11), +8, Ph+
UKALL 2003	229	16–24	97	76.4	72.3	-	5 year	
MDACC aBFM	106	22 (13–39)	93	53	-	60	5 year	t(4;11) or MRD+
NOPHO ALL 2008	221	26 (18–45)	-	78	74	-	5 year	D29 MRD >5% or D79>0.1%
GRAALL 2005	787	36 (18–60)	92	58.5	52	-	5 year	High risk or MRD+
CALGB10403	295	24 (17–39)	89	73	59	66	3 year	
ALLRE08 PETHEMA	89	20 (15–29)	95	74	62	65	5 year	MRD+
ALL06	86	22 (15–39)	90.2	74.9	-	72.8	3 year	t(4;11), MRD+, poor steroid response or WBC >100
GIMEMA LAL-1308	76	23 (18–35)	92	60.3	-	60.4	4 year	No CR at D33, pro-B ALL or WBC >100, t(4;11), MRD+

alloHCT, allogeneic hematopoietic stem cell transplantation; MRD, measurable residual disease.

Adapted from Carobolante et al.[2]

78% despite CR rates of 85% to 95%.[10] The cause of this outcome difference is multifactorial, including nonrelapse mortality from an increased risk of treatment-related toxicities compared to pediatric patients and the prevalence of chemoresistant disease due to the particular B-ALL mutational profile seen in AYA patients.[11,12] Among early efforts to improve B-ALL outcomes was the introduction of the CD20 monoclonal antibody rituximab to up-front therapy based on the GRAALL-2005/R randomized trial showing improved EFS and decreased rates of relapse in CD20+ adult B-ALL patients.[13] This is not standard practice in pediatric regimens, which do not incorporate rituximab into up-front treatment due to a lack of significant difference in measurable residual disease (MRD) response rates in patients receiving rituximab and those who do not receive it.[14]

Currently, given the emergence of novel agents (Figure 2) and the success of inotuzumab, blinatumomab, and CD19 chimeric antigen receptor (CAR) T cells in the relapsed/refractory (R/R) B-ALL setting,[15] the preferred up-front treatment for Ph− B-ALL is clinical trial enrollment, when available and accessible, investigating the incorporation of these treatment modalities.

Inotuzumab

For AYA patients treated in pediatric centers, the up-front COG trial AALL1732 randomizes Ph− B-ALL patients who are end of

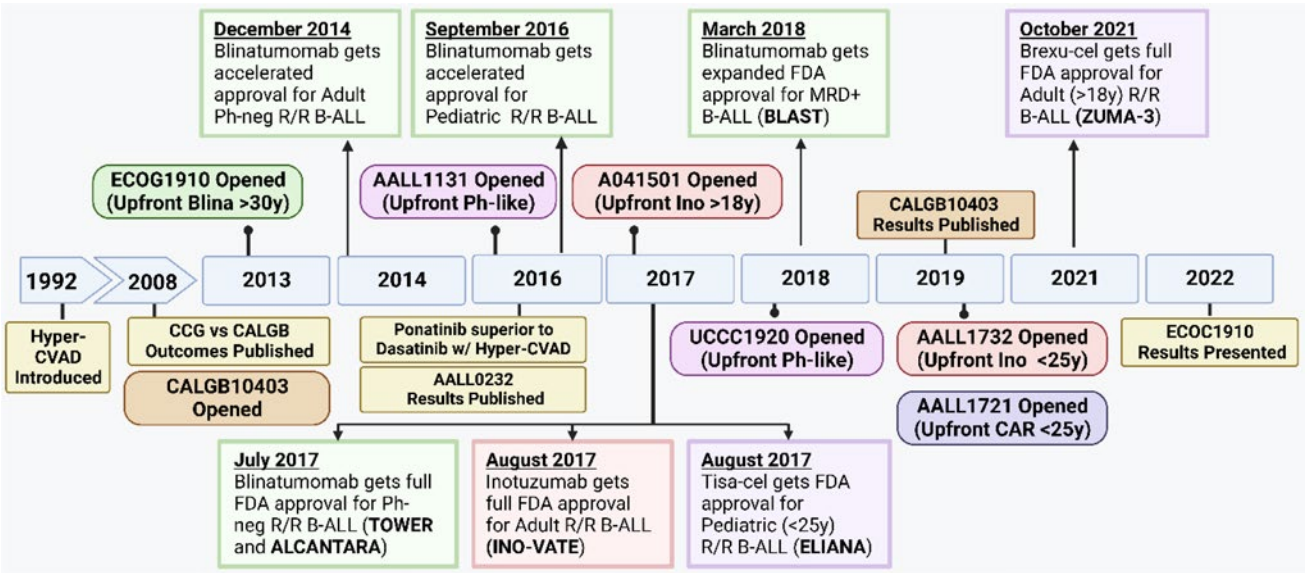

Figure 2. Time line of treatment approvals in B-ALL. Brexu-cel, brexucabtagene autoleucel; Car, chimeric antigen receptor; MRD, measurable residual disease; Ph-neg, Philadelphia negative; R/R, relapsed/refractory; Tisa-cel, tisagenlecleucel.

Table 2. Up-front inotuzumab and blinatumomab trials

Trial	Planned patients	Ages	Intervention	Status	Clinical trials
Inotuzumab					
COG AALL1732	4772	1–25	2 cycles of Ino in consolidation	Recruiting	NCT03959085
ALLIANCE A041501	310	18–39	2 cycles of Ino in consolidation	Suspended	NCT03150693
ALLTogether	6430	0–45	2 cycles of Ino prior to maintenance	Recruiting	NCT04307576
GRAALL (B-2022)	600	18–65	1 cycle of Ino in delayed intensification	Planned	Pending[9]
Blinatumomab					
Jiangsu Institute of Hematology	35	15–59	Reduced-intensity induction + blina	Recruiting	NCT05557110
HOVON146ALL	71	18–70	Steroid + blina prephase; blina for 2 cycles in consolidation	Closed to accrual	NCT03541083
Blina-CELL (CZECRIN)	45	18–65	1 cycle of blina + chemo for induction	Closed to accrual	NCT04554485
GIMEMA (LAL2317)	149	18–65	2 cycles of blina added to chemo	Closed to accrual	NCT03367299
GRAALL (B/2014-QUEST)	95	18–59	5 cycles of blina added to consolidation/ maintenance for MRD+	Closed to accrual	NCT03709719
GMALL (MolAct-1)	84	18+	Up to 2 cycles of blina added to chemo	Completed, results pending	NCT03109093
Inotzumab + blinatumomab					
MDACC	80	14+	Blina cycles 5–8 + Ino in cycles 6 and 8	Recruiting	NCT02877303

Blina, blinatumomab; Ino, inotuzumab; MRD+, MRD-positive.

consolidation (EOC) MRD-negative to receive 2 cycles of the anti-CD22 antibody drug conjugate inotuzumab (NCT03959085). A comparable study at adult centers is the successor to CALGB10403, Alliance trial A041501, which is investigating the use of 2 cycles of inotuzumab postinduction for MRD eradication (NCT03150693). Patients aged 18 to 25 would be eligible for either trial depending on treatment location.

Inotuzumab was chosen for these trials based on the results of the adult phase 3 INO-VATE trial showing both an improved CR rate (73.8% vs 30.9%; *P*<.0001) and MRD-negative CR (78.4% vs 28.1%; *P*<.001) in R/R B-ALL compared to chemotherapy,[16] as well as an acceptable safety profile from the R/R pediatric B-ALL trial COG AALL1621.[17] While the most frequent grade 3 adverse event in prior inotuzumab trials was hepatic toxicity, including cases of veno-occlusive disease, both AALL1732 and A041501 have noted increased rates of sepsis during subsequent blocks of chemotherapy, requiring protocol modifications.[18] Although not temporally associated with inotuzumab infusion, concern exists for both prolonged myelosuppression with chemotherapy following inotuzumab exposure and inotuzumab-induced hypogammaglobinemia leading to increased risk of bacterial infection.

In addition to AALL1732 and A041501, numerous ongoing trials including AYA patients are also exploring the role of inotuzumab as part of postinduction therapy and its efficacy for MRD eradication in up-front therapy (Table 2).

Blinatumomab

In addition to inotuzumab, the CD3-CD19 bispecific T-cell engager blinatumomab is being evaluated in the up-front treatment setting based on its efficacy in R/R (TOWER[19]) and MRD-positive (BLAST[20]) adult B-ALL trials. Unlike inotuzumab, blinatumomab response and outcomes are superior with low pretreatment tumor burden.[19,20] This has led to its superior efficacy as a consolidative treatment rather than salvage therapy, as illustrated by the results of 2 pediatric phase 3 trials in patients up to the age of 30 years.[21,22] Based on the initial results of the randomized phase 3 ECOG-ACRIN E1910 trial (which included patients as young as 30 years), the standard of care going forward for patients achieving an MRD-negative remission following induction will likely include the incorporation of blinatumomab.

Utilizing a BFM-based regimen adapted from the E2993/UKALLXII that incorporated an extended remission induction, rituximab for CD20+ patients, and pegaspargase for patients younger than 55 years, E1910 randomized patients achieving MRD-negativity at the end of induction (EOI) to receive 4 cycles of blinatumomab. With a median follow-up of 43 months, a significant improvement in median OS was seen in the blinatumomab arm (not reached vs 71.4 months; *P*=.003).[23] Currently, only AYA patients with Down syndrome are included in the up-front pediatric blinatumomab trial AALL1731 (NCT03914625), but several other ongoing trials are assessing the efficacy of blinatumomab with or without the addition of inotuzumab in the up-front setting (Table 2).

CAR T-cell products

Similar to blinatumomab, the 2 US Food and Drug Administration (FDA)–approved CAR T-cell products for R/R B-ALL, tisagenlecleucel (tisa-cel) and brexucabtagene autoleucel (brexu-cel), create a cytotoxic T-cell response against CD19-expressing B-ALL cells, resulting in high rates of MRD-negative CR (Table 3).[24] Tisa-cel is FDA approved for patients up to 25 years of age with refractory disease or those in a second or later relapse. It is being evaluated in the up-front setting for AYA patients younger than 25 years who are EOC-positive as part of COG AALL1721

Table 3. CAR T-cell therapies FDA approved for AYA B-ALL

Trial	No. of patients	Median age (range), years	CR/CRi (%)	MRD-negativeCR in responders (%)	Median RFS (mo)	Median OS (mo)	Unique toxicity
Tisa-cel							
ELIANA[26]	75	11 (3–23)	81	100	EFS at 12 months=50%	19.1	CRS (any): 77% Gr. ≥3: 35% ICANS (any): 40% Grade ≥3: 13%
Brexu-cel							
ZUMA-3, phase 1/2[28]	78	42.5 (18–84)	73	97	11.7	25.4	CRS (any): 93% Gr. ≥3: 31% ICANS (any): 78% Gr. ≥3: 38%

CRi, complete remission with incomplete count recovery; CRS, cytokine release syndrome; Gr, grade, ICANS, immune effector cell-associated neurotoxicity syndrome.

(NCT03876769). The use of CAR T cells earlier in therapy may be more efficacious at this time point given the effects of high disease burden and greater number of prior lines of therapy on outcomes.[25] Tisa-cel, which utilizes a 4-1BB costimulatory domain, also has the potential for durable remission with a single CAR T-cell infusion.[26,27] Based on the ability of NGS MRD assessment and loss of B-cell aplasia to predict relapse post CAR T cell, the proposed CAR-CURE BMT-CTN trial will seek to establish if CAR therapy alone is sufficient or whether a subset of patients may benefit from post-CAR consolidative hematopoietic stem cell transplantation (HSCT) (NCT05621291). Currently, no equivalent up-front trials exist for brexu-cel, which utilizes a CD28 costimulatory domain,[28] or other CAR T-cell products.

Philadelphia-positive B-ALL

Compared to pediatric B-ALL, where it accounts for 1% to 2% of cases, BCR-ABL1 rearrangement or Philadelphia-positive (Ph+) B-ALL is the most frequent genetic subcategory in adults, including an incidence in young adult patients of approximately 25% to 30%. Although previously carrying a dismal prognosis with an OS of less than 25% in adults and less than 50% in pediatric patients, the introduction of tyrosine kinase inhibitors (TKIs) has significantly altered overall outcomes.[29] Ongoing trials including AYA patients seek to understand both the ideal TKI selection and overall treatment intensity, as well as the need for postremission HSCT following the incorporation of third-generation TKIs and/or blinatumomab into up-front therapy.

Despite remission rates of 95% to 100% with the integration of TKIs into pediatric-inspired or hyperCVAD regimens,[29] relapse is common and is associated with kinase domain mutations that can be detected at the time of diagnosis.[30] Compared to the first- and second-generation TKIs, ponatinib can overcome mutations like *T315I* and was shown to have a 3-year OS superior to dasatinib when combined with hyperCVAD for frontline therapy (83% vs 56%; *P*=.03).[31] While it may become the preferred TKI in adult B-ALL, ponatinib is currently used in pediatrics only for relapsed disease or if a resistance mutation is detected. The efficacy of ponatinib in younger patients is being tested in the R/R setting as part of the phase 1/2 COG trial AALL1922, given the lack of pharmacokinetic and safety data for patients less than 18 years (NCT04501614).

Similar to Ph− B-ALL, current up-front trials are incorporating blinatumomab in combination with TKIs in hopes of improving EFS/OS and allowing chemotherapy dose reduction (Table 4).

Philadelphia-like B-ALL

Philadelphia-like (Ph-like) B-ALL expresses a gene signature that is quite similar to Ph+ B-ALL but portends significantly worse outcomes due to its increased resistance to asparaginase and daunorubicin and poor sensitivity to glucocorticoids.[32] Ph-like B-ALL is more prevalent in AYA patients (25-30%) compared to children (10-15%), with an increased prevalence in the Hispanic/Latino and Native American population.[33-35] Patients with Ph-like disease have high rates of persistent MRD-positivity and OS rates of only 20% to 30%, necessitating HSCT for long-term remissions.[36] Ongoing efforts to improve outcomes in AYA patients with Ph-like B-ALL have focused on targeting the JAK/STAT signaling pathway and ABL-class fusions (Table 3) and/or incorporating immunotherapy approaches.

Phase 2 studies exploring the efficacy of incorporating the JAK inhibitor ruxolitinib into induction regimens for Ph-like ALL are still ongoing (NCT03117751 and NCT03571321), including COG AALL1521 (NCT02723994) for patients with *CRLF2* rearrangements or JAK pathway mutations. Although ruxolitinib in combination with a traditional chemotherapy backbone has been shown to be safe and tolerable, it is still unknown whether outcomes are better than historical controls.[32] A trial adding ruxolitinib to hyperCVAD was terminated early due to low accrual and lack of efficacy (NCT02420717).

For Ph-like patients with *ABL* rearrangements, prior reports have suggested the benefit of BCR-ABL–specific TKIs for patients with rearrangements of *PDGFRB*, which are associated with a high risk of induction failure.[37] COG AALL1131 (NCT02883049) was amended to test the benefit of dasatinib in patients with *ABL* class fusions, but results are pending.[34] An interim data analysis of MDACC's phase 1/2 trial of hyper-CVAD plus dasatinib showed safety and efficacy in R/R, suggesting overall tolerability of the regimen.[38]

Given the high response rates in R/R Ph-like B-ALL with inotuzumab, blinatumomab, and CAR T,[39] AYA patients are also eligible for the up-front Ph− trials (Table 2).

Table 4. Newly diagnosed Ph+ and Ph-like clinical trials

Trial	Planned patients	Ages	Intervention	Status	Clinical trials
Ph+					
EsPhALL2017/ COG AALL1631	475	1–21	**Standard risk:** imatinib with EsPhALL vs COG high-risk pre-B ALL backbone **High risk**: alloBMT with imatinib maintenance	Recruiting	NCT03007147
EA9181	330	18–75	Dasatinib or ponatinib/blinatumomab vs dasatinib or ponatinib/Hyper-CVAD	Recruiting	NCT04530565
GIMEMA ALL2820	236	18+	Ponatinib/blinatumomab vs imatinib/ chemotherapy	Recruiting	NCT04722848
U Chicago	25	18+	Inotuzumab + dasatinib + steroid Induction	Recruiting	NCT04747912
Ph-like					
COG AALL1131	22	1–31	Dasatanib with chemotherapy for ABL-class fusions	Closed to accrual	NCT02883049
COG AALL1521	171	1–21	Ruxolitinib with chemotherapy for CRLF2 rearrangements and other JAK pathway alterations	Closed to accrual	NCT02723994
Total Therapy XVII	790	1–18	**Dasatinib:** patients with Ph+ and those with ABL-class fusion **Ruxolitinib**: patients with activation of JAK/STAT signaling	Closed to accrual	NCT03117751
UCCC 1920	15	18–39	**Ruxolitinib:** patients with activation of JAK/STAT signaling	Recruiting	NCT03571321

alloBMT, allogeneic bone marrow transplantation.

CLINICAL CASE (continued)

Fluorescence in situ hybridization testing reveals Ph+ B-ALL, and the patient is found to be *IKFZ*^{pos} on mutational profiling. Dasatinib is added to his induction therapy as well as rituximab for CD20+ disease. His EOI MRD testing on day 29 is positive by BCR-ABL polymerase chain reaction (PCR) and NGS.

Postinduction management of AYA patients

MRD monitoring and treatment modification

Regardless of B-ALL subtype or frontline regimen used, the most important prognostic factor for long-term outcomes in ALL remains the persistence of MRD following treatment.[40] While acceptable MRD testing should consist of a standardized, validated assay with a sensitivity of 10^{-4}, the optimal time point (EOI vs EOC, or prior to HSCT) and preferred method (MFC, reverse transcriptase PCR, or NGS) remains a point of debate.[41]

Compared to pediatric patients, the GRAALL-2003 and 2005 trials showed a delayed rate of MRD clearance by MFC in adult B-ALL patients. While only 36% of patients achieved MRD-negative CR at EOI, 76% of patients were in MRD-negative CR by the EOC.[42] Despite the potential for later MRD clearance, postinduction MRD-positivity in Ph− AYA patients treated on CALGB10403 was an independent predictor of OS, suggesting that earlier MRD clearance may be more important for durable remissions in AYA patients.[5] Currently, for both pediatric and adult treatment approaches for Ph− B-ALL, end consolidation MRD-positivity necessitates treatment modification and remains an indication for HSCT in patients able to obtain a first CR.[43,44] Future studies incorporating up-front cellular and immunotherapy approaches may redefine the acceptable timing of MRD clearance.

In addition to the need to determine the importance of when MRD clears, there has been a shift from MFC to utilizing the more sensitive immunoglobulin or T-cell receptor clonotype based NGS, which has a sensitivity of up to 10^{-6}. A recent analysis of discordant MFC and NGS outcomes showed a 5-year cumulative incidence of relapse of 0% in patients MRD-negative by both NGS and MFC compared to 36% in MRD-negative by MFC only. Patients found to be NGS negative at CR had a 5-year OS of 89% compared to 63% for NGS-positive patients, and achieving NGS MRD-negativity at a later time point did not predict low relapse risk.[45] For Ph+ ALL, NGS may be superior to reverse transcriptase PCR and allow identification of patients with detectable BCR-ABL1 but low risk for relapse.[46] In regard to HSCT, NGS-based MRD both pre- and post-HSCT has been shown to be predictive of transplant outcomes with a high concordance rate between peripheral blood and bone marrow sampling.[47]

Consolidative HSCT in the first CR

The introduction of pediatric-inspired regimens, the use of second- and third-generation TKIs for Ph+ disease, and the advent of improved MRD monitoring for treatment response have created a shift away from HSCT in the first CR (CR1) for the many AYA B-ALL patients. This is more in line with the pediatric approach where consolidative HSCT in CR1 is no longer indicated for several previously defined high-risk subtypes—hypodiploid ALL, Ph+, IKZF^{plus}—if patients achieve an MRD-negative CR.[48]

While once considered the standard of care for Ph− AYA patients based on results from the MRC UKALL XII/ECOG E2993 trial,[49] recent retrospective analysis comparing continued treatment per CALGB10403 to consolidative HSCT in CR1

showed a superior 5-year OS (66% chemo vs 47% HCT; $P<.001$) and DFS (58% chemo vs 44% HCT; $P<.004$) with chemotherapy alone.[50] HSCT also had higher nonrelapse mortality (29% HCT vs 8% chemo; $P<.001$), and a separate analysis found HSCT to be the only factor associated with decreased OS on multivariable analysis.[51]

For Ph+ B-ALL, HSCT is no longer the standard of care for pediatric patients with chemotherapy plus TKI alone and has shown to be noninferior to HSCT.[52] While transplant remains a recommendation for AYA patients in CR1 with Ph+ B-ALL with a suitable donor in adult settings,[53] the addition of second- or third-generation TKIs to intensive chemotherapy can produce deep molecular responses for which HSCT has not been shown to provide additional benefit.[31] For those patients proceeding to HSCT, maintenance therapy with a TKI remains the standard of care, although its benefit remains unknown with EsPhALL2017/COGAALL1631 (NCT03007147) prospectively evaluating the feasibility, toxicity, and outcomes of post-HCT TKI maintenance in the pediatric and young adult population.

Currently, proceeding to HSCT for AYA patients in CR1 is predominately reserved for patients with specific high-risk mutations (ie, Ph-like and *KM2TA*r) or individuals with persistent MRD with induction therapy able to achieve a remission.

CLINICAL CASE (continued)

The patient's TKI is switched to ponatinib, and he achieves a complete molecular remission at the EOC evaluation with negative MRD by MFC and NGS. He does not proceed to HSCT in CR1, and the patient remains in an ongoing remission during maintenance therapy with serial monitoring by BCR-ABL PCR.

Conclusion

Significant advances in the up-front treatment of AYA B-ALL have occurred with the adoption of pediatric-inspired regimens as a standard of care. Hope remains to further close the survival gap with the incorporation of immunotherapy and targeted agents in the up-front setting. Time will tell if the current, ongoing up-front trials in combination with more sensitive MRD testing can further improve OS/EFS, avoid the need for HSCT in CR1 for the majority of AYA patients, and lead to decreased treatment-associated toxicity through further dose reductions of chemotherapy.

Despite this optimism with improved treatment approaches, AYA B-ALL patients remain affected by significant financial and treatment-related toxicities that may lead to excessive long-term mortality and the negation of improvement in 5-year OS.[54-57] Outcomes also remain disproportionately worse for minority patients,[11] and substantial barriers remain for AYA clinical trial enrollment.[58] In addition to improved up-front treatment regimens, attention must remain on the unique needs of this patient population, which often remain underrecognized or overlooked.[59]

Conflict-of-interest disclosure

John C. Molina: no competing financial interests to declare.
Seth Rotz: no competing financial interests to declare.

Off-label drug use

John C. Molina: The off-label use of blinatumomab, inotuzumab ozogamicin, tisagenlecleucel, ponatinib, ruxolitinib, dasatinib is discussed.
Seth Rotz: The off-label use of blinatumomab, inotuzumab ozogamicin, tisagenlecleucel, ponatinib, ruxolitinib, dasatinib is discussed.

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Adolescents and young adults (AYAs) vs pediatric patients: survival, risks, and barriers to enrollment

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Adolescents and young adults (AYAs; ages 15–39 years) with acute lymphoblastic leukemia (ALL) have worse outcomes than pediatric patients with ALL. Multiple factors contribute to this differential survival. AYAs are more likely to have higher-risk leukemia biology than children with ALL. AYA patients have more choices for treatment facility and treatment protocol, as well as barriers to clinical trial enrollment, both of which can affect survival. AYAs must also navigate psychosocial factors inherent to their unique developmental stage. Furthermore, AYAs typically sustain more treatment-related toxicities than pediatric patients. Treatment on pediatric or pediatric-inspired ALL protocols at pediatric cancer centers has been associated with improved outcomes for AYAs with ALL, but there is still variation in the treatment that AYAs with ALL receive. Clinical trials focused on AYAs with ALL and individualized decision-making regarding choice of treatment facility and treatment protocol are needed to optimize the survival and long-term outcomes of this patient population.

LEARNING OBJECTIVES

- To identify key factors contributing to relatively worse outcomes in adolescents and young adults with ALL as compared to pediatric patients with acute lymphoblastic leukemia
- To compare and contrast treatment toxicities faced by adolescents and young adults vs pediatric patients with acute lymphoblastic leukemia
- To describe barriers to clinical trial enrollment faced by adolescents and young adults with acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is the most common cancer in children ages 0 to 14 years of age and has a survival rate above 90%.[1–3] However, adolescents and young adults (AYAs; ages 15–39 years) with ALL have worse outcomes than younger children, with 5-year overall survival (OS) rates for AYAs ranging between 54% and 74%.[4,5] Younger AYAs often fare better than older AYAs.[4–6] Moreover, when including patients up to 29 years of age, AYA patients with diagnoses of any leukemia have the highest mortality rate of any cancer.[4] This discrepant survival between pediatric and AYA patients with ALL is similar to AYAs with many other cancers as well.[2,5,7–9] Additionally, survival for AYAs with cancer has not improved over time at the same pace as that for other age groups, causing what has been termed the "AYA gap."[5] This AYA gap has been an area of increasing concern in the pediatric and adult oncology communities over the past several decades. Reasons for this gap are multifactorial and include clinical, biological, and psychosocial factors, as well as barriers to clinical trial enrollment. Further, AYAs with ALL are particularly unique because while ALL is the most common cancer in children, it is relatively rare in adults. The treatment of AYAs with ALL therefore requires careful consideration of protocol type and treatment center.[9–11] This review will use 2 clinical cases to explore discrepancies in survival, risks, and challenges with clinical trial enrollment for AYAs with ALL and to describe areas for optimization of care and quality of life of this unique population.

CLINICAL CASES

- Case 1: A 10-year-old boy presents to an off-therapy oncology clinic for his annual follow-up appointment. He presented to the emergency room at 6 years of age

with 2 weeks of fever and leg pain. He had a white blood cell (WBC) count of 60×10⁹/L, hemoglobin of 7 g/dL, and platelet count of 55×10⁹/L. He was diagnosed with B-cell ALL with ETV6-RUNX1 translocation. His cerebral spinal fluid was negative for leukemia. He was treated at a children's hospital and was enrolled onto the currently open Children's Oncology Group (COG) clinical trial for high-risk ALL, as his WBC count of ≥50×10⁹/L classified him as high risk. His end of induction bone marrow aspirate testing was negative for minimal residual disease (MRD) assessed through flow cytometry, with negative MRD defined as <0.01. His treatment complications included mild vincristine-induced neuropathy that improved with physical therapy and 1 admission for febrile neutropenia in delayed intensification, during which he was diagnosed with influenza A and his blood culture was negative. He recovered uneventfully from this. He was well supported by his family, school, and the hospital's child life team. His parents diligently ensured that he adhered to oral maintenance therapy. He is overall doing well off-therapy. His parents and teacher have concerns about academic difficulties, especially in math, for which he is scheduled to undergo neuropsychological testing.

- Case 2: A 28-year-old man presents to an oncology clinic. He initially presented at 23 years of age with fatigue and fevers. He had a WBC count of 80×10⁹/L, hemoglobin of 11 g/dL, and platelet count of 40×10⁹/L. He was diagnosed with B-cell ALL with IKZF1 deletion. His cerebral spinal fluid was negative for leukemia. He was treated at an academic adult hospital per a pediatric-inspired protocol (not on study) as there were no ALL trials open for his age. His bone marrow aspirate studies showed MRD positivity at end of induction (course I) and MRD negativity at end of consolidation (course II) assessed through flow cytometry, with negative MRD defined as <0.01. His treatment complications included asparaginase-associated pancreatitis in induction and delayed presentation for fever in delayed intensification (course IV) due to insurance concerns regarding mounting hospital bills, which resulted in intensive care unit admission for *Escherichia coli* septic shock. He has lived alone since time of diagnosis and has minimal psychosocial support, frequently missing appointments due to worries about losing his job. He had poor compliance to oral antimetabolite therapy and antimicrobial prophylaxis throughout treatment. At his most recent appointment 15 months off-therapy, he was found to have relapsed ALL and was referred for allogeneic hematopoietic stem cell transplantation. He has developed avascular necrosis of his knees and depression.

Introduction

Survival

Rates of remission for AYAs with ALL are similar to those for pediatric patients at greater than 90%. However, OS for AYAs is 54% to 74% as compared to greater than 90% in children.[3-6,8,9,11-14] Underlying differences that increase risk and contribute to the differential long-term survival include the unique biology of ALL in AYAs, choice of treatment protocol and center, increased susceptibility to therapy-related toxicities, and psychosocial challenges.

Table 1. Common genetic changes in pediatric and adolescent and young adult acute lymphoblastic leukemia

Genetic change	Prognostic significance	ALL patient population in which genetic change is commonly found
ETV6-RUNX1	Favorable	Pediatric
Hyperdiploidy*	Favorable	Pediatric
Philadelphia chromosome-like	Unfavorable	AYA
IKZF1	Unfavorable	AYA
BCR-ABL1	Unfavorable	AYA
Hypodiploidy†	Unfavorable	AYA

*Leukemia blasts containing >50 chromosomes.

†Leukemia blasts containing ≤45 chromosomes.

Risks

Unique cancer biology

AYAs have unique cancer biology, with different prevalence of genetic mutations compared to pediatric patients.[7,15] In ALL, AYAs are less likely to have leukemias with favorable features such as hyperdipoidy or ETV6-RUNX1 translocation; ETV6-RUNX1 translocation, which our pediatric patient had, has been identified in 10% of AYAs compared to nearly half of pediatric patients.[7,8] Further, AYAs with ALL, as compared to pediatric patients, are more likely to have high-risk features, including T-cell ALL with the unfavorable HOX subtype, KMT2A/MLL or BCR-ABL translocations, CRLF2 mutations, and hypodiploidy (Table 1).[7,8,16]

Choice of treatment protocol and treatment center

Differences in survival between AYA and pediatric patients can be addressed in part by using pediatric-inspired regimens in AYAs with ALL.[2,9,10,14] In 2008, Stock and colleagues[9] performed a retrospective cohort study of 321 AYAs (ages 16-20 years) with ALL and found that while the AYAs treated on pediatric and adult protocols both had remission rates of 90%, AYAs treated on a Children's Cancer Group (now COG) protocol had improved 7-year event-free survival (EFS) and OS. AYAs treated on pediatric protocols had 7-year EFS and OFS of 63% and 67%, respectively, as compared to 7-year EFS and OS of 34% and 46% for AYAs treated on the Cancer and Leukemia Group B (CALGB) adult protocol. This paved the way for CALGB 10403, a prospective study of 295 AYAs with ALL aged 17 to 39 years that mirrored the control arm of COG protocol AALL0232. CALGB 10403 demonstrated superior outcomes for AYAs treated on the pediatric-inspired protocol as compared to a standard adult ALL protocol. Median EFS was 78.1 months for those treated on CALGB 10403 vs 30 months for historic controls. Treatment-related mortality was 3%.[2] Together, these studies, along with several other national and international studies, demonstrated that the pediatric backbones are effective and generally well tolerated in AYAs.[2,17-20] Importantly, AYAs treated on pediatric-inspired protocols continued to have poorer outcomes than their pediatric counterparts, which may be due to inherent differences in leukemia biology and treatment response. A notable difference between pediatric and adult protocols is the types of chemotherapies used.[14] Pediatric protocols

Table 2. Treatment approaches in pediatric and adolescent and young adult acute lymphoblastic leukemia by representative protocols

	Pediatric protocol for ALL*	Pediatric-inspired ALL protocol for AYAs	Adult ALL protocol for AYAs
Representative protocol used	AALL1732†,[37]	CALG B10403‡,[2]	Hyper-CVAD[38,39]
Induction chemotherapy agents used	DXM (<10 years old)/PDN (≥10 years old) VCR DNR ASP	PDN VCR DNR ASP	Hyperfractionated CPM VCR DOX DXM HD-MTX ARAC ±Rituximab if CD20+
Approach to CNS prophylaxis	IT ARAC at diagnosis, then IT MTX throughout treatment 18 Gy CRT only if CNS3	IT ARAC at diagnosis, then IT MTX throughout treatment Prophylactic CRT in any patient with T-ALL 18 Gy CRT if CNS leukemia at diagnosis	Alternating IT MTX and IT ARAC in induction and consolidation 30 Gy CRT to whole brain (frank leukemia) or to skull base (cranial nerve involvement)
Use of HSCT	EOC MRD >0.01	If persistent MRD at EOI; if high-risk cytogenetics in CR1	If persistent MRD at EOI; if high-risk cytogenetics in CR1
Immunotherapy	InO given postconsolidation in experimental arm	Not used	Rituximab if CD20+
Key regimen differences compared to a pediatric protocol	—	Extended remission induction (PDN, DNR, VCR, ASP) One IM phase (uses escalating-dose MTX) while pediatric protocol has 2 IM phases (HD-MTX, then escalating-dose MTX) Patients with T-ALL who were CNS negative at presentation receive prophylactic CRT, which is not done in pediatric protocols for most patients with T-ALL More likely to proceed to HSCT Does not use novel immunotherapy agents	ASP not used in induction Less frequent and less aggressive IT CNS prophylaxis Higher doses of myelosuppressive drugs More likely to proceed to HSCT Use of rituximab if CD20+

Protocols described are for Philadelphia chromosome-negative acute lymphoblastic leukemia.

ARAC, cytarabine; ASP, asparaginase; CNS, central nervous system; CPM, cyclophosphamide; CR1, first complete remission; CRT, cranial radiation therapy; CVAD, cyclophosphamide, vincristine sulfate, doxorubicin hydrochloride, dexamethasone; DNR, daunorubicin; DOX, doxorubicin; DXM, dexamethasone; EOC, end of consolidation; EOI, end of induction; Gy, Gray; HD, high dose; HSCT, hematopoietic stem cell transplant; IM, interim maintenance; InO, inotuzumab ozogamicin; IT, intrathecal; MTX, methotrexate; PDN, prednisone; VCR, vincristine.

*A high-risk pediatric protocol is used as the representative pediatric regimen, as adolescents treated on pediatric protocols are considered high risk at time of diagnosis due to age.

†COG trial AALL1732 was selected for use in this table for the high-risk pediatric protocol as this is the current ongoing pediatric trial in the COG. CALGB 10403 is based on the COG trial AALL0232 control arm.[40] AALL0232 included 2 randomizations (dexamethasone vs prednisone for induction steroids and high-dose MTX vs Capizzi MTX in IM 1) while AALL1732 uses dexamethasone in induction if <10 years of age and prednisone in induction if ≥10 years of age, high-dose MTX in IM 1 with Capizzi MTX in IM 2, and randomization of the novel agent InO.[37,40]

‡Protocol CALGB 10403 was selected for the pediatric-inspired representative regimen for adult ALL as this is the trial discussed throughout the article. Current ongoing Alliance (formerly CALGB) trial A041501 is also testing the novel agent InO.

include extended durations of high-dose glucocorticoids, higher doses of asparaginase and vincristine, and earlier and repeated administrations of frequent central nervous system prophylaxis. Conversely, adult ALL protocols typically use significantly myelosuppressive agents and administer central nervous system prophylaxis later during therapy and less frequently.[2,9,14,21] Table 2 summarizes key differences in treatment protocol approaches.

The type of treatment center is also closely linked to also closely linked both to outcome and to procotol selected to be used.[10] AYAs with ALL may be treated at a children's hospital on a pediatric protocol, an adult hospital (academic or community) on a pediatric-inspired protocol, or an adult hospital (academic or community) on an adult protocol. Gupta et al[14] reviewed 271 AYAs aged 15 to 21 years treated between 1992 and 2011. They found that from 1992 to 2005, when most AYAs at adult hospitals received adult protocols, 56% of AYAs were treated at an adult hospital with 5-year EFS and OS of 56% and 64%, respectively. For AYAs treated at pediatric centers, 5-year EFS and OFS were 72% and 82%, respectively. From 2006 to 2011, however, 66% of AYAs treated at adult hospitals received pediatric-inspired ALL protocols. Outcomes were better than those for AYAs treated at adult hospitals from 1992 to 2005 but worse than those for AYAs treated at children's hospitals from 2006 to 2011. The authors concluded that survival differences are driven by both lack of universal use of pediatric ALL protocols and factors inherent to children's hospitals. For example, as seen in case 1, there is

Table 3. Proportion of patients with high-risk acute lymphoblastic leukemia with adverse events in induction on pediatric protocols by age[25]

	Cohort, No. (%)*			*P* value
	Overall (N=235)	<15 years (n=176)	≥15 years (n=59)	
Any adverse event	190 (80.9)	139 (78.9)	51 (86.4)	.21
Infection†	83 (35.3)	62 (35.2)	21 (35.6)	.96
Hypertension	72 (30.6)	52 (29.6)	20 (33.9)	.53
Hepatotoxicity	72 (30.6)	45 (25.6)	27 (45.8)	**<.01**
Fever†	58 (24.7)	44 (25.0)	14 (23.7)	.84
Hypoxia	46 (19.6)	35 (19.9)	11 (18.6)	.84
Hyperglycemia†	42 (17.9)	26 (14.8)	16 (27.1)	**.03**
Sepsis	28 (11.9)	21 (11.9)	7 (11.9)	.98
Hypotension	27 (11.5)	18 (10.2)	9 (15.3)	.29
Thromboembolism†	21 (8.9)	12 (6.8)	9 (15.3)	**.04**
Neuropathy	11 (4.7)	6 (3.4)	5 (8.5)	.11
Hyponatremia	8 (3.4)	6 (3.4)	2 (3.4)	1
Pancreatitis	8 (3.4)	6 (3.4)	2 (3.4)	1
Seizure	6 (2.6)	4 (2.3)	2 (3.4)	.64
Ileus	5 (2.1)	4 (2.3)	1 (1.7)	1
Constipation	3 (1.3)	3 (1.7)	0 (0.0)	.6
ARDS	3 (1.3)	1 (1.7)	2 (1.1)	1
Stroke	2 (0.9)	1 (0.6)	1 (1.7)	.44
Anaphylaxis	2 (0.9)	1 (0.6)	1 (1.7)	.44

Bold values indicate statistically significant results.

Permission to use data was obtained from the authors of the primary manuscript. All patients in the cohort were treated on pediatric protocols for high-risk acute lymphoblastic leukemia. Patients ranged from age 1.0 to 19.8 years. Adverse events are grade ≥3 unless otherwise specified.

ARDS, acute respiratory distress syndrome.

*Percentages represent column percentages.

†Clinically significant grade 2 to 5 adverse event.

typically more supervision in pediatric settings by pediatric oncology care teams and parents to ensure patients are compliant with medications and appointments, as well as more psychosocial support, such as child life services. Further, adult oncologists in both community and academic centers may be less familiar with pediatric-inspired ALL protocols and may favor the use of adult protocols when selecting treatment regimens.[1,22]

Susceptibility to toxicities

Even when AYAs are treated with pediatric-inspired protocols, there are differences in successful receipt of protocol-directed therapy. The rapid physical growth and hormonal changes of puberty alter drug metabolism, which may render AYAs more sensitive than pediatric patients to pediatric regimens.[7,15] Pediatric protocols employ higher doses of glucocorticoids and asparaginase than adult protocols.[2,8] While these drugs are not as myelosuppressive as the agents used in adult ALL protocols, they can still cause toxicities. Asparaginase is of specific concern. Alacacioglu et al.[23] showed that adult patients with ALL aged 18 to 50 years treated on pediatric asparaginase-containing Berlin-Frankfurt-Munster regimens had similar rates of complete remission but higher 5-year OS and relapse-free survival as compared to patients aged 18 to 59 years on non-asparaginase-containing combination chemotherapy regimens with cyclophosphamide, vincristine sulfate, doxorubicin hydrochloride, and dexamethasone (hyper-CVAD). While crucial to therapy, there is age-dependent susceptibility to asparaginase toxicity. AYAs incur more frequent and higher grades of asparaginase-related toxicities, specifically hepatotoxicity, pancreatitis, and venous thromboembolism.[21,24] Furthermore, when rates of adverse events during induction were compared between children and adolescents treated on pediatric protocols for high-risk ALL (Table 3), AYAs had higher rates of multiple toxicities, including hyperglycemia, hepatotoxicity, and thromboembolism.[25] While this single-institution study did not demonstrate higher rates for all toxicities, the cohort included only induction and patients aged 1.0 to 19.8 years, thus not capturing the full range of AYA ages. Advani et al[26] also compared toxicities during induction for AYAs treated on CALGB 10403 and COG study AALL0232 and found a direct association between toxicities and increasing age. More grade 3 to 4 toxicities were experienced by those on the CALGB 10403 protocol, which had an older overall age than the AALL0232 cohort. Studies evaluating all courses have demonstrated similar results, including describing higher rates of avascular necrosis (AVN) in

AYAs.[8] Our AYA patient developed bilateral AVN as a late effect of therapy and also had acute asparaginase-related pancreatitis. While use of adult protocols might mitigate these toxicity risks, this may result in undertreatment and poorer outcomes, as adult protocols often incorporate dose reductions that account for comorbidities found in older patients that AYAs may not have.[7,9,15]

Psychosocial challenges

The psychosocial changes of adolescence and young adulthood can be similarly challenging and lead to barriers to treatment adherence and clinic attendance in AYAs compared to children. The autonomy of the AYA period can be threatened by a cancer diagnosis; some AYAs need to rely more on their parents/guardians during a developmental stage that typically includes assertion of independence. Other AYAs may strive to remain autonomous by rebelling against treatment recommendations or inadvertently missing medication doses due to difficulty with properly self-managing complicated regimens. Bhatia and colleagues[27] demonstrated lower adherence to oral 6-mercaptopurine chemotherapy in patients aged 12 and older. Furthermore, AYAs may be concerned about treatment negatively affecting their fertility, which may also lead to declining treatment or treatment nonadherence.[28] These scenarios can all increase the risk of relapse.[29] AYAs with cancer are also faced with significant financial burdens as they balance attendance at appointments with pressures of maintaining employment. These financial pressures are exacerbated by AYAs transitioning onto their own insurance plans and by potential expenses of fertility preservation.[30] Our AYA patient faced several of these challenges while our pediatric patient benefited from significant psychosocial support and optimal medication adherence, with his parents' help.

Importantly, some these challenges disproportionately affect certain racial/ethnic minorities. For example, the poor-prognosis CRLF2 mutation has higher a prevalence in AYAs and Hispanic patients.[2] Wolfson et al[11] found that in 1870 patients with ALL and acute myeloid leukemia, AYAs aged ≥22 years who had either public or no insurance (odds ratio, 0.1; *P*=.004) or were African American or Hispanic (odds ratio, 0.3; *P*=.03) were less likely to receive treatment at a pediatric or academic adult site. This may exacerbate underlying health disparities.

Barriers to enrollment

There has been a historical paucity of available and accessible trials for AYAs, and despite attempts at addressing these barriers, availability remains an ongoing challenge.[5,15,31] This is concerning because in addition to providing data for future patients, some trials demonstrate improved outcomes for enrolled patients.[32-35] In 2006, only 14% of AYAs were enrolled on a trial, while 20% to 38% of pediatric patients were enrolled on a trial. These discrepancies have persisted over time.[5] Jacob and Shaw[31] aimed to determine if AYA enrollment at a large children's hospital would improve after inception of a formalized AYA program in 2006. From 2001 to 2006, pediatric and AYA enrollment rates were 38% and 27%, respectively. Between 2010 and 2014, rates of pediatric and AYA trial enrollment remained significantly different (34% and 24%, *P*=.0017), primarily due to a lack of open trials for AYAs. Unfortunately, AYAs may be too old for pediatric trials and too young for older adult trials. Furthermore, even when a trial exists, AYAs are often not eligible. AYAs may not have had adequate, required pretrial studies if they were referred from, and began treatment at, community-based cancer centers that do not participate in the trials.[31] Further, misdiagnosis, such as steroid pretreatment for presumed asthma rather than the mediastinal mass, and delays to diagnosis can affect trial eligibility.[31] AYAs may provide vague descriptions of symptoms that challenge timely diagnosis. Additionally, AYAs may be aging out of their insurance plans or between insurance providers, which can cause delays to care, subsequent clinical decline, and associated low performance scores, rendering these AYAs ineligible for trial enrollment.[5,30,31] Finally, even when an open age-matched trial exists, there are unique reasons why AYAs may not opt to enroll, including desire to choose a treatment rather than be randomized, competing activities such as school or work, and disinterest in research.[36]

Conclusion

AYAs with ALL are a unique subpopulation of patients with ALL and AYAs with cancer, as they can be treated at either pediatric or adult centers. Most AYAs benefit from treatment at a pediatric center or at least on pediatric-inspired protocols. The contrasting clinical cases illustrate the challenges AYAs must navigate. AYA patients have an increased likelihood of high-risk clinical factors that portend worse outcomes and increased toxicities, encounter psychosocial challenges that threaten therapy adherence, and face barriers to enrollment on trials. The disparate outcomes between children and AYAs with ALL have garnered significant attention, and efforts to address these are under way. Areas of focus include creation of AYA-specific biorepositories to facilitate improved AYA-specific research, advocating for use of pediatric-inspired protocols at adult centers, and education to empower providers to consider referral to pediatric centers to optimize survival and health outcomes for these patients.

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Off-label drug use

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Acute lymphoblastic leukemia in young adults: which treatment?

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Despite improvements in survival among pediatric patients with acute lymphoblastic leukemia (ALL), survival outcomes for adolescents and young adults (AYAs) with ALL have lagged. The reasons for the inferior outcomes among AYAs are multifactorial, each presenting unique challenges and requiring novel solutions. First, adverse disease biology is more common among AYAs with ALL. Ongoing trials are investigating novel approaches to treatment, such as incorporating JAK inhibitors for Philadelphia chromosome–like ALL, menin inhibitors for *KMT2A*-rearranged ALL, and BCL2/BCLXL inhibition for T-cell ALL. Poorer adherence to therapy also impedes improvements in survival outcomes for AYAs with ALL, but early data suggest that technology, both for monitoring and interventions, may be useful in increasing adherence among this population. Finally, better access to clinical trials and collaboration between pediatric and adult centers is critical in advancing the care of AYAs with ALL. Significant improvements have been made over the past decade, but recognizing, understanding, and addressing each of these unique challenges provides hope that the outcomes for AYAs will continue to improve even further.

LEARNING OBJECTIVES

- Identify challenges to improving outcomes in AYAs with ALL
- Describe novel strategies to overcome adverse disease biology in AYAs with ALL
- Evaluate measures and interventions to improve adherence among AYAs with ALL
- Compare health care delivery models between pediatric and AYA patients with ALL
- Identify challenges and potential solutions to clinical trial enrollment among AYAs with ALL

CLINICAL CASE

A 26-year-old woman presents with 4 weeks of fatigue. Peripheral blood laboratory analysis shows a white blood cell count of 6.5 K/uL, with 57% blasts, along with anemia (hemoglobin level, 5.8 g/dL) and thrombocytopenia (platelet count, 39 K/uL). A bone marrow biopsy is consistent with B-cell acute lymphoblastic leukemia (B-ALL). Karyotype is normal, but fluorescence in situ hybridization reveals a *CRLF2* rearrangement, consistent with Philadelphia chromosome–like (Ph-like) ALL. She lives with her parents approximately 1 hour away from the hospital and is currently working part-time as a piano teacher, without health insurance. A clinical trial for young adults with ALL is discussed, but she and her family are concerned about potential side effects and how they will manage the frequency of hospital visits.

Introduction

Over the past decade, significant gains have been made in the survival outcomes of adolescent and young adults (AYAs) with ALL. This progress has been due in large part to improved treatment approaches, including the adoption of pediatric-inspired regimens. Unfortunately, despite these improvements, outcomes remain significantly worse compared to pediatric patients, the so-called survival cliff for AYA patients.[1] Unique challenges in the AYA population include increased frequency of adverse disease biology, differences in treatment setting, and psychosocial factors that can impact adherence to care and access to clinical trials. In this review we describe some of these barriers to improved outcomes and highlight potential solutions to overcome them.

Challenges with adverse disease biology

One of the key drivers of poorer outcomes in AYAs with ALL, compared to their pediatric counterparts, is the

Table 1. Select trials in Ph-like ALL

Target	Agent	Clinical trial #	Phase	Age (years)	Disease status	Results
JAK	Ruxolitinib	NCT02723994	2	1–21	Newly diagnosed	Tasian et al[55]
JAK	Ruxolitinib	NCT03571321	1	18–39	Newly diagnosed	N/A
JAK ABL	Ruxolitinib Dasatinib	NCT02420717	1/2	10+	R/R	Jain et al[56]

increasing frequency of adverse disease biology. While favorable-risk subtypes, such as *ETV6/RUNX1* and hyperdiploidy, are more common among younger patients, adverse-risk subtypes, such as Ph-like ALL; *KMT2A*-rearranged (*KMT2Ar*) ALL, also known as mixed-lineage leukemia-rearranged ALL; and early T-cell precursor ALL, are more common in AYAs and adults.[2] However, as our understanding of the mechanisms underlying these adverse-risk subtypes has increased, novel treatment strategies are emerging to improve outcomes for AYAs with ALL.

Ph-like ALL

Ph-like ALL is a high-risk subtype of B-ALL, defined by a gene expression profile similar to Ph+ ALL but lacking the hallmark BCR-ABL1 fusion.[3] The prevalence of Ph-like ALL increases with age, from 10% to 15% of pediatric cases to 20% to 30% of AYA patients,[4-6] and is associated with adverse outcomes, including increased rates of minimal residual disease (MRD) at end induction and higher rates of induction failure and relapse.[4,5,7-9] Although a heterogenous disease, JAK/STAT pathway alterations are observed in the majority of AYAs with Ph-like ALL and have a particularly poor prognosis.[2,6] Ongoing studies, such as the Children's Oncology Group (COG) study ALL1521, are investigating JAK/STAT pathway inhibition with ruxolitinib for AYAs with newly diagnosed Ph-like ALL.[10] The phase 1 portion enrolled 40 patients and showed that the combination of ruxolitinib with intensive multiagent chemotherapy was well tolerated, with the predominant adverse effects being cytopenias and abnormal liver function tests.[10] Another ongoing phase 1 trial for patients aged 18 to 39 years with Ph-like ALL and a JAK-targetable genetic signature is using ruxolitinib during postinduction phases of the C10403 chemotherapy regimen (NCT03571321), with efficacy data pending (Table 1).

While JAK inhibitors offer promise for Ph-like ALL, emerging data suggest there are alternative biologic dependencies capable of mediating resistance. For instance, preclinical data have demonstrated that PI3K, mTOR, and BCR pathway activation, along with BCL6 and MYC upregulation, may confer resistance to ruxolitinib monotherapy.[11-14] In xenograft models, targeting these additional pathways prolongs survival and prevents resistance, but whether these combination approaches are more effective than ruxolitinib alone requires additional clinical studies. There is also promise in using recently approved therapies, such as blinatumomab and chimeric antigen recepter (CAR) T-cell therapy, for Ph-like ALL. A subgroup analysis of the TOWER study of blinatumomab for relapsed/refractory (R/R) B-ALL demonstrated an efficacy of blinatumomab comparable between patients with and without Ph-like ALL.[15] Similarly, outcomes of anti-CD19 CAR T-cell therapy in pediatric and young adult patients did not differ significantly between Ph-like ALL and other subtypes.[16] It remains to be determined whether these therapies sufficiently overcome the adverse prognostic effects of Ph-like ALL in the up-front setting.

KMT2A-rearranged ALL

KMT2Ar ALL is another high-risk subtype, characterized by rearrangements involving the long arm of chromosome 11 band q23.3 (11q23.3) and numerous fusion partners, with the most frequent being *AFF1* on chromosome 4q21.[17] *KMT2Ar* ALL follows a bimodal distribution, with a high incidence in infants, which declines during childhood and increases again in the AYA and adult population and is associated with increased chemotherapy resistance and higher relapse rates.[2,18] Unfortunately, unlike Ph-like ALL, novel agents such as blinatumomab, CAR T cells, and inotuzumab ozogamicin appear to be less effective in *KMT2Ar* ALL. For CD19-directed therapies, this is partially due to the propensity for lineage switching (transformation into mixed phenotype or acute myeloid leukemia) following CD19 CAR T-cell therapy and blinatumomab.[19,20] *KMT2Ar* ALL also has decreased CD22 expression and antigen density, resulting in lower response rates to CD22-directed therapy such as InO.[21] As such, novel therapies are needed for *KMT2Ar* ALL (Table 2).

In *KMT2Ar* ALL, *KMT2A* and its cofactor menin bind to *HOX* gene promoters, leading to overexpression of HOX genes, and block in hematopoietic differentiation and leukemic transformation. As such, small-molecule inhibitors disrupting the

Table 2. Select trials in *KMT2Ar* ALL

Target	Agent	Clinical trial #	Phase	Age	Disease status	Results
Menin	SNDX-5613 (revumenib)	NCT04065399	1/2	>30 d	R/R	Issa et al[22]
Menin	SNDX-5613 (revumenib)	NCT05326516	1	>30 d	R/R	N/A
Menin	BMF-219	NCT05153330	1	18+ y	R/R	Ravandi et al[57]
Menin	DSP-5336	NCT04988555	1/2	18+ y	R/R	Daver et al[58]
Menin	JNJ-75276617	NCT05521087	1/1b	30 d–30 y	R/R	N/A

KMT2A-menin interaction leukemias are currently being studied. In the phase 1 AUGMENT-101 trial of the menin inhibitor revumenib (SNDX-5613), 4 of 11 patients with R/R *KMT2A*r ALL achieved morphologic remissions.[22] Overall the treatment was well tolerated, with asymptomatic QT interval prolongation as the only dose-limiting toxicity, and the phase 1/2 study is ongoing (NCT04065399). However, the duration of remission (DOR) in this study was only 9.1 months,[22] and recent reports have demonstrated that menin inhibitor monotherapy may induce mutations in the *MEN1* gene that prevent drug binding and result in resistance.[23] Thus, combination approaches are likely needed. Interestingly, *KMT2A*r ALL exhibits elevated expression of antiapoptotic protein BCL2, and in preclinical studies, treatment with the BCL2 inhibitor venetoclax decreased leukemic growth.[24] Whether BCL2 inhibition is synergistic with menin inhibition and will prolong remission in *KMT2A*r ALL must be investigated in future trials.

T-cell ALL

T-cell ALL (T-ALL) accounts for only 10% to 15% of pediatric cases but represents up to 25% of diagnoses of ALL in adults, predominantly affecting young adults.[25] Previous studies have demonstrated similar remission rates and overall survival between B-ALL and T-ALL, but patients with R/R T-ALL have poor outcomes, with limited treatment options.[4] As such, there is a critical need for improvements in up-front therapy to prevent relapse and novel therapies for R/R T-ALL (Table 3).

One strategy is to incorporate nelarabine, a soluble prodrug of ara-G (9-β-D-arabinofuranosyl-guanine) that is currently approved for treatment of R/R T-ALL, into up-front treatment.[26] In a phase 3 COG trial (AALL0434) of 1596 pediatric patients with newly diagnosed T-ALL, the addition of nelarabine to a pediatric chemotherapy backbone significantly improved 5-year disease-free survival (88.2% vs 82.1%; $P = .029$), mainly related to a decrease in rates of central nervous system relapse (1.3% vs 6.9%; $P = .0001$).[27] Although the UKALL14 trial in adults did not demonstrate similar benefits,[28] ongoing studies (NCT02619630, NCT02881086) will help determine whether nelarabine should be incorporated into treatment of AYAs with newly diagnosed T-ALL.

Efforts to eradicate MRD are also being utilized in T-ALL to mitigate the increased risk of relapse.[25] Preclinical studies have demonstrated that malignant T lymphoblasts express high levels of CD38 and that targeting CD38 with monoclonal antibodies, such as isatuximab and daratumumab, reduced tumor burden and improved MRD-negativity in patient-derived xenograft models.[29,30] Clinical trials of daratumumab for MRD-positive T-ALL are underway, including EA9213, a single-arm phase 2 study administering 4 doses of weekly daratumumab to patients in complete remission/complete remission with incomplete count recovery (CR/CRi) with detectable residual disease (MRD $\geq 10^{-4}$) (NCT05289687). It is hoped that eradicating MRD early in the treatment of AYAs with T-ALL will result in decreased relapse rates and improved survival.

Another strategy exploits the dependence of T-ALL on antiapoptic pathways.[31] In a phase 2 trial of 53 pediatric and adult patients with R/R ALL, dual BCL2/BCLXL inhibition with venetoclax and navitoclax demonstrated an overall response rate of 59.6%, although the DOR was only 10 months.[32] Ongoing trials are investigating novel BCL2/BCLXL inhibitors, with the hope of improving response rates and increasing the DOR (NCT04771572). Future trials will investigate the combination of nelarabine and dual BCL2/BCLXL inhibition with venetoclax and navitoclax for AYAs with newly diagnosed T-ALL. Interestingly, recent work has demonstrated that tyrosine kinase inhibitors, such as dasatinib and ponatinib, may have activity in T-ALL through inhibition of lymphocytic-specific kinase and that this may be synergistic with BCL2/BCXL inhibition.[33] Studies of these combinations are ongoing (NCT05268003). Numerous ongoing trials are also investigating the use of CAR T-cell therapy for R/R T-ALL, and the results appear promising, although more long-term data are needed.[34,35]

CLINICAL CASE (continued)

Concerned about the visits and travel involved, the patient chooses not to enroll in the clinical trial and to receive her care closer to home at a community-based center. She undergoes induction chemotherapy per C10403, a pediatric-based regimen, and achieves a complete remission at the end of induction therapy, although MRD is not measured.

She continues on treatment per C10403 but has to frequently reschedule appointments due to a lack of transportation, resulting in delays in treatment. She often forgets to take her oral chemotherapy. Eventually, tired of feeling that the treatment and ongoing clinic visits are interfering with her life, she stops coming to her appointments and is lost to follow-up.

Table 3. Select trials in T-cell ALL

Target	Agent	Clinical trial #	Phase	Age (years)	Disease status	Results
N/A	Nelarabine	NCT02619630	2	18–59	Newly diagnosed	
N/A	Nelarabine	NCT02881086	3	18–55	Newly diagnosed	Goekbuget, et al[59]
CD38	daratumumab	NCT05289687	2	18+	MRD+ CR/CRi	N/A
CD3-CD38	XmAb18968	NCT05038644	1	18+	R/R	Murthy et al[60]
BCL2/BCLXL	LP-118	NCT04771572	1	13+	R/R	N/A
BCL2 + LCK	Venetoclax-ponatinib	NCT05268003	2	18+	R/R	N/A
CD7	WU-CART-007	NCT04984356	1/2	12+	R/R	Ghobadi et al[34]

LCK, lymphocytic-specific kinase.

Psychosocial challenges

Often diagnosed at a critical juncture between childhood and adulthood, AYAs with ALL face complex and unique psychosocial challenges. These issues are numerous and varied but include financial stressors such as a lack of health insurance and cost of health care, difficulty obtaining or maintaining employment and higher education, and fertility concerns, as well as long-term toxicities and survivorship. AYAs with ALL experience substantial psychological morbidities, which often go unrecognized by the providers.[36] Research is ongoing to identify how to best address these complex challenges, but a multidisciplinary approach is likely required.

Adherence to therapy

Among AYAs with ALL, poor adherence to the prolonged oral maintenance therapy is common and associated with an increased risk of relapse.[37-41] Tracking medication adherence can be challenging, with subjective methods of adherence monitoring, including self-report and provider surveys, tending to overestimate adherence.[42] Medication Event Monitoring Systems cap technology, which tracks the date and time of pill bottle opening, has been increasingly used as an objective measure of adherence. Adherence is calculated as the ratio of the number of days with Medication Event Monitoring Systems cap opening to the prescribed days, with nonadherence typically defined as less than 90% or less than 95%.[42] In several previous trials, this measure appeared to correlate well with pharmacologic drug-level monitoring and is currently being evaluated as a measure of adherence in AYAs with ALL (NCT03150693).[39,40]

Recently, several studies have leveraged technology to improve adherence.[43] A pilot study of 18 AYAs with leukemia assessing adherence to oral chemotherapy demonstrated that using a daily text message survey to monitor adherence was feasible, although there was no significant correlation between the text message survey and electronic pill bottle adherence.[44] In a study of 444 children and young adults with ALL, patients aged 12 and above who received personalized text message reminders to prompt directly supervised therapy had significantly higher adherence rates than those who received education alone (93.1% vs 90.0%; $P = .04$).[45] While these approaches hold promise, other studies have demonstrated significant individual variability among barriers to adherence, suggesting that a personalized approach to improve adherence may be needed,[46] in addition to ongoing study of novel adherence measures and interventions for AYAs.

Clinical trial enrollment

Another driving factor of poor outcomes among AYAs with ALL may be the "accrual cliff" in clinical trial enrollment. AYAs have superior survival outcomes when enrolled on clinical trials,[47-49] yet there is a marked decrease in the proportion of AYA patients enrolled onto clinical trials above the age of 15 years.[50] In a survey administered to providers by the COG, barriers to clinical trial enrollment are multifactorial, identified as logistical issues (45%), disparate enrollment practices (42%), lack of communication between pediatric and medical oncologists (27%), and limited trial availability (27%).[51]

Despite these challenges, there have been efforts to expand access to clinical trials through the National Cancer Institute Community Oncology Research Program (NCORP), with emphasis on clinical trials specifically for the AYA population. In a recent retrospective analysis of over 3000 AYAs with cancer, clinical trial enrollment increased significantly, from 14.8% to 17.9% between 2006 to 2012 and 2013, with the most significant improvements seen in patients aged 25 to 29 years, and notably doubled among uninsured patients, increasing from 5.7% to 12.9%.[52] Unfortunately, clinical trial enrollment remained low for older patients and those treated by adult oncologists, suggesting that further interventions are needed.

Leveraging communication technology may be another strategy to improve clinical trial enrollment for AYAs. A consensus statement recently released by the COG recommends the use of remote patient eligibility screening, electronic informed consent, virtual tumor boards, remote study visits, and remote patient monitoring to increase AYA access to trials and decrease the burden of participation.[53] Challenges to this approach include reimbursement, communication between electronic health record systems, and safeguards to maintain patient privacy and protect this vulnerable patient population. Future studies are needed to investigate whether these strategies are feasible and improve enrollment among AYAs.

Treatment setting

Several retrospective studies have demonstrated improved outcomes among AYAs with ALL treated at specialized, experienced centers. In a retrospective analysis of 1380 pediatric and AYA patients with ALL in the Los Angeles County Cancer Registry, patients treated at a National Cancer Institute–designated cancer center or COG center had significantly higher overall (HR, 0.80; 95% CI, 0.66-0.96) and leukemia-specific (HR, 0.80; 95% CI, 0.65-0.97) survival.[54] A separate retrospective analysis of 1473 AYAs with ALL treated in California between 2004 and 2014 demonstrated that over two-thirds received care at an adult institution, with the majority (60%) at a community-based center,[54] and that patients treated at a pediatric institution had significantly improved overall (HR, 0.53; 95% CI, 0.37-0.76) and leukemia-specific (HR, 0.51; 95% CI, 0.35-0.74) survival compared to those treated at adult institutions. Treatment preferences between institutions clearly contribute to these differences in outcome, with patients treated at community-based adult centers more likely to receive an adult-based regimen, but other facility and provider-related factors likely play a role.[54] It has been speculated that these factors may include differences in utilization of MRD testing, less experience with complex pediatric-based regimens, or decreased psychosocial support, but to date limited data exist. Ongoing studies aim to identify specific facility and provider-related factors contributing to poorer outcomes and to propose solutions in hopes of improving the outcomes for AYAs with ALL treated in a wide range of settings (NCT03204916). Nonetheless, these studies highlight the importance of treatment of AYAs with ALL at experienced, specialized centers with multidisciplinary psychosocial support.

Conclusions

Significant progress has been made in the treatment of AYAs with ALL, but many challenges remain. These challenges are multifaceted, including differences in disease biology, psychosocial issues, variation in treatment approaches, and disparities in enrollment on clinical trials. Collaboration between pediatric and adult groups is critical to improving outcomes of AYAs with ALL.

Conflict-of-interest disclosure

Annabelle Anandappa: no competing financial interests to declare.

Emily Curran: advisory board member: Kite, Amgen, Incyte, Pfizer, Servier, Jazz Pharmaceuticals.

Off-label drug use

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The dos, don'ts, and nuances of thrombophilia testing

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Considerable progress has been made in elucidating genetic and biologic risk factors for venous thromboembolism (VTE). Despite being able to identify heritable defects in a substantial proportion of patients with VTE, testing has not, in general, proven useful in management. Despite efforts to reduce inappropriate testing, it often falls to the hematologist to consult on patients having undergone thrombophilia testing. Through a series of cases, we discuss how D-dimer testing can be helpful in VTE recurrence risk stratification in younger women as well as how to approach patients with persistently elevated D-dimer levels in the absence of thrombosis. While elevated factor VIII coagulant activity levels are a significant risk factor for a first episode of VTE, its biologic basis is not fully understood, and studies have not shown it to be a useful predictor of recurrence. Abnormal results of genetic tests for methylene tetrahydrofolate reductase or plasminogen activator 1 promoter polymorphisms may be encountered, which carry little if any thrombotic risk and should never be ordered. We also discuss protein S deficiency, the most difficult of the hereditary thrombophilias to diagnose due to a wider "normal" range in the general population as compared with protein C, the presence of both free and bound forms in plasma, and the characteristics of the various assays in use. We also present a rare type of protein C deficiency that can be missed by functional assays using an amidolytic rather than a clotting end point.

LEARNING OBJECTIVES

- Identify patients with venous thromboembolism in whom D-dimer can be useful for VTE risk stratification
- Understand why an elevated FVIII:C level and testing for polymorphisms in PAI-1 and MTHFR lack clinical utility in the evaluation of VTE
- Know the criteria for diagnosing clinically relevant protein S deficiency and a rare subtype of protein C deficiency

Introduction

Thrombosis is a multicausal disease that is associated with acquired and inherited risk factors. Abnormalities have been identified in 5 genes that increase the risk of venous thromboembolism (VTE), but there is limited clinical utility in testing for the defects. Testing is also done for FVIII coagulant activity (FVIII:C) as well as variants in other genes for which the thrombotic risk is uncertain. The utility of measuring D-dimer has been evaluated for predicting recurrence risk in unprovoked VTE. Through a series of cases, we discuss the utility or lack thereof of measuring D-dimer and FVIII:C and testing for polymorphisms in the plasminogen activator 1 (*SERPINE1*) and methylene tetrahydrofolate reductase (*MTHFR*) genes. Issues in diagnosing deficiencies of protein C and protein S are also addressed.

Role of D-dimer testing for VTE risk stratification

CLINICAL CASE

A 22-year-old woman presented with chest pain and shortness of breath and was diagnosed with bilateral pulmonary emboli (PE) by computed tomography (CT) pulmonary angiography. She was started on apixaban and her symptoms resolved over several weeks. An etonogestrel implant, a progestin contraceptive that had been in place for 3 years, was removed per recommendation in the Food and Drug Administration (FDA) label. She then developed iron deficiency anemia due to menorrhagia. There was no family history of VTE; screening tests for

Table 1. Screening laboratory testing for hereditary thrombophilia in patients with venous thromboembolism

Hereditary thrombophilia
Factor V Leiden mutation (using PCR-based assay)
Prothrombin G20210 mutation (using PCR-based assay)
Protein C deficiency (functional amidolytic assay)
Free protein S antigen
Antithrombin deficiency (heparin cofactor assay, save for patients on oral factor Xa inhibitors in whom antithrombin antigen is recommended to prevent "false normal" levels)

PCR, polymerase chain reaction.

thrombophilia were normal (Table 1). She completed 6 months of rivaroxaban and was referred regarding the need for long-term anticoagulation.

This case illustrates a common question as to whether to extend anticoagulation beyond 3 to 6 months. The first consideration is whether etonogestrel constitutes a transient risk factor for VTE. Despite the information on the Food and Drug Administration label, studies have not shown an increased risk in users of progestin-only contraceptives. A population-based study showed that the use of oral low-dose progesterone, levonorgestrel intrauterine devices, and levonorgestrel implants was not associated with an increased risk of VTE.[1]

This patient therefore had unprovoked VTE, for which the risk of recurrent VTE after discontinuation of anticoagulation is 10% in the first year, 25% at 5 years, and 36% at 10 years.[2] Men have a higher rate of recurrent VTE compared with women.[2] While long-term anticoagulation is now recommended for many such patients, individualized decision-making is appropriate, taking into account its benefits and risks as well as patient preference.

D-dimer testing could be a useful risk stratification tool in this young woman (Table 2). Based on the PROLONG study, patients with a first unprovoked VTE who completed 3 months of anticoagulation had a VTE recurrence rate of 4.4% per year if a D-dimer test was negative 1 month after stopping warfarin.[3] Women younger than 65 with non-hormone related VTE had a lower recurrence risk of 1.1%.[4] In the subsequent DULCIS study, patients with persistently negative D-dimer tests at 15, 30, 60, and 90 days after discontinuation of anticoagulation had a VTE recurrence rate of 3% per year.[5] The D-dimer Optimal Duration Study reported that women with a negative D-dimer while on anticoagulation and 1 month after discontinuation had a recurrence rate in the first year of 5.4%; the rate was 9.7% per year in men.[6] The cumulative risk of recurrence at 5 years was 29.7% in men and 17.0% per year in women with non-estrogen-related VTE.[7] A negative D-dimer result 1 month after discontinuing anticoagulation in this woman therefore translates to a recurrence rate of approximately 1.1% to 5.4% per year. With respect to bleeding, a meta-analysis of 14 randomized trials of indefinite anticoagulation for preventing recurrent VTE found annualized rates of major bleeding of 1.12% for direct oral anticoagulants and 1.74% for warfarin.[8] The predictive value of D-dimer is diminished

Table 2. D-dimer testing for recurrent VTE risk stratification

	Population	N	D-dimer assay	Cutoff values	VTE recurrence rate after stopping anticoagulation (per year)	
					Negative D-dimer	Positive D-dimer
PROLONG[3,4]	First unprovoked VTE	608	Clearview Simplify (whole blood; qualitative)	Positive	**Overall: 4.4% (1st year)** 0.4% (women, age <65) 6.7% (women, age >65) 5.1% (men, age <65) 8.2% (men, age >65)	**Overall: 10.9%** 5.4% (women, age <65) 8.9% (women, age >65) 12.9% (men, age <65) 11.8% (men, age >65)
DULCIS[5]	First unprovoked VTE or VTE with minor risk factors	1010	5 quantitative assays	Predefined age- and sex-specific values	**Overall: 3.0% (median 1.93 years)** 2.1% (age ≤70) 8.9% (age >70) 4.7% (men) 4.8% (women)	**Overall: 8.8%** 14.3% (age ≤70) 12.5% (age >70) 10.0% (men) 20.5% (women)
DODS[6]	First unprovoked VTE	410	Clearview Simplify® (whole blood; qualitative)	Positive	**Overall: 6.6% (1st year)** 9.7% (men) 5.4% (women, non-estrogen) 0% (women, estrogen)	—
DODS extended follow-up[7]	First unprovoked VTE	293	Clearview Simplify® (whole blood; qualitative)	Positive	**Overall: 5.1% (median 5 years)** 7.5% (men) 3.8% (women, non-estrogen) 0.4% (women, estrogen)	—

VTE, venous thromboembolism.

in men and older women (ages >65-70) who have high recurrence rates despite low D-dimer levels (ie, 7.5% per year in men, 6.6% per year in older women).[4,7] Of note, the D-dimer "cutoff" (negative vs positive) varies among assays that have reported the risk of recurrent VTE; this should be considered in interpreting results.[9]

CLINICAL CASE (continued)

D-dimer levels on rivaroxaban and 1 month after discontinuation were 228 ng/mL and 235 ng/mL (reference range, 0–500 ng/mL FEU), respectively. Following discussion of the benefits and risks of long-term anticoagulation, she preferred to discontinue rivaroxaban. She was advised of the need for prompt medical attention for symptoms or signs of VTE and appropriate thromboprophylaxis in high-risk situations.

Persistently elevated D-dimer levels in the absence of thrombosis

CLINICAL CASE

A 28-year-old woman was referred for a persistently elevated D-dimer. A level of 604 ng/mL was noted on presentation to the emergency department with chest pain; evaluation including CT pulmonary angiography and ultrasound of both lower extremities was negative for VTE. Despite being in good general health, follow-up testing showed persistently elevated D-dimer levels (>2,000 ng/mL).

D-dimer is a product of fibrin formation and degradation. In cases of suspected VTE, clinical assessment using validated tools (ie, Wells criteria) along with a negative D-dimer assay can exclude a diagnosis of VTE. When D-dimer is elevated and imaging is negative for VTE, it is unclear whether further evaluation should be done. Aside from VTE, elevated D-dimer levels are observed in a variety of physiologic and pathologic conditions, such as increased age, hormonal use, pregnancy, infections including COVID-19, cancer, inflammatory diseases, atrial fibrillation, acute aortic dissection, and disseminated intravascular coagulation.[10,11] Clinical evaluation, including a detailed medical history, physical examination, routine laboratory tests (ie, complete blood count, basic metabolic profile, liver function tests), and age-appropriate cancer screening, are reasonable to identify such conditions.

D-dimer levels vary among healthy individuals, and a small percentage have very high levels.[11] Studies of healthy populations have shown that elevated D-dimer levels are associated with an increased risk of VTE and overall mortality.[12,13] These effects, however, are small and should not prompt extensive laboratory testing (eg, testing for thrombophilia) and imaging to exclude occult disorders that are not suspected clinically. It should be noted that interference with D-dimer assays can lead to spurious elevations (eg, presence of heterophilic antibodies).[8]

CLINICAL CASE (continued)

Evaluation did not reveal any causes of a persistently elevated D-dimer level, and no further diagnostic testing was deemed necessary. The patient was reassured.

Testing for elevated FVIII:C

CLINICAL CASE

A 53-year-old woman was diagnosed with PE 4 weeks after sustaining a left fibula fracture while on an estrogen-containing oral contraceptive. She remained active following the fracture. Her mother had a history of recurrent VTE following surgeries after age 60. She was initiated on apixaban and the oral contraceptive was discontinued. She was seen by her primary care physician 2 weeks later, at which time she was asymptomatic. Laboratory testing for hereditary thrombophilia and APS was negative; FVIII:C was elevated at 234% (reference range, 57%-163%).

This hematology consultation resulted from an elevated FVIII:C found as part of a thrombophilia evaluation. Despite the Choosing Wisely campaign's recommendation against testing for thrombophilia in patients with VTE in the presence of transient risk factors, such testing and referrals are frequent.[14,15]

An elevated FVIII:C, defined as the top decile of the population, has been identified in 25% of patients with a first unprovoked venous thrombotic event.[16,17] Several factors affect FVIII:C, including von Willebrand factor level and ABO blood group. Individuals with non-O blood groups have von Willebrand factor and FVIII:C levels that are approximately 25% higher than type O.[18] Genetics also plays a role in an individual's FVIII:C, and studies have identified familial clustering of high levels.[19-21] However, the genetic basis and inheritance patterns have not been determined. Acute thrombosis can result in transient elevations in FVIII:C for up to 3 to 6 months. In some cases of venous thrombosis, the concomitant presence of an underlying inflammatory disorder is present in association with elevated C-reactive protein (CRP) and fibrinogen levels along with FVIII:C.[17,22] Other factors such as body mass index, age, glucose, and triglyceride levels have been associated with elevations.[19]

In the Leiden Thrombophilia Study, patients with FVIII:C >150 IU/dL had an increased risk of venous thrombosis compared with those with FVIII:C <100 IU/dL (OR 4.8, 95% CI 2.3-10.0).[16] Levels were obtained at a median interval of 18 months following the diagnosis of deep venous thrombosis (DVT) and at least 3 months after anticoagulation was discontinued. The risk of VTE increased with higher FVIII:C in a dose-dependent fashion. This finding was confirmed by several subsequent studies.[17,23,24] However, it is unclear whether an elevated FVIII:C has an impact on the risk of recurrent VTE. In a recent systematic review, 9 of 16 studies failed to identify FVIII:C as an independent risk factor for recurrence.[25] Thus, it is not our practice to obtain FVIII:C in patients who undergo testing for thrombophilia.

When FVIII:C is obtained as part of thrombophilia testing, there are additional challenges with respect to its interpretation. FVIII:C is generally measured using a one-stage clot-based assay; elevated FVIII:C measured by chromogenic assay and ELISA have also been associated with thrombosis.[26,27] There is potential for interference in one-stage FVIII:C assays by heparins and lupus anticoagulants.[28] Elevated FVIII:C is often defined as above the 90th percentile of the population.[25] However, there is uncertainty regarding what cutoff values should be considered "elevated," since cutoffs were >150% in the Leiden Study and >234% in the Vienna study.[16,29] Standardization of such values with the applicable population is needed.[16,29]

CLINICAL CASE (continued)

Based on the provoked nature of the PE, this patient was continued on apixaban for 3 months. In this case, FVIII:C was found to be elevated 2 weeks after she was diagnosed with PE. Even if persistently elevated, its role in predicting recurrent VTE is uncertain. Thus the elevated FVIII:C should not affect the duration of anticoagulation.

Don'ts: testing for 4G/5G variants in the promoter of the gene encoding *PAI-1* (*SERPINE1*) and other polymorphisms

CLINICAL CASE

A 42-year-old woman sustained ischemic strokes at ages 17 and 41. CT and magnetic resonance imaging showed old infarcts, but there was no evidence of vascular disease. Echocardiography (both transthoracic and transesophageal) did not show a patent foramen ovale, and cardiac telemetry was negative. She had no cardiovascular risk factors but developed PE following delivery at age 28 that was treated with 6 months of warfarin. There was no family history of thrombosis, and she did not have hereditary thrombophilia or markers of APS. Analysis of the *PAI-1* gene showed heterozygosity for 4G/5G in the promoter. Her current therapy includes aspirin and a statin. Hematology is consulted regarding the significance of the 4G/5G variant and the role of anticoagulation for preventing recurrent stroke.

This case highlights one of several tests that are ordered by some practitioners to evaluate patients with arterial and/or venous thrombosis as well as women with recurrent adverse fetal outcomes: these include *SERPINE1* 4G/5G, *MTHFR* polymorphisms, homocysteine levels, and a common *F13A* gene polymorphism resulting in Factor XIII Val34Leu. However, the association of these laboratory abnormalities with an increased or decreased risk of thrombosis is minimal or relatively small.

The 4G/5G polymorphism in the *SERPINE1* is associated with an increased plasma level of PAI-1.[30] As compared with the 5G allele, carriers of the 4G allele had marginally increased risks of VTE and myocardial infarction (odds ratio, ~1.2).[31-33] However, there is no evidence of an increased risk of stroke.[31,34] Some studies have shown a reduced risk in individuals carrying the 4G/4G genotype.[35] The *MTHFR* polymorphisms, C677T and A1298C, are common, with heterozygosity and homozygosity in 40% to 60% and 16% of the population, respectively.[36,37] These polymorphisms decrease function of the MTHFR enzyme, leading to elevated homocysteine levels; this effect is only relevant in folate-depleted states, which are rare in countries where folate-fortified food is mandated.[38] Randomized trials have shown that lowering homocysteine levels with vitamin B6, B12, and folic acid supplementation does not prevent arterial thrombosis, VTE, or mortality.[39-43] *MTHFR* polymorphisms are not associated with an increased risk of VTE or arterial thrombosis; testing for them or measuring homocysteine levels should not be done to evaluate for thrombophilia.[37,44]

CLINICAL CASE (continued)

The patient's history of PE was provoked as it occurred in the postpartum period; there is no evidence that anticoagulation will prevent recurrent stroke in this patient as a consequence of carrying the *SERPINE1* 4G allele. The situation would be no different if she had 1 of the 5 hereditary thrombophilias (Table 1).

Nuances: Testing for protein C deficiency and protein S deficiency

CLINICAL CASE

A 31-year-old woman is referred with a history of 2 first-trimester miscarriages but no history of thrombosis. Her mother had multiple venous thrombotic events following an initial DVT during pregnancy; her maternal grandmother and great-grandmother also had VTE during pregnancy. The patient's protein C activity and antigen levels were 55% (reference range, 70%-180%) and 111%, respectively. At her consultation with you regarding potential risks during a future pregnancy, protein C activity was 97%.

This patient had discrepant protein C activity levels measured by different laboratories. There are several potential explanations besides laboratory error or preanalytic factors. Protein C activity levels can be measured using either amidolytic (chromogenic) or clot-based assays (Table 3). Generally, amidolytic tests are preferred due to lower variability.[45,46] Clot-based assays for protein C are subject to interference by lupus anticoagulants or anticoagulants. The levels of protein C can also be affected by several conditions (Tables 3 and 4).[47] Another possibility is that the patient may have a rare type 2B variant of protein C deficiency with abnormalities in calcium, phospholipid, or cofactor binding.[48] This type of protein C deficiency is only detected using the clot-based activity assay.

Table 3. Diagnostic considerations in protein C deficiency and protein S deficiency

	Protein C deficiency	Protein S deficiency
Genetic mutation	*PROC* gene	*PROS1* gene
Types of deficiency	Type 1 quantitative defect, low PC antigen and activity Type 2 qualitative defect, normal PC antigen but low activity • Type 2A • Type 2B—not detected by amidolytic assays	Type 1 quantitative defect; low total and free PS antigen, low activity Type 2 qualitative defect; normal total and free PS, low activity Type 3 selective quantitative defect; normal total PS, low free PS antigen, low activity
Activity assays	PC activity: • Clotting end point • Amidolytic end point (uses a chromogenic substrate)	PS activity: • Clotting end point
Antigen assays	PC antigen: immunoassays • Helps distinguish deficiency type, not required for diagnosis	PS antigen: immunoassays Free PS antigen—ELISA, latex immunoassays; initial test for diagnosis Total PS antigen—helps distinguish deficiency type, not required for diagnosis
Diagnostic threshold	PC activity <65%-70%	Free PS antigen <33% (in general population) <40%-50% (with patients with prior VTE or strong family history)
Laboratory interference	Clot-based assays: lupus anticoagulants, heparins, direct thrombin inhibitor, oral factor Xa inhibitors, elevated FVIII:C, FVL mutation, and hyperlipidemia	Clot-based assays: lupus anticoagulants, heparins, direct thrombin inhibitor, oral factor Xa inhibitors, elevated FVIII, FVL mutation, and PC deficiency
Conditions with acquired deficiency	Liver disease, DIC, vitamin K antagonist, vitamin K deficiency, recent surgery or trauma, acute inflammatory illnesses, oral contraceptive pills, or acquired antibodies	Liver disease, DIC, vitamin K antagonist, vitamin K deficiency, nephrotic syndrome, L-asparaginase therapy, oral contraceptive pills, pregnancy, or acquired antibodies

DIC, disseminated intravascular coagulation; PC, protein C; PS, protein S.

Table 4. The effects of oral factor Xa inhibitors and oral thrombin inhibitors on tests for hereditary thrombophilia

Thrombophilia	Tests	Effect on test
Factor V Leiden mutation	PCR	Not affected
Prothrombin G20210A mutation	PCR	Not affected
Protein C deficiency	Protein C activity: clot-based assays	Interference by oral factor Xa inhibitors and dabigatran
	Protein C activity: amidolytic assays	Not affected
	Protein C antigen assays	Not affected
Protein S deficiency	Protein S activity: clot-based assays	Interference by oral factor Xa inhibitors and dabigatran
	Protein S antigen assays	Not affected
Antithrombin deficiency	Antithrombin activity: anti-Xa–based assays	Interference by oral factor Xa inhibitors
	Antithrombin activity: anti-IIa–based assays	Interference by dabigatran
	Antithrombin antigen assays	Not affected

PCR, polymerase chain reaction.

CLINICAL CASE (continued)

The low functional protein C level was done by a commercial laboratory that uses a clotting end point, while the normal level obtained by our laboratory used an amidolytic assay. Repeat testing using an assay with a clotting end point returned low at 62%; the patient is therefore likely to have heterozygous protein C deficiency. Arrangements were made to have sequencing done to determine the mutation in the protein C gene. Regarding the implications of protein C deficiency on recurrent miscarriage, an association has not been established,[49,50] nor is there evidence for a higher live birth rate in women treated with antepartum low-molecular-weight heparin.[51] However, based on her having protein C deficiency and a family history of VTE, postpartum thromboprophylaxis is recommended per the ASH Guidelines.[52] This, however, is an

individualized decision that the patient and her physicians need to make based on her thrombotic and bleeding risk following delivery.

Nuances: diagnosing protein S deficiency

CLINICAL CASE

A 34-year-old G3P0 woman was referred with a history of 2 first-trimester miscarriages but no personal or family history of thrombosis. As part of the evaluation for in vitro fertilization, protein S activity was 57% (reference range, >64%). She was started on enoxaparin 40 mg subcutaneously daily on the day following embryo transfer. Protein S activity at 4 months' gestation was lower at 31%. She is seen regarding the need for continued prophylaxis with low-molecular-weight heparin.

This case highlights the challenge encountered in accurately diagnosing clinically relevant protein S deficiency (ie, associated with an increased risk for VTE). Protein S circulates in the blood in 2 forms, 60% bound to C4b-binding protein and 40% free.[53] Tests for protein S deficiency include activity assays as well as free and total protein S antigen (Table 3). Free protein S antigen is the preferred method due to its lower variability and correlation with risk of VTE.[54] In a case-control study, levels of free protein S had to be <33% (<0.10th percentile) to indicate an increased risk of venous thrombosis. No association was observed with total protein S antigen, even at levels <53% (<0.20th percentile).[54] As with protein C, protein S levels are influenced by many factors that must be considered in interpreting results (Table 3).

CLINICAL CASE (continued)

This patient's mild reduction in protein S activity at baseline has no clinical significance, and the level of 31% was due to being 4 months pregnant. Hence it was recommended that prophylactic anticoagulation be discontinued; the patient and her obstetrician agreed with this plan.

Conflict-of-interest disclosure

Thita Chiasakul has nothing to declare.
Kenneth A. Bauer has served as a consultant to Abbott and Sanofi.

Off-label drug use

Thita Chiasakul: nothing to disclose.
Kenneth A. Bauer: nothing to disclose.

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Provoked vs minimally provoked vs unprovoked VTE: does it matter?

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Venous thromboembolism (VTE) is a multifactorial disease, and its risk depends on exposure to risk factors and predisposing conditions. Based on their strength of association with a VTE episode, risk factors are classified as major or minor and determined using a temporal pattern to be transient or persistent. All patients with VTE should receive anticoagulant treatment for at least 3 months in the absence of an absolute contraindication. Beyond this period, selected patients may be candidates for an extended phase of anticoagulation aimed at secondary VTE prevention. The risk of recurrent VTE if anticoagulation is discontinued is probably the main driver of decision-making regarding extended treatment. The risk of recurrence after VTE associated with major risk factors is low if the risk factor is no longer present. In this case, treatment can be discontinued. If the major risk factor is persistent, anticoagulation should be continued. After VTE occurring in the absence of risk factors, anticoagulation should probably be continued indefinitely if the risk for bleeding is low and preferably with minimal effective doses of anticoagulants. VTE occurring after exposure to minor risk factors is probably the most challenging situation, especially if the clinical manifestation was acute pulmonary embolism. Understanding the actual role of minor risk factors in the occurrence of VTE helps in estimating the risk of recurrence and avoiding the dangers associated with unnecessary anticoagulation. The availability of safer strategies for anticoagulation could allow personalized strategies for secondary prevention of VTE.

LEARNING OBJECTIVES

- Understand the strength of different risk factors for VTE that support the classification in provoked, minimally provoked, or unprovoked VTE
- Detail the risk of recurrence after the discontinuation of anticoagulant treatment for index VTE

CLINICAL CASE

A 33-year-old woman presents to the outpatient clinic. Three months before she had visited the emergency department for left lower limb edema. A complete compression ultrasonography was performed, and a deep vein thrombosis (DVT) had been diagnosed at the popliteal and trifurcation levels. Two weeks earlier, she had experienced a left ankle sprain and was prescribed brace immobilization without surgery. She has now completed 3 months of oral anticoagulant treatment. She has no relevant medical history, and her body mass index is 23; she was on a combined oral contraceptive for birth control at the time of the lower leg injury. Now she is asking for advice on the need to continue anticoagulant treatment. In fact, she would like to have a second baby. She also asks whether she should have further testing to assess a potential predisposition to thrombosis.

Introduction

Venous thromboembolism (VTE) includes DVT and pulmonary embolism (PE) that may occur as separate events or in combination. In the United States, the average annual rate of hospitalization in the adult population due to VTE was 239 per 100 000 during 2007 to 2009.[1] In Europe the incidence of VTE has been recently reported to be 131 per 100 000 person-years.[2] PE, either alone or in combination with DVT, accounts for 30% to 40% of VTE events.[3]

The clinical course of major VTE, which includes proximal DVT and/or PE, is characterized by the risk for recurrence and death in the acute phase and by the risk of recurrence and long-term sequelae such as chronic thromboembolic pulmonary hypertension or postthrombotic syndrome thereafter; these events account for a substantial burden of illness in terms of quality of life.[4,5] The risk of recurrence is reduced by over 90% by appropriate anticoagulation. However, anticoagulant treatment has the counterbalance of increased risk for bleeding events. The

duration of anticoagulant treatment after index VTE should be accurately evaluated by taking into account the risk for recurrence, the risk for bleeding, and the implications in lifestyle and occupational hazards for each individual patient.[6]

For all patients with a diagnosis of acute major VTE, anticoagulant treatment is composed of an initial and a long-term phase (primary treatment) and for selected patients by an extended phase for secondary VTE prevention.[7,8] The identification of candidates for extended anticoagulation should be based on the estimated risk for recurrent VTE once anticoagulant treatment is withdrawn. This risk is strongly related to the features of index VTE.

Epidemiology of index VTE

VTE is a multifactorial disease, and its risk depends on exposure to risk factors and predisposing conditions (Figure 1).[3,9,10] The attributable risk and the strength of association with VTE varies among individual risk factors; in addition, more than 1 risk factor or predisposing condition can coexist in each individual patient.

Based on exposure to risk factors or underlying predisposing conditions, VTE can be classified as associated or not associated with identifiable risk factors. Risk factors can be classified as major or minor based on the strength of association with VTE and as persistent or transient based on duration of exposure (Table 1).[11]

Trauma, surgery, and VTE

All major trauma and surgeries associated with extensive tissue damage, blood stasis due to immobilization, pneumoperitoneum or the use of tourniquets, and, potentially, the release of procoagulant factors are major risk factors for VTE. Landmark studies have reported rates of asymptomatic VTE as high as 50% after major trauma or major orthopedic surgery in the absence of antithrombotic prophylaxis.

The risk of postoperative VTE after major surgery (interventions longer than 30 minutes) varies also by type of surgery (abdominal, orthopedic, neurosurgery, etc), type of anesthesia, patient features (age and male sex), underlying conditions (obesity, active cancer, malnutrition), and occurrence of postoperative complications.[12]

The epidemiology of VTE after trauma is also multifactorial and depends on patient features, type of bone fracture (lower vs upper limbs, long-bone fractures, surgery for bone fractures), need for immobilization, and whether surgery is required. Isolated lower limb trauma requiring immobilization is associated with an 18.0% risk of asymptomatic VTE (95% CI, 12.9-23.1) and a 2.0% risk of symptomatic VTE (95% CI, 1.3-2.7).[13,14] The risk increases with patient-related risk factors, including coexisting medical conditions, age, obesity, previous VTEs, medications, pregnancy or the postpartum state, and procoagulant changes after surgery.

In adults requiring temporary immobilization (eg, a leg cast or brace in an ambulatory setting) for an isolated lower limb injury, the rate of major VTE without pharmacological thromboprophylaxis ranged from 0% to 11.7%, symptomatic VTE from 0% to 2.1%, and PE from 0% to 2.1%.[15] The role of minor injuries as triggers for VTE is debated. In fact, the relationship between these events is difficult to study systematically due to recall bias in retrospective studies and because most of these minor injuries may not require medical care. Overall, minor injuries were reported to be associated with the risk of VTE, particularly if located in the leg and in factor V Leiden carriers.

Medical risk factors for VTE

In nonsurgical settings, immobilization and cancer are probably the risk factors with a stronger association with VTE.[11,16] Immobility, defined as confinement to bed for more than 72 hours or more than 7 days or bedridden or nonambulatory status, was associated with a 3-fold increased risk for VTE (odds ratio [OR], 3.17; 95% CI, 2.18-4.62); a similar risk was shown for paresis (OR, 2.97; 95% CI, 1.20-7.36).[16]

Active cancer is a strong risk factor for VTE, for either out- or inpatients, mainly in those receiving chemotherapy; in fact, up to 15% to 20% of cancer patients experience VTE during the course of their treatment.[17]

Critical illness, defined as requiring an intensive or coronary care unit or a need for resuscitation (7 observational studies; OR, 1.65; 95% CI, 1.39-1.95) and acute infections including cellulitis,

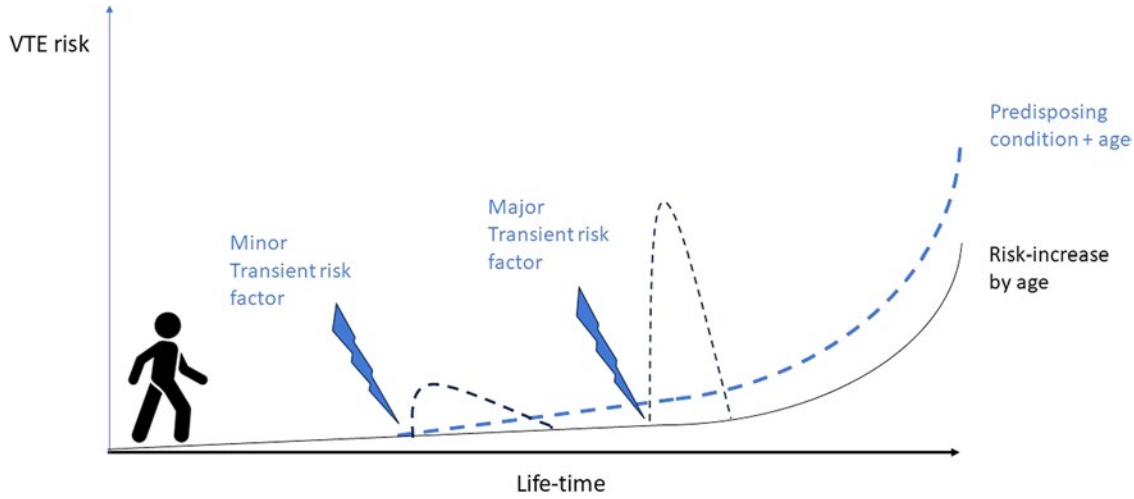

Figure 1. Lifetime course of VTE risk based on presence/absence of predisposing conditions and on exposure to major or minor risk factors.

Table 1. Risk factors and predisposing conditions for VTE

Risk factors	Surgical	Nonsurgical
Major transient risk factors	Orthopedic, general, urologic, or gynecologic surgery (duration >45 minutes)	Immobilization with/without paresis
	Trauma	Bedridden because of acute disease
		Critical illness
Major persistent	—	Cancer
		Neurologic disease with paresis
Minor transient risk factors	Orthopedic, general, urologic, or gynecologic surgery (duration ≤45 minutes)	Limb trauma with/without plaster cast
	Limb trauma requiring minor surgery	Pregnancy or postpartum
		Estrogen use for contraception or hormone therapy
		Long-haul air travel
		Acute infections
Minor persistent	—	Inflammatory bowel disease
		Autoimmune disease
Predisposing conditions	—	Increasing age
		Obesity
		Heart failure
		Prior VTE

pneumonia, and sepsis (OR, 1.48; 95% CI, 1.16-1.89) were associated with an increased risk of VTE.[16] Overall, hospitalization in general medical units is associated with an increased VTE incidence that varies as a function of both underlying medical conditions and immobility.

A number of conditions not related to hospitalization are also associated with an increased risk for VTE, but their association is probably weaker in comparison to those mentioned above. Inflammatory diseases, mainly rheumatoid arthritis, systemic lupus erythematosus, and inflammatory bowel disease, are associated with VTE, with low certainty of the evidence (OR, 2.33; 95% CI, 1.13-4.83).[16] Another intriguing association is that between long-haul air travel—a duration of at least 4 hours—and VTE, the so called economy class syndrome. By studying 8755 employees of international organizations accounting for a total time of exposure to long-haul flights of 6872 patient-years, the incidence rate of symptomatic VTE was shown to be 3.2 per 1000 patient-years, as compared to 1.0 per 1000 patient-years in individuals not exposed to air travel (incidence rate ratio, 3.2; 95% CI, 1.8-5.6).[18]

Elevated body mass index has been identified as a risk factor for VTE in most observational population-based studies.[19] Combined oral contraceptive use increases the risk of VTE by approximately 2-4-fold, but the absolute risk is still lower than 0.1%.[20] Hormone replacement therapy is associated with a slightly increased risk of VTE compared to no hormone exposure (OR, approximately 1.5-2).[21]

Classification of VTE

Based on the epidemiology of the index event, VTE is usually categorized as occurring in the absence of any identifiable risk factor (unprovoked or idiopathic VTE) or in association with major transient or minor transient or persistent risk factors (provoked VTE).[7,8,22] However, classification of VTE is still controversial, mainly due to a paucity of evidence for the risks associated with some specific conditions.[23] In fact, when the strength of association between a risk factor and VTE decreases, as is the case for minor or even minimal risk factors, the sample size required to demonstrate the association dramatically increases, and high-quality studies are scarce. Overall, exposure to a risk factor like major trauma, major surgery, or active cancer may be a sufficient cause for VTE. However, exposure to minor risk factors may only provide a partial explanation for the index VTE (Figure 1). The threshold of attributable VTE risk to consider exposure to a risk factor a sufficient cause of VTE is undefined. According to expert consensus, a major risk factor is associated with a greater than 10-fold increase in the risk of first VTE, while a minor risk factor is associated with a 3- to 10-fold increase.[22]

The classification of VTE may have implications on the risk of recurrence (Table 2). In fact, an inverse correlation exists between the attributable risk of each individual transient risk factor for an index VTE and the risk of recurrence after discontinuation of anticoagulant treatment. A direct correlation exists between the attributable risk of persistent risk factors at index VTE and the risk of recurrence, at least while the risk factor is present. In this view, the definition of the specific attributable VTE risk for each condition could facilitate the prediction of recurrence.

Epidemiology of recurrent VTE

As for index VTE, the risk for recurrent VTE is also multifactorial, based on patient features, epidemiology of the index event, and the exposure to upcoming risk factors or predisposing conditions.[24]

Based on evidence from landmark studies, VTE not associated with identifiable risk factors has a high risk for recurrence in the first 2 years after discontinuation of anticoagulant treatment.[25] The risk declines in the following 3 years, then reaches

Table 2. Current guidelines on VTE: recommendation on secondary prevention

Risk factor at index VTE	ESC 2019[30]	ASH 2020[7]	NICE[31]	CHEST 2021[8]
Unprovoked	**Extended oral anticoagulation of indefinite duration should be considered.**	**Suggests indefinite antithrombotic therapy** over stopping anticoagulation, except for high-risk of bleeding. In certain circumstances . . . clinicians and patients may use prognostic scores, or tests . . . to aid in reaching a final decision.	**Consider continuing anticoagulation**, taking bleeding risk, risk of recurrence, and patient preference into account. In low bleeding risk patient the benefits of continuing anticoagulation treatment are likely to outweigh the risks.	**We recommend offering extended-phase** anticoagulation. Patient preference and predicted risk of recurrent VTE or bleeding should influence the decision.
Transient risk factor	**Major transient** risk factor, **discontinuation of oral anticoagulation is recommended** after 3 months. **Extended oral anticoagulation of indefinite duration should be considered** after a first PE associated with a **minor transient** risk factor.	**Temporary risk factors discontinue anticoagulant therapy** after completion of the primary treatment. **Chronic risk factors**[a] suggests indefinite antithrombotic therapy over stopping anticoagulation.	**Consider stopping anticoagulation treatment** at 3 months following a provoked DVT or PE if the provoking factor is no longer present and the clinical course has been uncomplicated.	**Major transient risk factor**, **we recommend** against offering extended-phase anticoagulation. **Minor transient risk factor**, **we suggest** against offering extended-phase anticoagulation.
Persistent risk factor	**Extended oral anticoagulation of indefinite duration should be considered** for patients with a first episode of PE associated with a persistent risk factor.	**Chronic risk factors may** continue anticoagulant therapy indefinitely for secondary prevention after completion of the primary treatment.		**We recommend offering extended-phase** anticoagulation.

[a]Cancer patients are excluded from this recommendation.

a plateau of about 3% per year and never falls to 0. The risk of recurrence after an initial event of major VTE provoked by a temporary risk factor is expected to be about half that of unprovoked VTE, with no evidence that this effect can be modified by the length of anticoagulant treatment or the type of VTE (DVT vs PE). VTE associated with surgical risk factors seems to have a lower risk for recurrence in comparison with VTE associated with medical risk factors.

Of note, limited data are currently available on the risk for recurrence after exposure to specific risk factors, except for cancer.[17,26-29] Concerning the risk for recurrence after oral contraceptive–associated VTE, a meta-analysis of 19 studies including 1537 women found that the incidence of recurrence after the discontinuation of anticoagulation was 1.22% per person-year (95% CI, 0.92-1.62; I^2, 6%) during 5828 person-years of follow-up.[27] Similarly, the risk for recurrent VTE after an index pregnancy–associated VTE seems to be low and mainly related to subsequent pregnancy.

The recurrence risk in healthy patients with travel-associated VTE in the absence of other risk factors is unknown. There is controversy as to whether travel-associated VTE should be regarded as provoked vs unprovoked (especially since it is quite transient in duration). This controversy exists for other minor risk factors such as short immobilizations or mild trauma and suggests the potential for a further categorization of minor risk factors into minor and minimal. However, while awaiting further evidence on the risk for recurrence, this further categorization would have no or minimal clinical implications.

Making decisions about anticoagulation

Secondary prevention of recurrences should be tailored based on the estimated risk for recurrent VTE after discontinuation of anticoagulation and the estimated risk for bleeding if anticoagulation is continued.[7,8,30,31] The risk of recurrence decreases over time after discontinuation of anticoagulation for the index event, while the risk of bleeding during anticoagulation remains constant over time. Of note, case-fatality rates of recurrent VTE and major bleeding events are expected to be similar during the initial period of VTE treatment.[32] Over time, the case-fatality rate of recurrent VTE declines, while the case-fatality rate of major bleeding remains stable. Case-fatality rates of recurrent VTE have been reported to be higher in patients initially presenting with PE than with DVT.[32]

Direct anticoagulants are associated with a reduced risk of bleeding in comparison with vitamin K antagonists. The risk of major bleeding was estimated to be 1.92% (95% CI, 1.57-2.33) per year with vitamin K antagonists, with about one-fourth of events caused by intracerebral hemorrhage.[33] DOACs are associated with a reduced risk of major and intracerebral bleeding in comparison to vitamin K antagonists, though the risk of nonmajor clinically relevant bleeding is not negligible. The safety of these agents may encourage physicians to reduce the threshold of recurrence risk in patients who are candidates for extended anticoagulation. In fact, time-limited prolongation of anticoagulant treatment beyond 3 months delays recurrences without reducing the absolute risk of recurrence after discontinuation of treatment. This evidence, mainly derived from patients suffering

unprovoked VTE, should discourage the prolongation of anticoagulation for time-limited periods.

Recent studies have shown that a consistent proportion of patients who experienced VTE associated with transient risk factors receive extended anticoagulation for prevention of recurrences. In a large international registry, 36.7% of patients with transient provoking risk factors were still receiving anticoagulant treatment 12 months following the index VTE.[34,35] However, reducing the threshold for extended anticoagulation potentially exposes a consistent proportion of patients to an unnecessary risk of bleeding. In addition, extending anticoagulation may limit everyday life activities and sports.[36]

As a further option for secondary prevention of VTE, rivaroxaban and apixaban also have shown a favorable efficacy to safety profile when used in prophylactic regimens.[37,38] In patients treated for an index VTE for whom there was clinical equipoise regarding their need for continued anticoagulation, prophylactic doses of apixaban reduced the risk of recurrent VTE by an extent similar to therapeutic dose, with a promising safety profile.[37] This study, in which 90% of patients were treated for a first unprovoked VTE, was not powered for safety outcomes, but its results changed international guidelines and clinical practice. In the Einstein Choice study, the risk of recurrent VTE was significantly lower with rivaroxaban at either a treatment or a prophylactic dose than with aspirin, without a significant increase in bleeding rates.[38] About 60% of the patients entered the Einstein Choice study after an index VTE associated with risk factors. Overall, these studies paved the way for extended prevention of VTE with potentially safer anticoagulant regimens than the therapeutic regimens of vitamin K antagonists or DOACs. Reduced doses of rivaroxaban and apixaban represent the regimen of choice for secondary prevention of VTE in the majority of noncancer patients for their efficacy to safety profile. Unfortunately, patients with a high risk for recurrence were not included in these studies, and specific data are required before the reduced-dose regimen can be used in this setting.

In conclusion, secondary prevention of VTE is a challenging management issue; fatal recurrences may occur, mainly after an index PE, major bleeding continues to be associated with long-term anticoagulation, and clinically relevant nonmajor bleeding may impact quality of life as well as compromise everyday life activities.[36] Though the risk of recurrence is definitely higher after VTE occurring in the absence of risk factors, the cumulative incidence of recurrent VTE after an index episode associated with risk factors has been described to be about 15% at 10 years.[39]

Several scores and models have been proposed to support decisions on the duration of anticoagulation. However, despite extensive efforts, the accuracy of proposed models and scores continues to be suboptimal.[40] Clinicians should be aware that prediction tools can be used to support decision-making, but the final decision on treatment duration cannot skip holistic medical assessment.

CLINICAL CASE (continued)

All the above information is essential for providing our patient advice after the initial 3 months of anticoagulant treatment. The patient was on combined oral contraceptive therapy at the time of the index VTE. This makes the risk for recurrent VTE low after discontinuation of both anticoagulants and hormone therapies. In addition, the patient suffered VTE after nonsurgical ankle trauma. As this is not a major trauma, doubts remain on whether the VTE should be considered provoked after the exposure to this sole risk factor. However, the association of minor trauma and estro-progestin therapy is probably strong enough to convey a sufficient risk for VTE. In addition, as the patient suffered DVT, recurrence, if any, will probably occur as DVT, thus reducing the risk of fatal events. In this patient extended treatment is not required. After discontinuation of anticoagulation, an antithrombotic prophylaxis could be considered in the event of pregnancy, also based on patient features. Tests for thrombophilia could be considered, but their clinical implications are limited due to the low level of evidence supporting thrombophilia-guided anticoagulation strategy.[41]

Future perspectives

A randomized clinical trial is ongoing in patients who experienced VTE associated with a major provoking factor, including major surgery or major trauma, and have at least 1 persistent risk factor for VTE (such as persistent immobility, obesity, heart failure, or inflammatory/autoimmune disorders).[42] After completion of at least 3 months of standard-dose therapeutic anticoagulation, patients will be randomized to apixaban at 2.5mg twice daily or placebo for 12 months.

In addition, potential advances could come from clinical studies of factor XI inhibitors.[43] Should these agents turn out to be as effective and safer than currently available anticoagulants in reducing VTE, a new paradigm for extended treatment could emerge.

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How to diagnose and manage antiphospholipid syndrome

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Antiphospholipid antibodies (aPL) are autoimmune antibodies directed toward phospholipids or phospholipid-protein complexes, particularly those containing β2-glycoprotein I (β2GPI). Persistently positive aPL accompanied by arterial or venous thrombosis, or recurrent pregnancy loss, constitutes the antiphospholipid syndrome (APS). Several types of aPL with different specificities have been defined and may be detected in the clinical lab, including lupus anticoagulants (detected using clotting assays) and anticardiolipin, anti-β2GPI and anti-prothrombin/phosphatidylserine antibodies (detected by ELISA); each of the last 3 aPL may be either IgG, IgM, or IgA, though IgA antibodies are not included in criteria for APS. Due to the relative rarity of APS and the heterogeneity of aPL, thrombosis risk stratification is challenging, and randomized clinical trials for thrombosis treatment and prevention have been limited. This lack of high-quality data has made the clinical management of APS difficult, and existing guidelines are few and could not possibly cover many of the scenarios encountered in managing patients with APS. In this review, we present 3 patients with aPL and/or APS who highlight treatment dilemmas, and we discuss background information that may help guide clinical judgment in developing individualized treatment plans for patients with these enigmatic antibodies.

LEARNING OBJECTIVES

- Evaluate the significance of a mild, transient risk factor in defining APS treatment in a patient with IgM antiphospholipid antibodies
- Assess the need for transitioning a patient with APS from a DOAC to warfarin
- Assess the role of anticoagulation in asymptomatic aPL

Introduction

Antiphospholipid syndrome (APS) is among the most common causes of acquired thrombophilia and may be found in >10% of patients presenting with new thromboembolic events (TE).[1] Unlike most inherited thrombophilias, APS is associated with both venous and arterial thromboembolism (VTE and ATE, respectively). Moreover, the rate of thrombotic recurrence in APS is sufficiently high to lead to recommendations that patients with APS be placed on indefinite anticoagulation.[2,3] Antiphospholipid antibodies (aPL) occur in 20% to 30% of patients with systemic lupus erythematosus as well as in other autoimmune disorders, but most patients seen by hematologists have primary APS. The particular importance of APS is not only the risk of developing thrombotic disease but also its association with increased mortality and shortened survival. Moreover, there are currently no widely accepted biomarkers or risk stratification strategies to identify patients with high precision who may be able to discontinue antithrombotic medication.

Risk stratification approaches in patients with APS are limited primarily to assessment of aPL levels and whether one or more aPL tests are positive. While such assessments are useful, the hazard ratios derived from them are relatively small and the confidence intervals wide; this limits applicability to individual patients with aPL/APS and leads to uncertainty as to optimal management. Moreover, questions unique to patients with different clinical histories and aPL patterns are often not addressed by broad guidelines. In this review, we discuss 3 cases seen in our clinics that raise management questions and discuss the underlying rationale for our clinical decisions. While there is often no "right or wrong" approach to APS, some of the background data discussed here will provide information useful for approaching other APS patients with diverse presentations.

CLINICAL CASE 1

A 77-year-old female physician presents for an opinion concerning duration of anticoagulation. She had undergone cervical discectomy and fusion 6 months previously. She was not on prophylactic anticoagulation during surgery but was able to sit in a chair the day of surgery and was ambulatory upon discharge the following day. One week after discharge she developed pain in her right calf and chest discomfort and was diagnosed with acute deep vein thrombosis in the right peroneal and soleal vein and extensive pulmonary embolism involving lobar, segmental, and subsegmental arteries. She was treated with unfractionated heparin and transitioned to apixaban. When seen by a hematologist in follow-up, an evaluation for hypercoagulability revealed anti-β2GPI IgM antibodies of 87 standard IgM units (SMU) and anticardiolipin (aCL) IgM antibodies of 56 IgM phospholipid units (MPL). Lupus anticoagulant could not be determined due to apixaban. Based on these results, her apixaban was changed to warfarin. Studies repeated 3 months later showed β2GPI IgM antibodies of 105 SMU and aCL IgM antibodies of 63 MPL. She saw another hematologist who advised her that since she had a provoked thrombotic event, she had received an adequate course of anticoagulation (6 months) and could discontinue. She presents now for another opinion as to whether warfarin should be discontinued.

This case presents several questions that should be considered when developing a treatment plan, including:

- Did she experience a provoked or unprovoked thrombotic event?
- Has her course of anticoagulation been adequate?
- Do the presence of IgM antiphospholipid antibodies influence the decision on whether to continue or discontinue anticoagulation?

The definition of an unprovoked versus a provoked VTE is subjective, but a framework for classification has been provided by Kearon et al.[4] This patient's event was chronologically related to surgery. The exact duration of anesthesia was uncertain, but the patient was ambulatory upon hospital discharge the day after the procedure. We therefore considered this event likely to be provoked but by a minor, transient risk factor.

Delineation of a transient risk factor as a provocation for VTE supports the position that a relatively short-term course of anticoagulation therapy may be appropriate. Though this approach is advocated in guidelines,[5] recent opinion has suggested that the risk for rethrombosis should be based more on individual patient characteristics and risk factors rather than solely whether the event seemed provoked or nonprovoked.[6] This is supported by data from the Garfield-VTE study, a registry of treatment patterns and recurrent thrombosis in patients with VTE.[7] In a report from that study that included more than 10000 patients, the risk of recurrent VTE was similar in patients with unprovoked VTE and those with transient provoking factors (hazard ratio [HR] 0.84; 95% CI 0.62% to 1.14%), suggesting that the relationship between this patient's thrombotic event and her limited surgical procedure should not be the only factor influencing decisions regarding anticoagulation duration.

Important in this patient's history is the presence of persistently positive IgM anticardiolipin and anti-β2GPI antibodies; how these antibodies are perceived to have contributed (or not) to the patient's thrombotic event will change her risk assessment from that of a transient minor provoking factor (surgery) to one of a persistent risk factor. Although positivity for IgM antiphospholipid antibodies is included in the criteria for APS,[8] there remains disagreement about their significance (Table 1). In an observational study of 255 stroke patients under 55 years of age, Rodriguez-Sanz et al. observed a significant correlation between levels of IgM aPL measured within 48 hours of stroke and stroke severity.[9] Del Ross et al. reported on 106 patients with thrombotic APS, finding that 13 patients had isolated, persistently positive IgM aPL of medium to high levels.[10] In a large study of patients with neurological disorders, IgM aPL were the most common persistently positive aPL subtype in patients with cerebrovascular accidents.[11] Urbanski et al. reported that 14.3% of a series of 168 patients with APS had isolated IgM antibodies; these patients were older and had a higher incidence of stroke after adjusting for other cardiovascular risk factors.[12] They were more frequently treated with aspirin alone, though patients treated in this manner had an increased incidence of recurrent events. In contrast, Chayoua et al. analyzed anticardiolipin and anti-β2GPI antibodies in 1008 consecutive APS patients and controls.[13] They reported that isolated IgM aPL were present in only 3.5% to 5.4% of patients with thrombotic APS, compared with 5.7% to 12.3% of patients with obstetric APS. Combined positivity for lupus anticoagulant and IgG and IgM aPL was strongly associated with thrombosis, however. The lower frequency of isolated IgM aPL in this study may reflect greater discrepancies in solid phase assays for IgM antibodies.[14]

A recent study of relevance to this patient analyzed aPL profiles in patients >65 years with APS, finding that APS was more frequent in elderly males and more often associated with arterial events, including myocardial infarction.[15] The older cohort also had a significantly higher incidence of single positivity, particularly for IgM aCL, and increased mortality. While this report does not confirm a pathogenic role for IgM aCL, the implications of this study and others demonstrating a high incidence of arterial events in elderly APS patients are concerning.

Our recommendation for this patient was to continue warfarin, with periodic monitoring of antiphospholipid antibody levels.

CLINICAL CASE 2

A 53-year-old man was referred for evaluation of triple-positive antiphospholipid antibodies. He was diagnosed with medically intractable epilepsy six years ago and had a strong family history of coronary artery disease. Imaging studies to evaluate the site of his epileptic foci suggested an origin in the orbital/anterior-mesial temporal regions. He gave a history of a distant diagnosis of antiphospholipid antibodies, though he had never experienced a TE. A previous echocardiogram revealed no valvular thrombi, and several previous magnetic resonance imaging studies revealed no evidence of cerebral ischemia. Antiphospholipid testing revealed a lupus anticoagulant (positive dilute Russel's viper venom time, hexagonal phospholipid assay), anticardiolipin IgG and IgM each >150 GPL/MPL units, anticardiolipin IgA of 21.2 APL, and anti-β2GPI IgG and IgM each >150 standard IgG units (SGU)/SMU. He was referred for evaluation of the need for prophylactic anticoagulation.

Table 1. Selected studies of isolated IgM antibodies in APS

	Type of study	Patient selection	N	Findings
Rodriguez-Sanz[9]	Observational, cross-sectional	>55 years Acute brain infarct	255 (161 male)	• 22 APS (4 before IS, 18 after) • IgM aCL within 48 h of admission correlate with admission NIHSS by MVA • IgG aCL within 48 h of admission correlate with 3-month mRS by MVA
Del Ross[10]	Retrospective	APS/thrombosis	106 (81 female)	• VTE = 55, ATE = 48, small vessel = 3 • 13 single positive for IgM (12.3%) • Single IgM stable over mean of 10.4 yr • Single IgM were significantly older and had increased retinal vein thrombosis
Sahebari[11]	Cross-sectional	Neurological disorder and ≥1 aPL	100	• IgM anti-β2GPI positive in 100% of optic neuritis • IgM aCL was most common antibody in stroke patients
Urbanski[12]	Retrospective	Single-positive IgM aCL or anti-β2GPI vs IgM aPL with ≥1 other aPL	168	• 24 patients with isolated IgM APS (9 isolated aCL, 2 isolated anti-β2GPI) • IgM only were older • Isolated IgM more common in stroke (OR 3.1; 95% CI 1.2–1.9; P = 0.18) • Single IgM positivity persisted in 70%
Chayoua[13]	Retrospective	Multicenter study of APS samples	1008 (763 female)	By MVA • LAC: OR 2.3 (95% CI 1.6–3.3) to 2.4 (95% CI 1.7–3.4) • IgG aCL or anti-β2GPI: OR 2.3 (95% CI 1.6–3.5) to 3.2 (95% CI 2.0–5.0) • IgM aCL or anti-β2GPI: NS • Isolated IgM aCL or anti-β2GPI in 3.5%-5.4%

aCL, anticardiolipin antibodies; aPL, antiphospholipid antibodies; APS, antiphospholipid syndrome; ATE, arterial thromboembolism; β2GPI, β2-glycoprotein I; h, hours; IS, ischemic stroke; LAC, lupus anticoagulant; mRS, modified Rankin scale; MVA, multivariate analysis; NIHSS, NIH stroke scale; NS, not significant; OR, odds ratio; VTE, venous thromboembolism.

This patient presents a common dilemma as to whether anticoagulation is indicated in a patient with aPL who has not experienced a prior TE (Figure 1).

It should first be considered whether aPL levels in this patient are persistently positive. Levels of aPL as high as seen here, however, remain positive in >95% of cases, so repeat studies may not be necessary.[16]

A prospective study by Pengo et al. of 104 patients with triple-positive aPL who had not experienced a prior TE reported 25 first TEs over 4.5 years, for an annual incidence of 5.3%.[17] Male sex and risk factors for venous thrombosis (oral estrogen/progesterone, pregnancy, family history, other thrombophilias) imparted relative risks of TE of 4.4% and 3.3%, respectively. In another prospective study, Ruffati et al. assessed the development of a first TE in a cohort of 248 patients with aPL and no prior TE history, with a mean follow-up interval of 39 months.[17] The frequency of different types of aPL were not reported and patients were not classified by single, double or triple positivity. The annual incidence of new TE in this population was 1.86% per patient year. The only significant TE risk factors were the presence of a lupus anticoagulant and hypertension. Taken together, these studies suggest that consideration of prophylactic anticoagulation for a triple-positive aPL patient is quite reasonable.

Aspirin provides one option for TE prophylaxis in asymptomatic aPL carriers.[18] However, in both studies mentioned above, chronic prophylaxis, almost always with aspirin, was not found to decrease the incidence of TE.[17,19] In a randomized study of low-dose aspirin in asymptomatic patients with aPL, outcomes were compromised by the small sample size and lower than expected event rate (2.75/100 patient-years in aspirin-treated patients, 0/100 patient-years in the placebo arm), resulting in an HR for thrombosis of 1.04 (95% CI 0.69%, 1.06%; *P* = 0.83) in aspirin-treated patients.[20] However, a meta-analysis by Arnaud et al. found a decreased incidence of primary thrombosis in patients treated with aspirin (odds ratio 0.50; 95% CI 0.27%, 0.93%), though 10 of the 11 series in the meta-analysis were observational.[21] The use of aspirin as primary prophylaxis in asymptomatic patients with high-risk aPL profiles (lupus anticoagulant, double or triple positivity for aPL, or the presence of persistently high positive aPL levels) is endorsed by guidelines of the European Alliance of Associations for Rheumatology (EULAR).[22] Proceedings of the 16th International Congress on Antiphospholipid Antibodies also suggest that low-dose aspirin be considered in such patients, while recognizing the need for a randomized trial.[23]

Statins provide another alternative for primary prophylaxis in high-risk aPL patients. In a mechanistic study, 1 month of treatment with fluvastatin reduced the expression of monocyte procoagulant proteins, including tissue factor, protease-activated receptors 1 and 2, and flt-1, due to inhibition of p38 mitogen-activated protein kinase and reduction in NF-κB/Rel DNA binding activity.[24] In another study, fluvastatin significantly reduced inflammatory cytokines levels in patients with aPL, including IL-6, IL-8, Il-1β, and TNFα.[25] A retrospective analysis of statins in

patients with aPL with or without SLE showed a modest reduction in thrombosis in the primary aPL group (HR 0.12; 95% CI 0.01, 0.98).[26] Additional studies are needed to confirm utility of statins in patients with aPL, but they might be considered in patients with risk factors for cardiovascular disease.

Another potential alternative for prophylaxis is hydroxychloroquine (HCQ). HCQ may reduce aPL-mediated thrombosis risk through several mechanisms, including (1) reducing the binding of aPL-β2GPI complexes to phospholipid bilayers,[27] (2) reversing the effects of aPL on annexin V displacement from phospholipid bilayers and cells,[28] and 3) inhibiting aPL-induced endothelial cell dysfunction.[29] In a prospective nonrandomized study, 20 patients with APS received oral anticoagulation with HCQ, while an additional 20 received oral anticoagulation alone.[30] Recurrent thrombosis occurred in 6 patients treated with oral anticoagulants alone and in no patients receiving additional HCQ ($P = 0.0086$ by time-to-event analysis). Similar results were obtained in another study of primary APS (HR 0.09; 95% CI 0.01% to 1.26% for anticoagulation and HCQ vs anticoagulation alone); this study also suggested that HCQ may be associated with reduction in aPL IgG levels over time. However, the only study to use HCQ in asymptomatic aPL carriers was stopped early due to poor enrollment.[31]

Finally, a randomized study compared low-dose aspirin in combination with warfarin adjusted to an international normalized ratio (INR) of 1.5 with low-dose aspirin alone in aPL carriers. This study revealed no reduction in thrombosis in the aspirin plus warfarin arm, with subjects in the latter arm having an increased incidence of bleeding.[32]

CLINICAL CASE 2 (continued)

Based on these studies, we discussed the pros and cons of each type of intervention with the patient. Given his triple positivity and high antibody levels, we suggested that he consider low-dose aspirin prophylaxis.

CLINICAL CASE 3

A 36-year-old woman presented to the emergency department with an acute ileofemoral deep vein thrombosis. She has no significant past medical history and denied provoking factors. She is not obese and does not smoke. She was started on apixaban upon discharge. At 3-month follow-up, she is found to have β2GPI antibodies of IgG 143 SGU and anticardiolipin IgG 56 GPL. Testing for a lupus anticoagulant could not be completed due to direct oral anticoagulant (DOAC) interference. She is referred to hematology for a discussion of anticoagulation and the need for transitioning to a vitamin K antagonist (VKA). She expresses hesitation about initiating warfarin due to dose monitoring requirements and dietary limitations and asks about continuing on apixaban.

In thrombotic APS, VKAs are the preferred therapy for secondary thrombosis prevention. However, when APS is not diagnosed on initial presentation and patients are initiated on a DOAC, subsequent identification of aPL requires a decision of continuing DOAC or transitioning to warfarin for long-term anticoagulation. DOACs are attractive due to fixed dosing, lack of routine monitoring, minimal drug-to-drug interactions, and generally higher patient satisfaction. Warfarin requires more frequent monitoring, which can be complicated in APS due to artefactual prolongation of point-of-care INRs in patients with lupus anticoagulants.[33] However, warfarin therapy does not commonly require significant dietary modification or elimination as often assumed by patients.[34] The major concern with DOAC use in APS is efficacy. Four major prospective randomized clinical trials have compared DOACs with warfarin (Table 2).[35-38] Collectively, these failed to demonstrate noninferiority of DOACs for secondary prevention of TEs and safety in APS. The first of these was the RAPS trial, which assessed thrombin generation as a global measure of thrombotic risk and anticoagulation effectiveness in APS patients randomized to rivaroxaban or warfarin.[35] Endogenous thrombin potential was significantly higher in the rivaroxaban group, thus rivaroxaban did not meet the primary noninferiority end point. However, peak thrombin generation, a secondary end point, was lower with rivaroxaban. These results were interpreted as consistent with a similar overall thrombotic risk in both arms and attributed to differences in anticoagulant mechanisms. However, the trial was not powered to assess clinical end points, and there were no thrombotic episodes in either arm.

The TRAPS trial compared rivaroxaban with warfarin for prevention of TEs, major bleeding, and vascular death in high-risk, triple-positive APS patients. This trial was stopped prematurely after an excess of events (4 ischemic strokes, 3 myocardial infarctions) in the rivaroxaban group versus none with warfarin.[36] In another prospective, randomized trial by Ordi-Ros, rivaroxaban was associated with nearly twice the incidence of recurrent thrombosis as warfarin, with stroke again more common in rivaroxaban-treated patients. Post hoc analysis, while underpowered, suggested an increased risk in patients with prior arterial thrombosis, livedo racemosa, or APS-related cardiac valvular disease, while a significant association with triple positivity was not identified. Taken together, these trials suggest that rivaroxaban offers inferior protection from thrombosis compared with warfarin in thrombotic APS, especially for individuals with triple-positive disease and prior arterial thrombosis. The fourth trial, ASTRO-APS, compared apixaban with warfarin and was challenged by multiple protocol modifications, dose intensification, and an amendment to exclude patients with prior arterial events, before terminating prematurely.[38] The authors reported 6 ischemic strokes in apixaban-treated patients compared with none with warfarin. Strokes occurred in patients with single-, double-, and triple-aPL positivity, though most patients with recurrent events had a history of prior stroke.

Whether the findings of these trials represent a class effect or are drug specific remains unknown. Few cohort studies have looked at the use of other DOACs, and insufficient data are available to extrapolate strong conclusions.[39,40] Based on trial data, regulatory agencies in the US and Europe changed the labeling of DOACs to advise against their use in patients with APS, especially triple-positive. The 2019 European Society of Cardiology and 2020 American Society of Hematology guidelines similarly recommend against DOAC in APS patients.[41,42] However, other societal guidelines, including those of the British Society for Haematology, EULAR, and the International Society on Thrombosis and Hemostasis, offer a conditional

Table 2. Randomized control trials of direct oral anticoagulants in APS

Trial	Author	Patient selection	N	Triple positive (%)	Findings
RAPS	Cohen	APS, prior ATE excluded	116 (54 rivaroxaban)	28	• Endogenous thrombin potential greater with rivaroxaban (primary end point) • No recurrent thrombosis either arm in 6 months • No difference in MB: 5% DOAC vs 4% VKA
TRAPS	Pengo	Triple-positive APS	120 (59 rivaroxaban)	100	• Higher recurrent thrombosis and MB with DOAC • 4 IS, 3 MI (12%) DOAC vs 0 events VKA • MB: 7% DOAC vs 3% VKA
EUDRA	Ordi-Ros	APS	190 (95 rivaroxaban)	60.5	• Higher recurrent thrombosis with rivaroxaban • 11 (11.6%) VTE DOAC vs 6 (6.3%) VKA, RR 1.83 [95% CI, 0.71–4.76] • Higher rate of IS with DOAC • Livedo, small vessel disease, cardiac valvular disease associated with increased risk of thrombosis • Triple positivity not associated with increased risk • MB: No difference
ASTRO-APS	Woller	APS, protocol modification to exclude prior ATE	48 (23 apixaban)	30.4	• Terminated prematurely • 6 strokes DOAC vs 0 events VKA • Strokes occurred in single-, double-, and triple-positive APS • MB: 0 DOAC vs 1 VKA

APS, antiphospholipid syndrome; ATE, arterial thromboembolic event; DOAC, direct oral anticoagulant; IS, ischemic stroke; MB, major bleeding; MI, myocardial infarction; RR, relative risk; VKA, vitamin K antagonist; VTE, venous thromboembolic event.

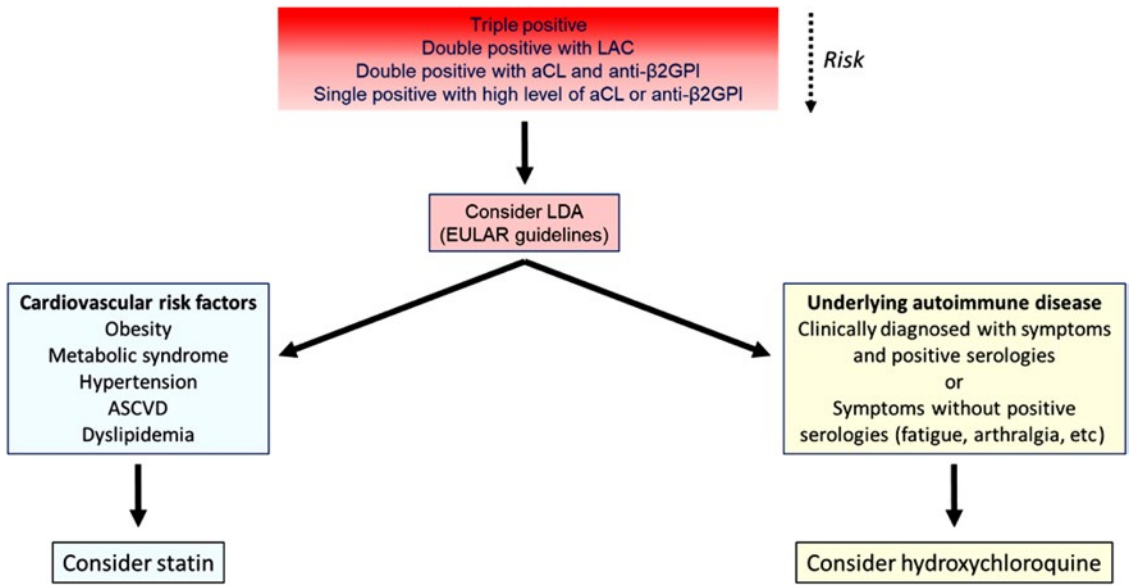

Figure 1. Decision tree for management of patients with asymptomatic aPL. Initial risk assessment is based primarily on the antibody profile, with triple-positive patients considered highest risk. Progressively less risk is depicted by less intense shading in the red box, though grading of this risk is imprecise. Patients with significant cardiovascular risk factors might also benefit from statins (blue box) and patients with rheumatologic symptoms, even without diagnostic serologies, from hydroxychloroquine. ASCVD, atherosclerotic cardiovascular disease; EULAR, European Alliance of Associations for Rheumatology; LAC, lupus anticoagulant; LDA, low dose aspirin.

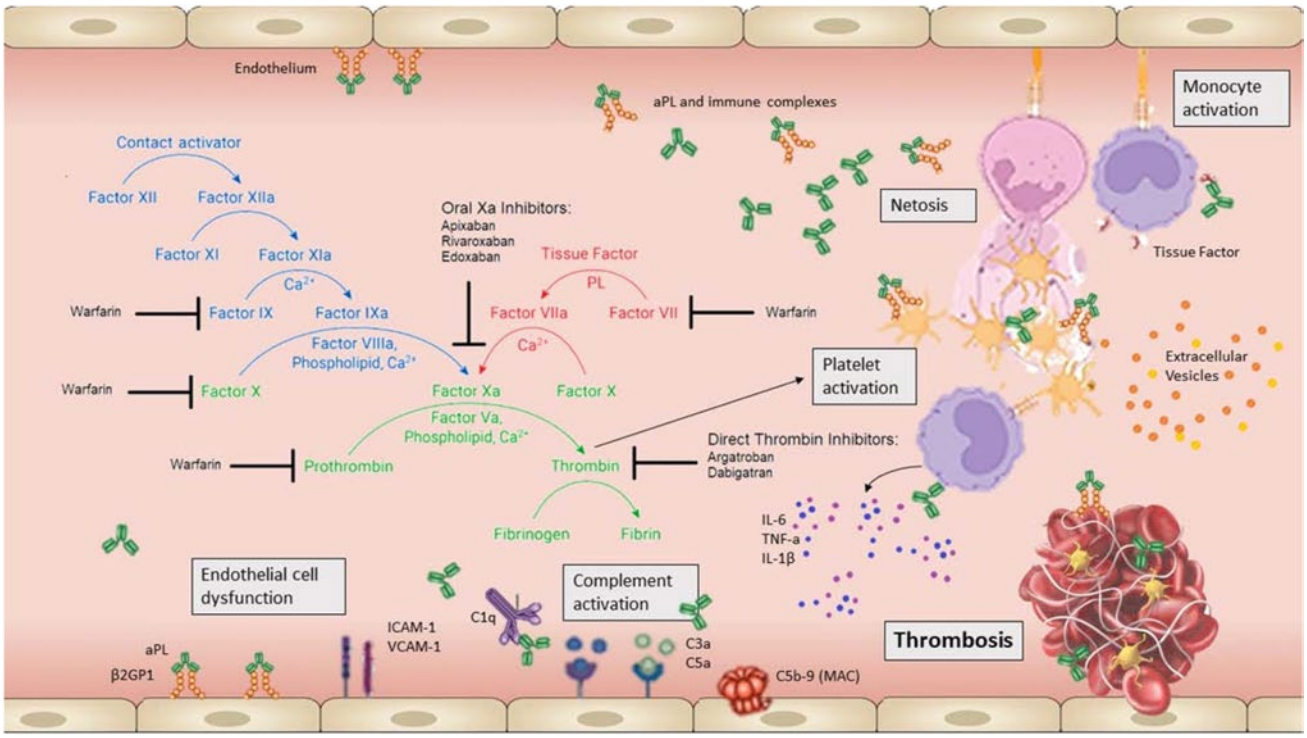

Figure 2. Mechanisms of aPL-mediated thrombosis and inhibition by warfarin or DOAC. This figure depicts multiple potential mechanisms underlying APS and the cell types that are affected by aPL. Cellular activation results in cell-specific responses that include releases of neutrophils extracellular traps (NETs), expression of cellular procoagulant activity, and extracellular vesicle release, among others. Warfarin inhibits γ-carboxylation of vitamin K–dependent coagulation factors, thus reducing the catalytic efficiency of coagulation complexes such as the phospholipid-dependent tenase and prothrombinase reactions.

approach. While recommending warfarin as the first-choice agent, collectively the guidelines suggest that DOACs may be considered in individuals (1) without high-risk APS features such as triple positivity, arterial thrombosis, small vessel thrombosis, organ involvement, or heart valve disease; (2) already stable on a DOAC; (3) unwilling to undergo INR monitoring; (4) with <60% time in therapeutic range with VKA; or (5) with contraindications to VKA.[22,40,43]

Limitations in risk stratification in APS and a lack of data pose a challenge to applying these recommendations. Moreover, deficiencies in understanding the driving pathophysiology of APS limit insight into DOAC failure in some patients. VKA and DOACs act upon different aspects of the coagulation system (Figure 2), but what mechanistically accounts for the observed disparity in anticoagulation effectiveness in APS is unknown. Several hypotheses have been raised, including differences in pharmacokinetics with lower trough levels of DOACs, the need for higher anti-Xa activity levels to prevent arterial versus venous events, and higher thrombin generation with DOACs due to more limited blockade of coagulation factors.

CLINICAL CASE 3 (continued)

After we discussed the evidence with this patient who has double-positive APS with unknown lupus anticoagulant status and has been doing well on a DOAC, she elected to transition to warfarin.

The management of APS patients is challenging. There are insufficient data concerning APS pathogenesis to formulate a mechanism-based approach. Given the variety of patient characteristics and potential diversity of aPL profiles, it is unlikely that guidelines will cover all situations. Thus, each patient must be assessed individually, with best clinical judgment used in some cases. A better understanding of APS through additional studies will hopefully inform more data-driven treatment approaches in the future.

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Conflict-of-interest disclosure

Anne Hubben: no competing financial interests to declare.
Keith R. McCrae: no competing financial interests to declare.

Off-label drug use

Anne Hubben: Anticoagulant therapy has an approved label for patients with aPL and thrombosis. There are no other medications with an approved label for APS, thus the use of all medications discussed in this review other than warfarin and DOAC should be considered off label.

Keith R. McCrae: Anticoagulant therapy has an approved label for patients with aPL and thrombosis. There are no other medications with an approved label for APS, thus the use of all medications discussed in this review other than warfarin and DOAC should be considered off label.

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EVIDENCE-BASED MINIREVIEW

Should older patients with low weight and CKD receive full-dose DOACs for treatment of acute proximal DVT?

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LEARNING OBJECTIVES

- Explain the age-related challenges involved in considering anticoagulation for acute VTE
- Identify the best treatment strategy for acute VTE in older patients with chronic renal disease and low weight

CLINICAL CASE

A 76-year-old woman with a past medical history of coronary artery disease and on chronic therapy with aspirin presents to the emergency room with right lower extremity pain and swelling. A venous duplex ultrasound showed an acute thrombus involving the right common femoral vein. Her hemoglobin is 11.5 mg/dL and platelet count is 158,000/uL. Her weight is 58 kg, and her serum creatinine is 1.3 mg/dL (estimated creatinine clearance [CrCl] of 36 mL/min). What anticoagulant and dosing are appropriate for this patient?

Introduction

A delicate balance exists between preventing morbidity and mortality from venous thromboembolism (VTE) and preventing associated bleeding complications in older patients. While older age is associated with increased risk of venous thrombosis,[1] it is also associated with increased risk of bleeding and ambiguity around the appropriate dosing of anticoagulation.[2] The evidence to support anticoagulation selection and dosing in older patients is limited since they are not well represented in clinical trials for the approval of direct oral anticoagulants (DOACs) in VTE.[3] Furthermore, common conditions in this population such as stage 4-5 chronic kidney disease (CKD), anemia, liver disease, hypertension, or concomitant use of dual antiplatelet therapy were excluded from registration trials.[4-7]

The problem

The incidence rate of VTE increases with age from 1 per 1000 person-years in the general population to 6-8 per 1000 person-years in those 80 and older.[1,8] There are physiologic and acquired risk factors that are responsible for this high incidence. Aging is associated with increasing levels of procoagulant factors, as well as impairment in the fibrinolytic pathway.[3] At the same time, cancer, immobility, cardiovascular disease, and chronic inflammatory diseases contributing to VTE risk are also more common with aging.[9]

The treatment of VTE in older patients is challenging. Studies have shown that adherence to treatment is lower in older patients across different diseases such as atrial fibrillation, leading to poor clinical outcomes and increase risk of VTE relapse.[10,11] Falls are also more common in this age group.[9] This has been a very well described concern of physicians when prescribing therapeutic anticoagulation,[2] even though our ability to predict future bleeding due to falls is very limited. Interactions with other drugs may also affect the absorption of DOACs[12] in the setting of frequent polypharmacy in this population.[9] For example, carbamazepine, a strong P-glycoprotein and CYP3A4 inducer sometimes prescribed for neuropathic pain, can decrease serum concentrations of most DOACs.

The case for full dose anticoagulation in older patients

The five landmark studies that led to the approval of apixaban, dabigatran, edoxaban, and rivaroxaban in VTE treatment[4-7,13] have a median age of 55-60 years, even though the majority of VTE occur in patients over 70 years[1] (Table 1). Albeit for low numbers or participants, the efficacy and safety reported on those trials remained favorable for DOAC vs vitamin K antagonists (VKA) in patients 75 and older.[14]

The prescriber's perception of high risk of bleeding attributed to frailty in older patients often leads to off-

Table 1. Evidence on use of therapeutic anticoagulation for treatment of VTE in elderly patients[3]

TRIAL	Drug and dose	Number of patients	Patients ≥75	Patients with CrCl ≤50 mL/min	Pertinent results for elderly population
EINSTEIN-DVT/PE[4,5]	Rivaroxaban 15 mg twice daily for 3 weeks followed by 20 mg daily	8281 Mean age = 57 years	1283 (18%)	664 (8%)	No differences in primary and safety outcome in different age, weight, and CrCl groups Excluded patients with "high risk of bleeding" (not defined)
RE-COVER[6]	Dabigatran 150 mg twice daily	2564 Mean age = 55 years	290 (11.3%)	133 (5.2%)	No differences in primary and safety outcome in different age, weight, and CrCl groups Excluded patients with "high risk of bleeding" (not defined) 100 mg or less of daily aspirin was acceptable
AMPLIFY[7]	Apixaban 10 mg twice daily for 1 week, followed by 5 mg twice daily	5395 Mean age = 57 years	768 (14%)	327 (6.2%)	No differences in primary outcome in different age, CrCl, and weight groups. Safety outcome favored apixaban for age subgroups 65–75 and >75 Safety outcome favored apixaban on weight >60 kg Excluded patients with "high risk of bleeding" (not defined), Hb <9 g/dL, platelets <100 000/m^3, or patients on dual antiplatelet therapy Aspirin at a dose of 165 mg or less was accepted
HOKUSAI-VTE[8]	Heparin lead-in followed by edoxaban 60 mg, or 30 mg in patients with CrCl 30–50 mL/min or weight <60 kg	*n* = 8292 Mean age = 55.8 years	1004 (12%)	541 (6.6%)	No differences in primary outcome and safety outcomes in different age and weight groups Excluded patients with "high risk of bleeding" (not defined) and CrCl <30 mg/dL 100 mg or less of daily aspirin was acceptable

label use of lower doses of DOACs. One particular concern is low body weight, which can be seen in up to 20% of patients above age 85.[9] A recent survey showed that hematologists frequently reduced the dose of apixaban and rivaroxaban in older patients for the treatment of acute VTE, with only 50%, 35%, and 25% of responders using the label doses in patients between 65 and 74, 75 and 84, and >85, respectively.[15] This was also evidenced in a multicenter registry enrolling 3027 consecutive patients with acute, symptomatic VTE (COMMAND VTE),[16] in which patients 80 and older received less anticoagulation after the first 10 days (93%, 93%, and 90% for patients <65, 65-80, and >80, respectively, $P = 0.04$). This study also provided reassuring data on bleeding; even though patients older than 80 had significantly more anemia and lower body weight and were more commonly on antiplatelet agents and nonsteroidal antiinflammatory drugs compared to younger patients, they did not show a statistically higher risk of major bleeding.

Another important challenge in older patients is the high incidence of CKD occurring in up to 35% of patients above age 66.[17] Due to the varying level of renal clearance in different DOACs, kidney dysfunction is an important factor when selecting an anticoagulant. On this topic, there is confusion regarding dose reduction recommendations in older patients. While dose adjustment is recommended in patients with a serum creatinine >1.5 mg/dL along with age ≥80 or weight ≤60 kg for stroke prevention in those with atrial fibrillation, it has not been studied for the treatment of acute VTE.[18] Pharmacokinetics studies have shown that age alone has a small impact in DOAC exposure.[19-22] However, those same studies found that dabigatran clearance was affected by renal function more so than apixaban and rivaroxaban, while edoxaban had a labeled dose reduction for the treatment of VTE if CrCl was 15-50 mL/min, weight was ≤60 kg, or in the setting of a concomitant use of p-glycoprotein inhibitors. In a post-hoc meta-analysis comparing DOAC vs warfarin in registration trials, VTE recurrence and major bleeding were significantly lower in the pooled DOAC treatment arm in the subgroup aged ≥75 years and nonsignficantly lower in the subgroup with CrCl ≤50 mL/min.[14]

Future research

Due to the small number of older patients in randomized trials, there is an ongoing need for dedicated studies powered for this subgroup. To date, there is no convincing evidence to support a lower dose of DOAC for the treatment of acute VTE for older

patients based on age alone. We believe that age, frailty, dependency, and cognitive function should not determine changes in treatment doses of DOAC when there is a clinical indication for anticoagulation for VTE.

Recommendations

- We recommend approved dosing of DOAC for the treatment of acute symptomatic VTE per package insert regardless of patient age (Grade 1B).
- We suggest dose-reduced edoxaban for patients with CrCl 15–50 mL/min, weight ≤60 kg, or concomitant use of p-glycoprotein inhibitors regardless of patient age, when followed by 5 days of parenteral anticoagulation.
- We suggest addressing modifiable bleeding risk factors, including uncontrolled hypertension, avoid antiplatelet therapy when acceptable, nonsteroidal anti-inflammatory drugs or alcohol to reduce the risk of bleeding, rather than using an off-label dose of a DOAC.

Conflict-of-interest disclosure

Nicolas Gallastegui: no competing financial interests to declare.
Camila Masias: no competing financial interests to declare.

Off-label drug use

Nicolas Gallastegui: nothing.
Camila Masias: nothing.

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Sex, lies, and iron deficiency: a call to change ferritin reference ranges

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Iron deficiency is a very common and treatable disorder. Of all the tests available to diagnose iron deficiency, the serum ferritin is the most able to discriminate iron deficiency from other disorders. However, the reference range for ferritin in many laboratories will lead to underdiagnosis of iron deficiency in women. Studies have shown that 30%-50% of healthy women will have no marrow iron stores, so basing ferritin cutoffs on the lowest 2.5% of sampled ferritins is not appropriate. In addition, several lines of evidence suggest the body physiologic ferritin "cutoff" is 50 ng/mL. Work is needed to establish more realistic ferritin ranges to avoid underdiagnosing a readily treatable disorder.

LEARNING OBJECTIVES

- Understand why serum ferritin is the best test to diagnose iron deficiency
- Learn why iron deficiency in women is vastly underdiagnosed

CLINICAL CASE

A 24-year-old runner self-refers to hematology because of fatigue and decreased exercise performance. Over the past year, her running pace has slowed and she tires more readily with exertion. Now when she rock climbs, she also notes more muscle fatigue and has noticed diminished concentration at work. She is concerned her symptoms may be related to iron deficiency after reading an article in a running magazine. Upon taking a menstrual history, she states she had menarche at age 11 and has regular menses every 28 days, which last around 5 days. On her heaviest days she has to change her tampon every 2 hours and wakes up at night to change her pad. She experiences flooding around once per cycle. Initially, she presents to an urgent care clinic for further evaluation but was told not to worry as her hemoglobin was normal. Given her progressive symptoms, she is seen at another urgent care clinic, where a serum ferritin was obtained and returns at 10 ng/mL. Given the lower limit of normal of ferritin at this clinic was 8 ng/mL, she was reassured that her iron stores were normal.

Introduction

Iron deficiency is one of the leading contributors to the global burden of disease, disproportionately impacting women of reproductive age and people living in low- and middle-income countries.[1] It has become apparent that the prevalence of iron deficiency is much higher than what has been appreciated in the past, in part due to a lack of standardized guidelines to diagnose iron deficiency, as well as provider misconceptions on how to accurately interpret iron studies. As a result, iron deficiency is often overlooked because blood counts or ferritin fall within "normal range." This article reviews testing for iron deficiency, the wide array of symptoms associated with iron deficit even in the absence of anemia, and the need for more appropriate and standardized laboratory reference ranges for serum ferritin.

What is ferritin?

Ferritin is the cellular storage molecule for iron.[2] Ferritin consists of 24 subunits that form a hollow sphere with 6 openings that allow up to 5000 atoms of iron to be stored inside, permitting the cell to store a large amount of iron without concern of free iron catalyzing toxic reactions. The organs that contain the highest concentration of ferritin are the liver and reticuloendothelial system in the spleen and bone marrow.

The cellular production of ferritin is tightly regulated by total iron stores within the body[3] (Figures 1 and 2). The 5′ end of the ferritin messenger RNA (mRNA) contains a sequence known as the iron response element, upon which a protein called the iron regulatory protein (IRP) binds in the absence

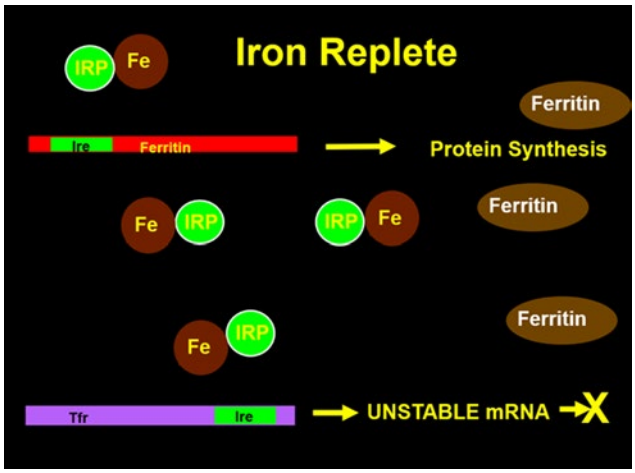

Figure 1. With cellular iron, the IRP binds iron and releases it from the ferritin IRE to allow translation of mRNA to ferritin protein while destabilizing the transferrin receptor mRNA.

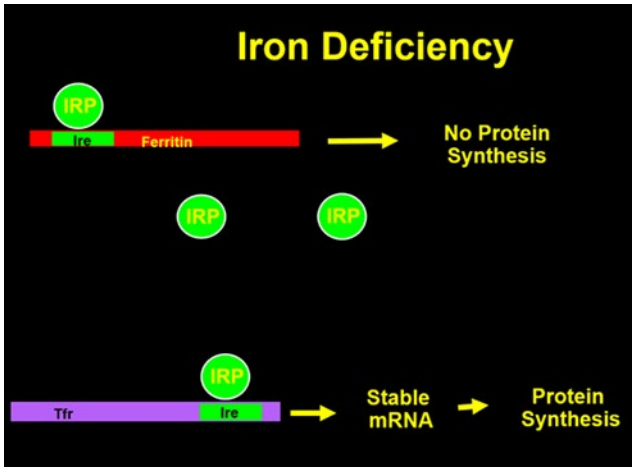

Figure 2. With no cellular iron, the IRP binds the ferritin IRE to blocking translation of mRNA to ferritin protein while stabilizing the transferrin receptor mRNA to allow protein synthesis.

of cellular iron. When this binding occurs, translation of ferritin mRNA into protein cannot occur, thus making iron more readily available for cellular use. When iron is present, it can bind to the IRP, which then dissociates from the IRE, allowing ferritin protein synthesis to occur. The regulation of the transferrin receptor (TfR) is also controlled by a 3′ IRE in its mRNA. Conversely, when the IRP is bound, the mRNA is stabilized, allowing for increased protein synthesis leading to mobilization and use of iron. With abundant cellular iron, the IRP is released, leading to destabilization of the TfR mRNA and decreased synthesis. By this elegant mechanism, iron stores can control the production of key iron metabolism proteins.

Why do we use ferritin as a measure of iron stores?

Traditional tests to diagnose iron deficiency have key limitations that affect their clinical utility (Table 1). For example, serum iron levels are low both in inflammation and iron deficiency, and levels can vary based on dietary intake. Total iron binding capacity, which is typically elevated in iron deficiency, is a specific but not sensitive finding, as inflammation, age, and malnutrition can falsely lower levels. Transferrin saturation is low both in iron deficiency and in inflammation, limiting its utility in differentiating 2 of the most common forms of anemia. Reticulocyte hemoglobin content is low with any iron-deficient erythropoiesis and therefore cannot discriminate between anemia of inflammation and iron deficiency.[4] Finally, a low mean corpuscular volume is a late finding of iron deficiency with several cofounders, such as liver disease, alcohol use, among others, that prohibit its specificity.

Table 1. Tests for iron deficiency

Test for iron deficiency	Limitations
Mean corpuscular volume	Decreases late in iron deficiency, confounded by liver disease, alcohol use, etc
Serum iron	Decreased in inflammation, daily variation, affected by diet
Total iron binding capacity	Not sensitive as in the setting of iron deficiency can be decreased by inflammation and malnourishment
Iron saturation	Low in inflammation
Free erythrocyte protoporphyrin	Affected by inflammation
Red cell distribution width	Not specific for iron deficiency
CHr (Ret-He)	Can also be abnormal in inflammation or thalassemia
Ferritin	Best performing test

For reasons that are poorly understood, small amounts of ferritin leak from the cytoplasm into the blood when synthesized to store cellular iron. Serum ferritin levels correlate with total body iron stores with 1 ng/mL of ferritin equating to 8-10 mg of storage iron.[5] While it is true that, as an acute phase reactant, ferritin can be markedly elevated in inflammation, this is only the case with good iron stores. Recall that ferritin protein synthesis is dependent on the presence of cellular iron. With concurrent inflammation and iron deficiency, lack of iron prevents the translation of ferritin mRNA into protein, thus blunting any extreme rise in serum ferritin (over 100 ng/mL).

In a systematic review of the literature, Guyatt et al concluded that serum ferritin "was by far the most powerful test" for diagnosing iron deficiency.[6] This test strongly outperformed other traditional tests of iron deficiency, including red cell protoporphyrin, mean corpuscular volume, transferrin saturation, and red cell distribution width. This review noted that a ferritin level of over 100 ng/mL ruled out absolute iron deficiency (absent iron stores), while a lower limit was dependent on the clinical scenario. A newer test of iron status, serum soluble transferrin receptor (sTR), has been proposed to differentiate anemia related to iron deficiency from chronic inflammation, in which sTR would be expected to be elevated in iron deficiency but not impacted by inflammation.[7] However, an important caveat is that sTR can be elevated in any condition associated with increased erythropoietic activity/ineffective erythropoiesis, leading to potential misinterpretation and diagnosis of iron deficiency.[8]

Table 2. Symptoms of iron deficiency

Alopecia
Decreased exercise performance
Fatigue
Impaired cognition
Impaired thyroid function
Increased bruising
Increased susceptibility to acute mountain sickness
Pica
Pruritus
Restless legs

Are low iron stores without anemia an issue?

While adverse effects of iron deficiency anemia have been recognized for over a century, it is increasingly appreciated that low iron stores with normal blood counts can also lead to clinical complications (Table 2). Three studies have shown that administering iron to women with normal blood counts and ferritin levels less than 50 ng/mL significantly improved symptoms of fatigue.[9-11] A systematic review and meta-analysis also demonstrated the detrimental effects of low iron stores and exercise, with the supplementation of iron leading to improved aerobic capacity (VO_2 max) and athletic performance.[12] Supplementing iron in nonanemic iron-deficient adolescent girls also improved tests of verbal learning and memory.[13] Finally, restless legs syndrome is another symptom resultant of low iron stores that resolves with iron supplementation.[14]

A potential explanation of the adverse effects seen with low iron stores in the absence of anemia is that anemia reflects an end stage complication of progressive iron deficiency. As iron stores fall, iron is stripped out of muscles and other tissue to maintain adequate erythropoiesis. Interestingly, a study supporting this hypothesis found a direct correlation with muscle iron depletion as serum ferritin fell from 75 ng/mL to 36 ng/mL.[15] Thus waiting for anemia to develop, instead of acknowledging the symptomatic implications of iron deficiency, will result in underdiagnosis of a very treatable and preventable disease.

How common are low iron stores in women?

Because of obligate menstrual losses, women are at higher risk of iron deficiency. Menstrual losses can average 35 mL of blood (16 mg of iron) per cycle, leading to higher dietary iron requirements, which are often not met by diet alone or hindered by inadequate gastrointestinal absorption. The recommended iron intake for women is 18 mg/day, but an Institute of Medicine Report shows that on average, the intake of iron ranges from only 12.6-13.5 mg/day.[16]

When iron losses chronically outpace iron intake, iron deficiency develops. In a 1966 study, investigators performed bone marrow studies on 114 healthy college women and found that 24% had no stainable iron in their marrow, and another 37% had depleted (1+) iron stores.[17] These remarkable findings have been further validated by Puolakka et al, who found absent marrow iron in 50% of healthy women,[18] and Hallberg et al, who showed absent iron stores in 34%.[19] Thus, due to the inability to keep up with iron losses, a substantial number of women are iron deficient.

Should women have a different ferritin reference range than men?

Established reference ranges are historically derived from values observed in 95% of individuals within a sample population.[20] Commonly cited flaws with this approach include the nonrepresentative sample populations, the assumption of a Gaussian distribution, and the fact that only 2.5% of values are pathologic. However, since ~30%-50% of women have absent iron stores in their marrow, deriving a cutoff at the lowest 2.5% will both dramatically underdiagnose iron deficiency and result in an inappropriately low lower limit of normal. There is no physiologic reason that ranges of normal serum ferritin should differ between men and women; rather, this reflects the fact many women have little to no total body iron stores. The use of population normal for ferritin's cutoff is reminiscent of decades ago when a "normal" cholesterol was said to be under 300 mg/mL. To reiterate, most laboratories use a descriptive statistical finding for their ferritin reference range instead of one that reflects the reality of the high prevalence of iron deficiency. Therefore, there is a critical need to develop a standardized lower limit of ferritin that more appropriately reflects physiology to assist in accurate diagnosis of low iron stores.

What should the ferritin lower limit be to diagnose iron deficiency?

Several studies have employed a physiologic approach to determine a more accurate ferritin lower limit. Since iron absorption in the gastrointestinal tract can increase severalfold in iron-deficient states, a study using absorption of a stable iron isotope showed this physiologic compensation does not return to baseline until the serum ferritin is over 50 ng/mL, perhaps reflecting a more precise threshold of iron deficiency.[21] Another study using sensitive biomarkers of iron depletion—soluble transferrin receptor and hepcidin—confirm the use of 50 ng/mL as a physiologic cutoff.[22] Importantly, this proposed threshold corresponds with the above-mentioned studies that show repletion of the serum ferritin to over 50 ng/mL reduces fatigue (Table 3).

What are the clinical implications?

Laboratory reference ranges are essential for the accurate interpretation of test results and clinical decision making; however, inappropriate ranges may inadvertently contribute to inequitable care, particularly among women. Clinical implications of using ferritin ranges that do not accurately reflect women's iron stores have led to systemic underdiagnosis and underrecognition of iron deficiency. Additionally, flawed reference ranges also limit eligibility for coverage of parenteral iron therapy. It is the unfortunate truth that many women are denied parenteral iron therapy until their untreated iron deficiency progresses to anemia, resulting in significant morbidity.

Those who disagree with raising the serum ferritin cutoff have voiced concern that this change will dramatically increase the

Table 3. Why the ferritin cutoff should be 50 ng/mL

• Gastrointestinal absorption of iron returns to baseline at 50 ng/mL
• Clinical trials show this cutoff is associated with fatigue
• Biochemical markers of iron deficiency normalize at 50 ng/mL

number of women diagnosed with iron deficiency. Ironically, however, this highlights the crux of the issue. Given resounding evidence that many women are iron deficient and symptomatic, greater emphasis on developing ferritin ranges using physiologic data can lead to more accurate estimation of the global burden of disease.

Another key question that clinicians should consider is if hematologic reference ranges for hemoglobin/hematocrit are appropriate, acknowledging the high prevalence of women with iron deficiency. While men have higher upper ranges for their hemoglobin due to the effects of testosterone, there is no physiologic explanation for why women should have a lower hemoglobin compared to men. And, as such, there are several studies to support that, among iron-replete women, the hemoglobin lower limit of normal does not differ. As people age, the reference ranges for men and women draw closer together.[23] A study conducted in 1936 showed that iron administration raised women's hemoglobin by 10%. Interestingly, the discussion included the prescient comment that "the accepted 'normal' for women's heamoglobin may not be a true normal, but should perhaps be regarded as mildly pathological."[24] A paper published in 1967 also showed that iron supplementation in a "normal control" group of women resulted in an increase in hemoglobin by –1 g/dL.[25] Taken together, these findings highlight sex-based inequities that lead to normalization of disease states and the critical need to update hematologic ranges truly reflective of iron repletion.

What should be done?

There are several ways to improve recognition and treatment of this common clinical scenario (Table 4). First, we propose broader implementation of educational programs highlighting the prevalence and clinical implications of undiagnosed and untreated iron deficiency. Second, hematologists need to collaborate with their hospital laboratory leadership to develop new ferritin reference ranges that allow for accurate diagnosis of iron deficiency. Finally, standardized guidelines to diagnose and treat iron deficiency may allow for more accessible, consistent, and widespread implementation. Ultimately, hematologists are in a prime position to substantially decrease the global burden of disease by more aggressively screening and correcting iron deficits. This is another example of how hematologists are on the front lines of combating structural inequality in medicine.[26]

Table 4. Action steps

• Educate your colleagues about
o The symptoms that can be seen with iron deficiency without anemia
o The very high incidence in women
o The need for appropriate laboratory ranges for ferritins
• Work with your hospital laboratory to provide accurate ferritin reference ranges
• Work to establish accurate reference ranges for women's hemoglobin/hematocrit cutoffs

CLINICAL CASE (continued)

The patient was appropriately diagnosed with iron deficiency and treated with oral iron supplementation. Within weeks, she noticed improvement in her exercise capacity and her ability to concentrate at work. In addition, her astute hematologist thoroughly asked about reversible causes of iron deficiency and diagnosed her with heavy menstrual bleeding as the likely cause of ongoing blood loss. She was referred to a gynecologist with prompt resolution of her heavy periods with the placement of a progesterone intrauterine device.

Conflict-of-interest disclosure

Kylee Martens: no competing financial interests to declare.
Thomas G. DeLoughery: no competing financial interests to declare.

Off-label drug use

Kylee Martens: nothing to disclose.
Thomas G. DeLoughery: nothing to disclose.

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IV iron formulations and use in adults

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Intravenous iron has become a major component of the therapeutic armamentarium for iron deficiency and iron deficiency anemia. The earliest formulations were associated with unacceptable toxicity. Newer formulations, with complex carbohydrate cores that bind elemental iron more tightly, allow the administration of full therapeutic doses in 15 to 60 minutes. Nonetheless, a folklore of danger, fueled by earlier formulations no longer available, continues to foment caution. Complement-mediated minor infusion reactions, referred to as complement activation-related pseudo-allergy, resolve without therapy. Inappropriate intervention with vasopressors and H_1 blockers converts these minor reactions into hemodynamically significant adverse events. Four new formulations, low-molecular-weight iron dextran, ferumoxytol, ferric carboxymaltose, and ferric derisomaltose, all approved for the treatment of iron deficiency in a host of conditions, are now widely used with an excellent safety profile. Herein, the administration, safety, indications, and management of infusion reactions are discussed. Treatment-emergent hypophosphatemia, a newly recognized side effect for some formulations, is also reviewed. Based on the preponderance of published evidence, intravenous iron should be moved up-front for the treatment of iron deficiency and iron deficiency anemia in those conditions in which oral iron is suboptimal.

LEARNING OBJECTIVES

- Debunk an antiquated folklore of danger associated with intravenous iron formulations
- Recognize infusion reactions and their management
- Become familiar with the administration of the 4 formulations of intravenous iron capable of complete replacement (total dose infusion) in 15 to 60 minutes

CLINICAL CASE

A nulliparous woman is referred for fatigue, pagophagia, and inability to sleep due to constant uncomfortable feelings in the legs while lying down. Menses have been intermittently heavy, lasting 7 days with clot passage and flooding. The hemoglobin concentration is 10 g/dL with a platelet count of 620 000/uL. Serum ferritin is 11 ng/mL, and transferrin saturation (TSAT) is 9%. Ferrous sulfate ($FeSO_4$) containing 60 mg of elemental iron was prescribed on alternate days. Gastric irritation and constipation occurred. She was referred to hematology, and 1000 mg of low-molecular-weight iron dextran (LMWID) was ordered over 60 minutes. Forty seconds after a slow start, chest pressure and flushing occur. She experiences no hypotension, wheezing, stridor, or periorbital edema.

Introduction

It has been nearly a century since Heath injected subcutaneous and intramuscular iron, reporting hemoglobin increments in hypochromic anemias (Figure 1).[1] In the early 20th century, an attempt to administer intravenous iron as colloidal ferric hydroxide resulted in toxicity so severe as to "preclude its use for therapeutic purposes."[2] Undoubtedly, the observed toxicity was provoked by prohibitive levels of labile-free iron after the administration of a formulation with virtually no ability to bind elemental iron. However, in 1954 Baird and Padmore introduced iron dextran for intramuscular and intravenous injections, observing rapid hematologic responses with few serious adverse events (SAEs).[3] Infusion reactions abounded, resulting in perceptions of anaphylaxis. Intravenous iron continued to be infrequently prescribed until the 1990s when recombinant erythropoietin was released for dialysis-associated anemia, requiring intravenous iron as an adjuvant for optimal response.[4]

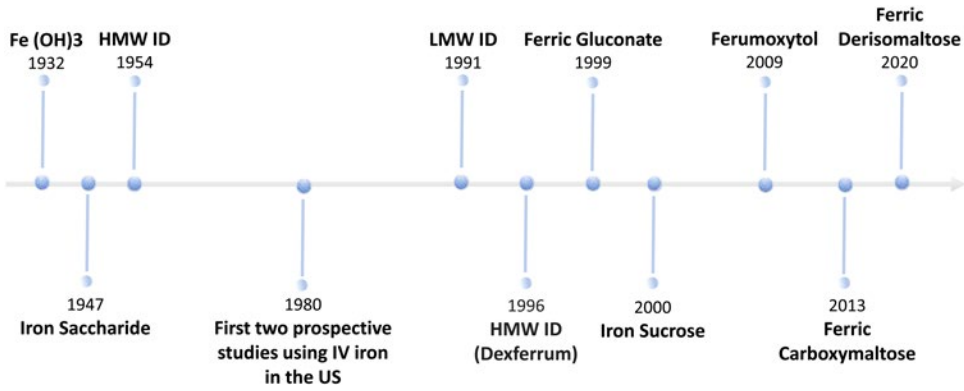

Figure 1. History of intravenous iron in the United States.

Today we know that the preponderance of AEs occurring with intravenous iron are minor reactions to labile-free iron, likely complement mediated and self-limited, resolving without therapy. Anaphylaxis is rare, occurring in fewer than 1 in 200 000 administrations.[5] A folklore of danger persists, driven by the alarming incidence of SAEs caused by a formulation of high-molecular-weight iron dextran (HMWID) no longer available, along with imprudent administration of vasopressors and antihistamines for minor reactions, converting them into hemodynamically significant SAEs. Publications using indirect surrogates for anaphylaxis such as spontaneous AE reporting,[6] medical claims,[7,8] and sales data perpetuate this concern.[9,10] These surrogates are unable to distinguish inappropriate intervention for minor reactions from SAEs. This position is supported by thousands of patients in head-to-head studies reporting no difference in safety or efficacy among the available products.[11,12]

Herein we report the pharmacology of 6 available formulations of intravenous iron, its administration, and its indications in a host of conditions associated with iron deficiency (ID). These include ferric gluconate (FG), iron sucrose (IS), LMWID, ferumoxytol, ferric carboxymaltose (FCM), and ferric derisomaltose (FDI) (Table 1). Following infusion, all share a similar fate. The iron carbohydrate complexes mix with plasma and are phagocytosed within the reticuloendothelial system, wherein the carbohydrate shell is degraded, and iron is stored as ferritin or transported out of the cell, bound to transferrin, which delivers iron to its destiny.[13]

The formulations are similar in structure, with an iron core surrounded by a carbohydrate shell, excluding FDI, which is a matrix structure. They differ in the physicochemical properties of size, labile iron content, and release of iron in the serum.[14] Labile iron derives from bound iron within the nanoparticle and is readily mobilized by chemical reactions or proteins.[14] All formulations have the potential to cause infusion reactions from labile-free iron dependent on dose, speed of infusion, and formulation stability (Figure 2).[15] FG and IS, with much smaller cores releasing larger amounts of labile-free iron, require lower doses and more frequent visits to achieve the therapeutic dose.[14] Accordingly, these formulations are not discussed further, and discussions of formulations are limited to the 4 able to be administered as full doses in 15 to 60 minutes. We hope these data debunk the myths of danger, leading to increased use of this necessary treatment for the most frequent maladies we as hematologists see in our work, ID and ID anemia.

Safety

The initial response to parenteral iron was once so negative that it is remarkable we have this opportunity to demonstrate the ease of administering new formulations that allow for the complete correction of ID in 15 to 60 minutes. The perception of danger was so ingrained that although the first large prospective study of intravenous iron reported only three SAEs without residua or hospitalization in 481 patients who received 2099 doses of intravenous HMWID, the authors concluded that intravenous iron should be reserved for situations in which oral iron cannot be used, and the need for replacement is urgent.[16]

A decade later the release of erythropoietin for dialysis-associated anemia fostered new interest in intravenous iron. LMWID (INFed) was released in 1991 and became standard for dialysis-associated anemia.[17] Unfortunately, a HMWID, Dexferrum (Vifor), was released as a less expensive alternative to LMWID and resulted in an alarming increase in reactions.[5] With this formulation's removal from the pharmacopoeia and the release of two iron salts, FG and IS, SAEs became rare. This position was corroborated by a systematic review and meta-

Table 1. Intravenous iron formulations

Trade name Manufacturer Carbohydrate	INFeD-US Cosmofer-Europe AbbVie low-molecular-weight iron dextran	Feraheme Covis Ferumoxytol	Injectafer-US Ferinject-Europe Daiichi Sankyo Carboxymaltose	Monoferric Pharmacosmos Derisomaltose
Total dose infusion **Test dose required** **Approved dose**	Yes Yes 100 mg per dose	No No 510 mg	Yes- Europe/No- US No 1000 mg Europe 750 mg US	Yes No 20 mg/kg (1000 mg if >66 kg)
Optimal dose **Infusion time**	1000 mg 60 minutes	1020 mg 30 minutes	1000 mg Europe/750 mg × 2 US 15 minutes	1000 mg 20 minutes

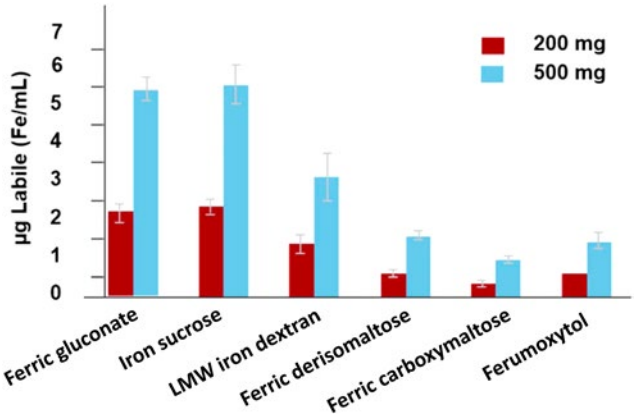

Figure 2. Labile iron by iron formulation. Labile-free iron is elemental iron that has been released from the core of the iron/carbohydrate nanoparticle and available to bind transferrin. All formulations have the potential to cause infusion reactions from labile-free iron. The higher the labile iron content, the greater the likelihood of CARPA. FG and IS, with much smaller cores, releasing larger amounts of labile-free iron, require lower doses and more frequent visits to achieve the therapeutic dose. Reprinted from Jahn et al.[14] with permission.

analysis reviewing 103 trials including 10 390 patients treated with IV iron compared with 4044 with oral iron, 1329 with no iron, 3335 with a placebo, and 155 with intramuscular iron (a route now proscribed).[12] While infusion reactions occurred, intravenous iron was not associated with an increased risk of SAEs or infections. This conclusion was proven by the largest and longest prospective trial ever performed. A cohort of 2141 adults undergoing hemodialysis were randomized to intravenous iron administered proactively (400 mg/mo targeting ferritin of >700 ug/L or TSAT >40%) or reactively (0–400 mg/mo when ferritin was <200 ug/L or TSAT <20%).[18] After a mean 2.1-year follow-up, the proactive regimen was not only noninferior but resulted in lower doses of erythropoiesis stimulating agents for the same clinical outcome, leading to significant cost savings. Cardiovascular outcomes were superior in the proactive arm. Corroborating the meta-analysis,[12] as expected no increase in infections was observed.

The preponderance of perceived SAEs are not immunoglobulin E (IgE)–mediated hypersensitivity reactions but complement activation–related pseudo-allergy (CARPA). CARPA is characterized by complement-mediated activation by nanoparticles of free or labile iron that do not bind quickly enough to transferrin.[19] Mast cells and basophils are triggered by compliment activation that resembles true IgE-mediated allergy.[20] This reaction is not specific to intravenous iron and has been recognized with monoclonal antibodies and liposomal medications.[21] CARPA can occur any time, does not require prior sensitization, and is dependent on infusion rate. This not infrequently occurring and quickly resolving reaction is non–life-threatening and characterized by flushing, myalgias and/or arthralgias, back pain, and/or chest pressure.[21] No symptoms of anaphylaxis are present.[20,22] These reactions are self-limited, and diphenhydramine should be avoided as it can worsen symptoms (Figure 3).[23] Rechallenge at a slower rate with the same formulation is appropriate,[24] as well as caution against intervening with vasopressors and antihistamines, which can convert minor reactions into SAEs.[21,25]

CLINICAL CASE (continued)

Within 4 minutes symptoms resolve. Methylprednisolone and famotidine are administered empirically as prophylaxis. The remaining planned dose is administered seamlessly over 30 minutes. The pagophagia disappears immediately, and the restless leg syndrome resolves that evening. Within 48 hours energy levels improve.

Formulations

Multiple head-to-head studies among the formulations have failed to report a significant difference in efficacy, infusion reactions, or safety. Subsequently, the discussion assumes equality.

Low-molecular-weight iron dextran

LMWID was approved in the United States in 1991. Millions of doses have been administered in dialysis-associated anemia,[5] non–dialysis dependent chronic kidney disease (CKD),[26] pregnancy,[27] heavy menstrual bleeding,[26] and a host of other conditions associated with ID without a safety signal. Nonetheless, unfounded concerns regarding anaphylaxis persist. LMWID can be administered as a single 1000-mg infusion over 1 hour.[26] LMWID was used in the first study of IV iron in cancer-associated anemia.[28] In this study 157 subjects were randomized to LMWID, oral, or no iron. The administration of LMWID resulted in greater hemoglobin (1 g/dL) and hematopoietic (2 g/dL) responses with reduced times to targets (with concomitant decrements in erythropoiesis stimulating agents) compared with oral and no iron. Intravenous iron resulted in consistently improved quality-of-life parameters.

LMWID was used in the first US prospective study evaluating IV iron in pregnancy.[27] Seventy-three gravidas received 1000 mg of LMWID. No SAEs were observed, and no negative infant outcomes were reported. The authors concluded that compared with oral iron, intravenous iron has less toxicity and is more effective in increasing hemoglobin, supporting moving it closer to frontline therapy in iron-deficient pregnant patients.

LMWID carries a black box warning of risk of severe, sometimes fatal anaphylactic reactions and requires a 25-mg test dose.[29] This warning stems from antiquated notions of danger that are discredited. Nonetheless, we recommend starting slowly and observing for 10 to 15 minutes. Following a brief observation, the remainder should be administered over the balance of 1 hour.

Ferumoxytol

Ferumoxytol was the first new formulation allowing rapid infusion (20-30 minutes) of a complete dose. In 1981 the company Advanced Magnetics, led by physicists and physical chemists, designed a compound intended as a magnetic resonance imaging (MRI) contrast agent.[30] Ferumoxytol is a superparamagnetic iron oxide linked to polyglucosesorbitol carboxymethylether.[31] A rapid injection of 510 mg in 17 seconds, consistent with the administration of radiologic contrast agents, was recommended.

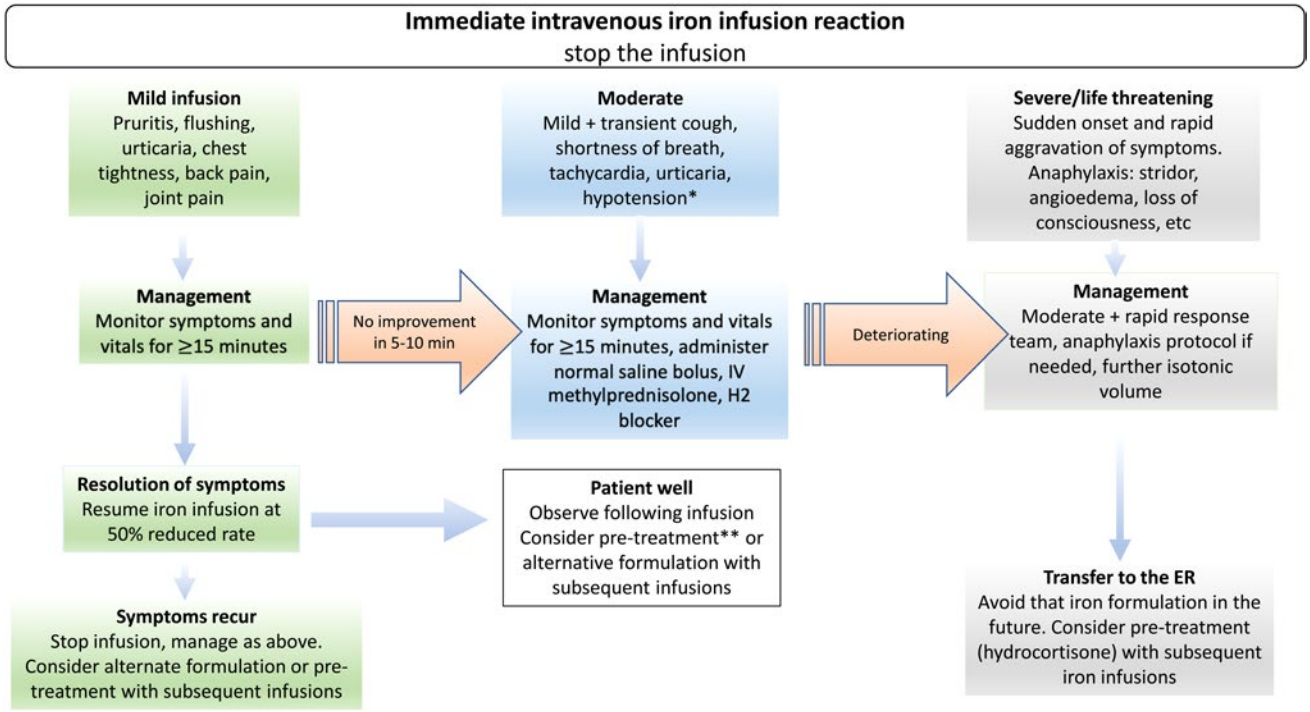

Figure 3. Management of acute intravenous iron infusion reactions. *Hypotension is defined as a drop of 30 mmHg or more in systolic blood pressure from baseline or systolic blood pressure of equal to or less than 90 mmHg. **Methylprednisolone 125 mg plus or minus H2 antihistamine. We avoid first-generation H1 antihistamines (eg, diphenhydramine), as this can cause somnolence, tachycardia diaphoresis, and sometimes hypotension, mimicking an anaphylactic reaction. The majority of SAEs can be attributed to the use of diphenhydramine and/or epinephrine for the management of immediate infusion reactions. Adapted from Rampton et al.[20]

Serendipitously, significant improvements in hemoglobin concentrations were observed, leading to its investigation as an iron replacement therapy. In 2009 it was approved for the correction of ID with CKD. As a result of its function as an MRI contrast agent, if an MRI is planned within 8 weeks of administration, radiologists must be notified of its presence so that interpretation is not confounded.[32]

The 17-second injection was implemented, and retrospectively, while labile-free iron is markedly reduced with ferumoxytol compared to IS and FG,[14] an alarming number of reactions occurred. Even allowing for the Weber effect (increased reporting whenever new products are launched), reactions were frequent and mistaken for anaphylaxis.[33] While we now know these were likely CARPA, the number of reports of AEs to the US Food and Drug Administration (FDA) was so high a black box warning was issued. This resulted in failure to obtain a broad label for other causes of ID, limiting its use. The label stipulated a minimum infusion rate of 510 mg over 15 minutes. Support for the imprudence of rapid administration comes from one of our practices (MA). The first 90 doses administered over 17 seconds were associated with 3 episodes of hypotension that resolved rapidly without sequelae. Serum tryptase levels were normal, suggesting these were not anaphylactic reactions.[34] Upon slowing the rate to 3 minutes, more than 2500 doses were administered without clinically significant AEs. Nonetheless, we recommend following the 15-minute infusion rate, consistent with recommendations for the other 2 new parenteral iron formulations, FCM and FDI.

To address hypersensitivity, in 2017 the manufacturer of ferumoxytol performed a randomized, double-blind comparison to FCM.[35] The methods, which were FDA mandated to approve a broad label for ferumoxytol for ID, required two 510-mg infusions of ferumoxytol 1 week apart compared with two 750-mg infusions of FCM 1 week apart, each over 15 minutes. While a 50% difference in dose may seem obtuse, this iteration compared 2 existing approved methods. Of 1997 patients, 997 received ferumoxytol and 1000, FCM. No difference in reactions was observed. As expected, there was more hypophosphatemia with FCM (discussed further under treatment-emergent hypophosphatemia). No clinical sequelae secondary to hypophosphatemia were reported. Hypophosphatemia was not observed with ferumoxytol. These results were also consistent with the randomized trial of ferumoxytol and IS, which evaluated 162 patients, reporting comparable efficacy and AE rates.[36]

Consistent with the data, ferumoxytol received broad FDA approval for the treatment of ID. The approval remained consistent with the existing label requiring 2 infusions of 510 mg over 15 minutes, 1 week apart. However, there was no reason to believe ferumoxytol could not be administered more conveniently, as a single infusion of 1020 mg. Several studies corroborate this position.[37,38] We routinely administer 1020 mg in 250-mL normal saline over 30 minutes with no observed SAEs in more than 2000 infusions and are currently conducting a randomized, double-blind, double-dummy trial of oral and IV iron in patients after bariatric surgery using the single-dose

method.[39] It is our hope that these data will foster the approval of 1020 mg of ferumoxytol as a single infusion.

Recently a generic of ferumoxytol (Sandoz) was approved. To date there are no safety data. In the initial filing with the FDA for generic approval, the results of 60 patients were submitted. Minor infusion reactions were increased compared with published data on the brand. Multiple institutions have reported increased infusion reactions with the generic. We recommend caution with its use.

Ferric carboxymaltose

Like ferumoxytol, FCM infusion results in limited labile-free iron.[40] FCM was first licensed in Europe as Ferinject (Vifor Pharma) in 2007. In its initial filing in the United States, concerns around hypophosphatemia and adverse cardiac events delayed the approval for nearly 2 years. It received broad-label approval from the FDA in 2013.[41] It is a macromolecular ferric hydroxide carbohydrate complex allowing a single dose of 1000 mg in Europe or 2 doses of 750 mg in the United States in 15 to 20 minutes.[42] FCM is effective in patients with heavy uterine bleeding,[43] inflammatory bowel disease,[44,45] chemotherapy-induced anemia,[46] and pregnancy.[47,48]

Notably, FCM was the first formulation to definitively report the benefits of intravenous iron in heart failure (HF) and ID.[49,50] The definitive FAIR-HF trial resulted in significant improvements with intravenous iron independent of hemoglobin, supporting the correction of ID in patients with HF. These data were corroborated by the CONFIRM-trial with a similar design, supporting the benefits of intravenous iron in this population with no safety signal.[49,50]

While FCM results in the least labile-free iron of the intravenous formulations,[14] treatment-emergent hypophosphatemia has come to be most associated with its use,[35,51] which is discussed below.

Ferric derisomaltose

FDI has a short linear structure of linked glucose units forming a unique carbohydrate matrix, binding elemental iron similarly to ferumoxytol and FCM, limiting the release of labile-free iron and allowing large doses to be administered in 15 to 20 minutes.[52] It was approved in Europe in 2009, Canada in 2017, and the United States in 2020 for administration in patients with ID intolerant of, or refractory to, oral iron.

In a randomized, open-label, multicenter trial, FDI compared with IS with coprimary end points adjudicated serious hypersensitivity reactions and a change in hemoglobin from baseline to week 8. The trial consisted of 1512 patients, who were randomized 2 to 1 to a single 1000-mg infusion of FDI or IS administered as 200-mg IV injections, up to 5 times.[53] No difference in safety was observed, and the coprimary efficacy end point for noninferiority in hemoglobin change was met, with a more rapid hemoglobin response observed with FDI. FDI, 1000 mg in 20 minutes, is a more convenient method of IV iron administration than IS, without sacrificing efficacy or safety.

Two similar randomized, open-label trials comparing the safety and efficacy of FDI and IS in non–dialysis dependent CKD randomized over 3000 patients to a single 1000-mg infusion of FDI or multiple infusions of IS.[54,55] The coprimary end points were safety (FDA recommended based on low risks of severe reactions with either) and a change in hemoglobin. Adjudicated AEs and hemoglobin concentrations at week 8 were similar, meeting the coprimary end points.

In a prospective comparison of FDI to usual care in 1137 patients with HF and reduced ejection fraction,[56] similar to that

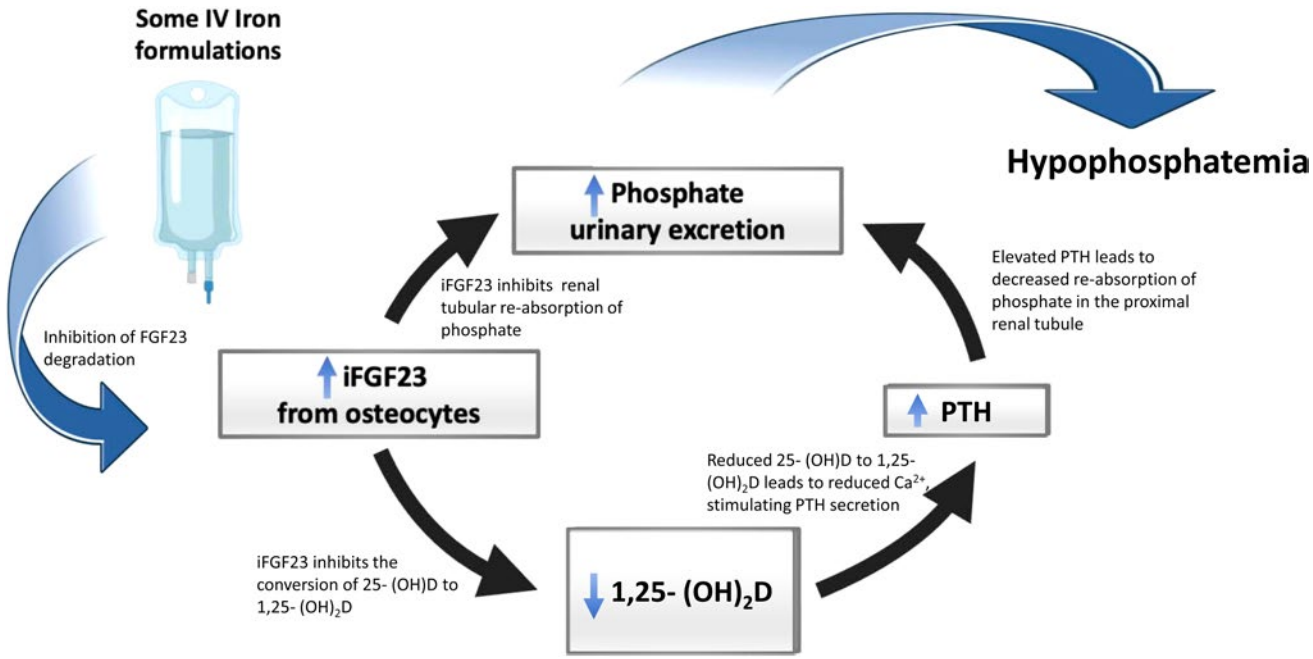

Figure 4. Mechanism of treatment-emergent hypophosphatemia. Following the administration of some intravenous iron formulations is a sharp rise in the plasma iFGF23, triggering a pathophysiological cascade of renal phosphate wasting, calcitriol deficiency, and secondary hyperparathyroidism. This frequently culminates in hypophosphatemia even after iFGF23 levels have normalized. PTH, parathyroid hormone.

reported with FCM, FDI administration resulted in fewer admissions to the hospital as well as a decrease in cardiovascular deaths. The data corroborate the benefit of intravenous iron for patients with HF and ID.

FDI administered as a single 1000-mg infusion has been shown to be safe and effective in ID with CKD,[55] in IBD after bariatric surgery,[57,58] for heavy uterine bleeding, and during both pregnancy and the postpartum period.[59,60] FDI should be administered as a 1000-mg infusion diluted in 100mL of normal saline over 20 minutes.

Treatment-emergent hypophosphatemia

Systematic reviews,[61] meta-analyses,[51,62] and clinical trials have associated some iron preparations with treatment-emergent hypophosphatemia, with incidence, severity, and duration of hypophosphatemia highest following administration of FCM.[35,63-65F] The mechanism of hypophosphatemia following administration is renal wasting regulated by the phosphoturic hormone fibroblast growth factor 23 (FGF23) (Figure 4).[66,67] Following administration, hyperphosphaturic hypophosphatemia triggered by high intact FGF23 (iFGF23) culminates in low 1,25 $(OH)^2$ vitamin D, hypocalcemia, and secondary hyperparathyroidism, which has been associated with osteomalacia, fracture, and other bone deformities.[68] The specific physicochemical properties of FCM likely trigger the sharp increases in iFGF23.[69] This "6-H syndrome" is characterized by 1) high FGF23, 2) hyperphosphaturia, 3) hypophosphatemia, 4) hypovitaminosis D, 5) hypocalcemia, and 6) secondary hyperparathyroidism.[57,70] In extreme cases, hypophosphatemia following FCM has been associated with osteomalacia, fractures, and other bone deformities.[68]

Providers should have a high suspicion of hypophosphatemia in patients who present with worsening fatigue following FCM administration and, consistent with its new label, monitor phosphorus.

Conclusion

The use of intravenous iron to treat a host of common ailments that cause ID and IDA has increased. These conditions include heavy menstrual bleeding and angiodysplasia (hereditary hemorrhagic telangiectasia) in which oral iron cannot keep up with the losses, second- and third-trimester pregnancy in which oral iron does not keep up with the requirements of the growing fetus, bariatric surgery in which oral iron is not absorbed after the procedure, inflammatory bowel disease in which oral iron worsens the underlying pathology, and comorbid conditions (cancer and chemotherapy-induced anemia, CKD) in which IV, and not oral iron, is able to bypass the hepcidin block. Medical personnel often cite safety as a barrier to the use of intravenous iron (internal data). There are an abundance of data supporting the safety of intravenous iron. The majority of infusion reactions are minor, which can be converted into SAEs with unnecessary intervention. It is time to embrace a total dose infusion of intravenous iron in 15 to 60 minutes as frontline therapy for most causes of iron deficiency.

Conflict-of-interest disclosure

Layla Van Doren: honoraria: Pharmacosmos, Daiichi Sanyko.
Michael Auerbach: honoraria: Pharmacosmos. I have received research funding for data management from Covis.

Off-label drug use

Layla Van Doren: There are two off-label uses (based on US label). These off-label uses are covered in Table 1: the recommendation to give LMW ID as a 1000 mg one hour infusion and the recommendation to administer ferumoxytol as a 1020 mg infusion in 30 minutes instead of the current label to give it as 510 mg twice on different days.
Michael Auerbach: There are two off-label uses (based on US label). These off-label uses are covered in Table 1: the recommendation to give LMW ID as a 1000 mg one hour infusion and the recommendation to administer ferumoxytol as a 1020 mg infusion in 30 minutes instead of the current label to give it as 510 mg twice on different days.

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Intravenous iron therapy in pediatrics: who should get it and when is the right time?

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Iron-deficiency anemia occurs most commonly in young children due to a low-iron diet and adolescent girls due to menstrual blood loss. However, children with gastrointestinal conditions such as intestinal failure, inflammatory bowel disease, celiac disease, and/or other chronic conditions, including chronic kidney disease and heart failure, also commonly have iron deficiency. Many patients with classic iron-deficiency anemia will improve with oral iron therapy. However, in children who have an incomplete response to oral iron, intravenous iron therapy is increasingly being used. Benefits of intravenous iron therapy include a rapid repletion of iron stores in addition to resolution of anemia, less gastrointestinal side effects, and relief for patients and families struggling with long-term iron supplementation. Indications for first-line therapy with intravenous iron in children with chronic conditions have also increased. Four intravenous iron formulations have approved indications in pediatrics, and many are increasingly used off-label in children as well. Here we discuss the indications and appropriate timing of intravenous iron therapy in children with a wide range of underlying etiologies.

LEARNING OBJECTIVES

- Identify the clinical indications for intravenous iron therapy in pediatric patients
- Select appropriate intravenous iron therapies based on presentation and underlying conditions
- Understand the clinical course and laboratory response in patients receiving intravenous iron

Overview of pediatric iron-deficiency anemia

Globally, iron deficiency remains a major cause of anemia in children. The incidence of pediatric iron deficiency is highest during 2 time periods: infancy and adolescence. In the United States, approximately 2% to 3% of infants and young children have iron-deficiency anemia (IDA) due to a low-iron diet, particularly in those without initiation of appropriate iron supplementation while breastfeeding and in toddlers with excessive cow's milk intake (>24 oz daily). Over 10% of adolescent females are iron deficient postmenarche, particularly in the setting of heavy menstrual bleeding (HMB). Prevalence is lower (<1%) in healthy school-aged children and adolescent males. Across all ages, inflammatory bowel disease (IBD) or other gastrointestinal (GI) illnesses, cardiac disease, and/or other chronic conditions place children at high risk for iron deficiency and iron dysregulation due to chronic inflammation. Unique to pediatric patients are the increased iron requirements to support ongoing growth and development, which results in a higher daily iron requirement compared to adults. While prolonged iron deficiency will cause symptomatic microcytic anemia, iron-deficient children with a normal hemoglobin can exhibit similar symptoms. Risk factors and underlying conditions associated with IDA are listed in Table 1.

While many children and adolescents with IDA will respond appropriately to oral iron, a number will have an incomplete response or fail to complete a full oral iron course due to intolerance of side effects or nonadherence. Children with persistent or refractory IDA, as well as those with severe or recurrent IDA, are often referred to hematology for ongoing evaluation and management, including consideration of intravenous (IV) iron therapy. Here we review when to consider IV iron and available data on IV iron options in pediatrics.

CLINICAL CASE 1

A 15-year-old female is referred to hematology for evaluation of persistent anemia in the setting of HMB. Laboratory evaluation demonstrates hemoglobin of 9.5 g/dL and ferritin of 4 ng/mL. She was prescribed a ferrous sulfate 325-mg

Table 1. Indications for IV iron therapy in pediatrics by etiology

IDA etiology and affected patient populations	Indications for IV iron
Nutritional IDA (low-iron diet)	
Infants Toddlers Children with restricted diets (vegan, vegetarian) Adolescents with eating disorders	Severe IDA causing hospital admission, hemodynamic compromise, or severe symptoms affecting daily functioning Failed oral iron therapy due to either inability to tolerate (poor taste or GI side effects) or poor adherence History of medication nonadherence or poor follow-up
Blood loss	
Menstrual (adolescent females with HMB) Gastrointestinal (IBD, other GI tract disease) Other (recurrent epistaxis in patients with bleeding disorders)	Brisk, ongoing, difficult to control bleeding Severe IDA causing hospital admission, hemodynamic compromise, or severe symptoms affecting daily functioning Inability to tolerate oral iron due to GI side effects (patients with IBD) Ongoing IDA secondary to medication nonadherence (adolescents) Recurrent IDA in a patient who previously required IV iron
Malabsorption	
History of GI surgery/tract alteration (intestinal failure or short gut syndrome)	Absence of duodenum for oral iron absorption
GI tract disease (celiac, *Helicobacter pylori*, atrophic gastritis)	Reduced soluble iron from stomach acid insufficiency
Chronic inflammation/disease states	
Chronic kidney disease Heart failure Rheumatologic/immunologic diseases	Renal replacement therapy on erythropoiesis-stimulating agents Heart failure with evidence of iron deficiency (to maximize cardiac function) Relative or absolute iron deficiency due to hepcidin activity in the setting of chronic inflammation and inability to correct iron deficiency with oral supplementation alone

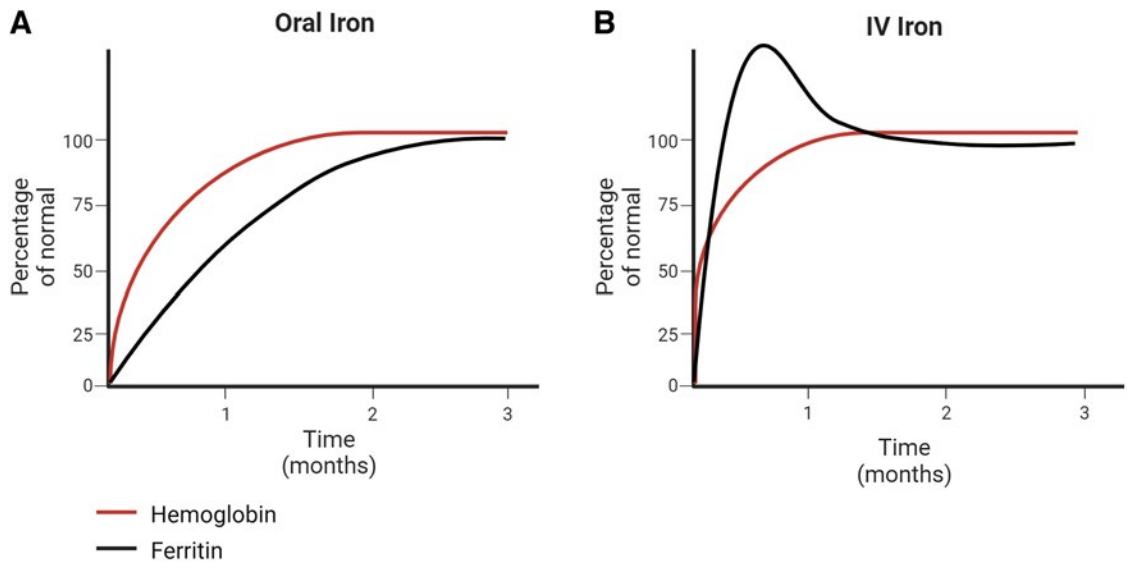

Figure 1. Laboratory changes following oral vs intravenous iron therapy. Expected changes in hemoglobin and ferritin in (A) patients with an optimal response to oral iron therapy and (B) patients treated with intravenous iron therapy.

tablet (65 mg elemental iron) once daily for the past 6 months but admits difficulty taking it consistently due to abdominal cramps that develop following tablet ingestion. Menarche was at 11 years of age, and her current menstrual cycles last 7 days and require frequent pad changing during the first 3 days, often accompanied by bleeding onto her clothing and bedsheets at night. She complains of fatigue, which is limiting her participation in sports activities and causing intermittent headaches.

Intravenous iron for classic iron-deficiency anemia

This patient represents a classic clinical scenario of IDA. Ongoing attempts to correct the iron deficiency with an alternative oral iron formulation or dosing regimen after 6 months of oral iron are unlikely to be successful. An appropriate response to oral iron is demonstrated by reticulocytosis within 5 to 7 days followed by an increment in hemoglobin within the first 1 to 2 weeks from initiation (Figure 1). In mild IDA, resolution of the anemia (ie, normalization of the hemoglobin) should occur within 1 month.

Children with moderate to severe IDA should have an improvement in the hemoglobin by at least 2 g/dL within a month, although many patients will demonstrate a more robust response with a hemoglobin increment of up to 4 to 5 g/dL within that same time frame. All patients require ongoing iron therapy beyond normalization of the hemoglobin to ensure iron stores are replenished (normalization of serum ferritin). Therefore, in this patient, due to the persistence of anemia well beyond 1 month of therapy, IV iron as a second-line strategy for her persistent IDA should administered.[1]

In any child with moderate to severe IDA and suboptimal response after 1 month of oral iron, IV iron should be strongly considered. For most insurance carriers in the United States, this is also the minimum amount of time required to justify consideration of IV iron in children outside an emergent setting such as the emergency department, during an inpatient admission, or with active, uncontrolled blood loss. Children with mild IDA who cannot complete a course of oral iron or remain iron deficient after normalization of hemoglobin may also be considered for IV iron therapy to mitigate ongoing long-term effects associated with prolonged iron deficiency.

Intravenous iron formulations

Four of the 6 available IV iron formulations in the United States have a Food and Drug Administration (FDA)–approved indication in pediatrics (Table 2). These include low-molecular-weight iron dextran (LMWD), iron sucrose (IS), ferric gluconate (FG), and ferric carboxymaltose (FCM).[2-11]

LMWD, the oldest available form of IV iron, is approved for use in children as young as 4 months of age. Approved dosing in pediatrics is 100mg per infusion. However, several case series in both adults and pediatrics have demonstrated the safety and efficacy of total dose infusions of up to 1000mg. A case series of 31 pediatric patients with diverse underlying etiologies of IDA, including nutritional IDA and IDA due to HMB, demonstrated good efficacy, although hypersensitivity reactions did occur.[3] Another larger series of 191 patients from 2021 demonstrated a hemoglobin increment of 2.1 g/dL and low rate of infusion reactions (4.7%).[12] Utilization of premedications should be considered with this formulation, particularly in children with atopic conditions or a history of hypersensitivity.

FG and IS were both approved over 20 years ago for children with chronic kidney disease (CKD). Dosing restrictions with FG result in limited utilization outside of children with CKD, for whom frequent infusions can be coupled with hemodialysis sessions.[13] Although also approved for children with CKD, the utility of IS has been demonstrated in diverse groups of children, including those with a poor response to oral iron and HMB.[6] IS allows for the administration of slightly increased doses compared to FG, although multiple infusions are required in adolescents to achieve the same total dose compared to newer formulations. A case series of 38 patients from 3 months to 18 years of age without CKD demonstrated that it was effective in raising the hemoglobin in all patient groups (median number of 3 infusions per patient). Of the 510 doses given, only 6 adverse events were recorded (5 mild; 1 serious event consisting of facial swelling and hypotension treated with epinephrine).[6] Today, IS remains one of the most commonly used IV iron formulations in children.

The FDA approved FCM for the treatment of children 1 year and older with iron deficiency who do not tolerate or have an unsatisfactory response to oral iron.[14] As one of the newer-generation IV iron formulations, large doses (15 mg/kg, maximum 750 mg [United States] or 1000 mg [Europe]) can be given in a single infusion over a shorter period of time compared to the older-generation formulations. Pediatric experience with FCM in patients with IDA refractory to oral therapy has demonstrated that patients with a hemoglobin less than 2 g/dL below the normal value for age have resolution of their IDA with 1 dose of FCM.[10] This is an advantage of FCM over other IV iron formulations that require multiple doses to resolve IDA.[2-4] Twenty-one patients with HMB within a larger cohort of children and adolescents with IDA refractory to oral iron therapy and treated with FCM demonstrated an improvement in median hemoglobin from 8.9 to 12.5, pre- and post-FCM infusion, respectively.[10] Patients with moderate to severe anemia (hemoglobin is >2 g/dL below

Table 2. Intravenous iron formulations with FDA-approved indications in children

Formulation	Approved pediatric indication	Approved dosing and administration notes*
Iron dextran†	Children over 4 months of age	Dose (mL) = 0.0442 (Desired Hgb − Observed Hgb) × LBW + (0.26 × LBW) Requires test dose prior to full therapeutic dose
Iron sucrose	Iron maintenance in patients ≥2 years with dialysis-dependent or non-dialysis-dependent CKD receiving erythropoietin therapy	Dose = 0.5 mg/kg, not to exceed 100 mg per dose every 2 weeks (for hemodialysis-dependent patients) or 4 weeks (for non-dialysis-dependent patients on erythropoietin therapy) for 12 weeks
Ferric gluconate	Treatment of IDA in pediatric patients ≥6 years undergoing chronic hemodialysis receiving erythropoietin therapy	Dose = 0.12 mL/kg (1.5 mg/kg of elemental iron) administered intravenously over 1 hour during 8 sequential dialysis sessions (maximum 125 mg per dose)
Ferric carboxymaltose	Treatment of children aged >1 year with IDA who are intolerant of oral iron or who have unsatisfactory response to oral iron	Patients <50 kg: 15 mg/kg/dose for 2 doses Patients ≥50 kg: 750 mg/dose for 2 doses Separate doses by at least 7 days Alternative dose is 15 mg/kg (maximum 1000 mg) as single infusion Associated with hypophosphatemia

*See product package insert for additional administration and dosing guidelines.

†Large intravenous doses associated with delayed reactions: arthralgias, fever, nausea, and chills 24 to 48 hours after administration.

Hgb, hemoglobin; LBW, lean body weight.

the lower limit for age) and those over 50 kg typically require 2 doses of FCM at least 7 days apart for a full treatment course.[10]

Two retrospective cohort studies in children refractory to oral iron treated with IV FCM reported no adverse effects experienced in 84% to 92% of patients. Pruritus and urticaria were the most common adverse events.[9,10] In pediatric studies, hypophosphatemia occurred in 11.3% to 14% of patients within 4 weeks of FCM administration, although no symptomatic hypophosphatemia was reported.[15,16] The typical nadir in phosphorus values occurs 2 weeks after infusion with subsequent improvement. It is recommended that patients with FCM have phosphorus levels monitored to ensure that no supplementation is required. In adults with underlying comorbidities receiving repeated treatment courses of FCM (median 17 infusions), osteomalacia causing fractures and pseudo-fractures has been reported. Switching IV iron formulations resolved the adverse skeletal symptoms in most patients.[17] The long-term effect on bone health in children and adolescents receiving a short treatment course of FCM is unclear, although vitamin D deficiency should be identified and treated in those receiving it.

Two additional newer-generation formulations, ferumoxytol and ferric derisomaltose, are FDA approved for utilization in adults but do not have a pediatric indication at this time. One case series of 110 ferumoxytol infusions administered in 54 pediatric patients (median age 11.7 years) with underlying GI conditions and/or as part of a patient blood management program for surgical patients demonstrated an improvement from baseline hemoglobin of 9.2 to 11.5 g/dL and 11.8 g/dL at 1 week and 4 weeks postinfusion.[18] Despite tolerance of previous doses, infusion reactions may occur with ferumoxytol upon repeat infusion.[19] No pediatric data on ferric derisomaltose are yet available.

Infusion reactions can occur with IV iron therapy, although severe allergic reactions such as anaphylaxis are rare. Rather than an IgE-mediated hypersensitivity reaction, most reactions to IV iron are likely complement-mediated pseudo-allergic responses triggered by the iron nanoparticles.[20] Historically, a test dose has been required for LMWD before full-dose administration. Recent adult studies, however, have demonstrated that the test dose may be omitted for certain patients.[21,22] Care should be taken to monitor patients during the initiation of an iron infusion for symptoms of hypersensitivity. Although not a vesicant, painless extravasation into the subcutaneous tissues with any formulation of IV iron can result in skin staining, and families should be counseled about this risk. The intensity and size of staining may be related to the concentration of the formulation (diluted administration with lighter stain) and volume distributed into the subcutaneous tissue. Over time, the iron may be absorbed, lessening the intensity of the stain, but there is no other known treatment, and laser therapy has not been demonstrated to be effective. Preventive measures such as IV placement away from joint spaces (including the antecubital fossa) may decrease the likelihood of extravasation, and patients who report pain around the IV site during an infusion should result in immediate cessation of the infusion to determine if a new IV must be placed.

Laboratory changes following intravenous iron

Hematologic response post-IV iron follows a similar timeline to patients closely adherent to oral iron, with a brisk reticulocytosis at 5 to 7 days followed by increase in hemoglobin. However, most patients receiving IV iron may see a greater improvement (compared to oral) since adherence to ongoing daily supplementation is unnecessary. In contrast to oral iron, wherein iron parameters normalize well after the anemia resolves, changes in iron values occur immediately with the administration of IV iron (Figure 1). Ferritin values may become markedly elevated within the weeks following iron infusions. As infused iron is used for red cell production and stored within other organs, levels trend down and plateau to a new, typically higher baseline value. In adults, observed persistence of very high serum ferritin levels 1 to 2 weeks after IV iron was not associated with any evident clinical consequence.[1] One case series of pediatric patients with underlying GI conditions demonstrated a rise in ferritin from 51 to 192 ng/mL at 1 week with trend down to 89 ng/mL at 4 weeks postinfusion.[18]

Intravenous iron as first-line therapy for severe IDA

Increased efforts promoting patient blood management emphasize consideration of upfront IV iron for individuals who have severe IDA (hemoglobin <7 g/dL). The American Society of Hematology–American Society of Pediatric Hematology Oncology and American Association of Blood Banks Choosing Wisely Campaigns recommend avoiding red cell transfusion in hemodynamically stable individuals with IDA and no active bleeding.[23,24] Two adult studies have demonstrated that implementation of an emergency department protocol for FCM infusion reduced red blood cell transfusions, hospital admissions, length of emergency department stay, and overall cost compared to preprotocol implementation.[25,26]

Although oral iron may be a reasonable option for children and adolescents with severe IDA in the absence of active bleeding, the ability to administer a total dose correction of the iron deficit in a single infusion reduces the disease burden to the patient and family. In patients with severe IDA and active bleeding, upfront administration of IV iron can mitigate prolonged anemia due to oral iron nonadherence, which is commonly seen in adolescence. Young children with severe IDA who have difficulty with oral medications should also be considered for upfront IV iron. Most oral iron formulations are not palatable, and in severe IDA, the risk of serious complications includes heart failure, stroke, and death.[27] In all patients with IDA, identification and elimination of the underlying cause is essential. This most commonly includes hormonal supplementation to limit uterine blood loss and increasing dietary iron content (along with limiting milk intake) in young children.

CLINICAL CASE 2

A 12-year-old previously healthy male presents with moderate anemia and progressive weight loss. Initial laboratory stores demonstrate hemoglobin of 7.8 g/dL, mean corpuscular volume of 74, ferritin of 60, transferrin saturation of 10%, and C-reactive protein of 10. Stool occult is negative but subsequent endoscopic studies are consistent with Crohn disease. He is initiated on oral iron as well as immune-modulating therapy but has not yet achieved remission. Repeat laboratory values after 1 month demonstrate no significant change, with a hemoglobin of 7.6 g/dL.

Intravenous iron for children with anemia due to iron deficiency and inflammation

In contrast to classic cases of IDA in otherwise healthy children, those with active inflammation, like this case, benefit from first-line therapy with IV iron. Inflammation results in upregulation of the iron regulatory hormone hepcidin, which both limits iron absorption from the duodenum and restricts the utilization of stored body iron. This iron-restricted state limits the effectiveness of oral iron. In contrast, IV iron bypasses the need for absorption and can partially overcome the restrictive effects of hepcidin on iron utilization, particularly at higher doses. Such benefits have been demonstrated in children and adolescents with IBD,[28] epidermolysis bullosa,[29] and heart failure.[30-34] Treatment of the underlying cause of inflammation is key to fully addressing the anemia.

In addition to the limitations of oral iron therapy with ongoing inflammation, children with disorders that affect the GI tract may have reduced absorptive capacity due to either anatomic changes (history of intestinal failure or other bowel surgery) or IBD resulting in inflammation, reduced absorption, and GI blood loss.[35] Patients with celiac disease in remission may continue to have inadequate oral iron absorption and may benefit from periodic IV iron.[36] Children receiving total parenteral nutrition (TPN) are also at risk for iron deficiency due to limitations in the amount of iron compatible within the TPN. In the United States, most TPN has no iron included. The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition Special Interest Group recommends IV iron for both children with intestinal failure and IDA and those with iron deficiency without anemia who cannot tolerate or are unresponsive to enteral supplementation.[37]

Intravenous iron for symptomatic low ferritin

Increasingly, children with symptomatic fatigue, restless legs syndrome, and other neurologic and sleep conditions with low ferritin in the absence of anemia are referred to hematology for consideration of IV iron. Data in these patient populations are limited, and variable target ferritin thresholds can further complicate management. A meta-analysis published in 2022 demonstrated that IV iron reduced fatigue scores in women with iron deficiency (defined as serum ferritin <100 ng/mL).[38] Most studies of adult women with fatigue and low ferritin have demonstrated greatest improvement of fatigue scores post-IV iron in the setting of confirmed iron deficiency (<20 ng/mL) with variable response when ferritin values are between 20 and 50 ng/mL.[39,40] One small prospective study in adolescent girls with fatigue demonstrated improved fatigue scores after a course of IS.[41] The role of IV iron in endurance athletes is unclear at this time. Benefits have been demonstrated in athletes with confirmed iron deficiency,[42] and in distance runners without iron deficiency, IV iron may improve perceived fatigue and mood.[43] Yet, compared to oral iron supplementation, IV iron has not demonstrated superiority in improving oxygen transport capacity or erythropoietic response.[44] Due to the lack of strong evidence yet potential benefit, a shared decision-making approach that reviews the risks and benefits of iron therapy (both oral and IV) with patients and families is recommended.

Summary

IV iron therapy can be considered in a wide variety of pediatric patients. Patients with classic IDA should receive a trial of oral iron, but a poor initial response (as early as 4 weeks) may prompt consideration of IV iron therapy. Patients with persistent, refractory, or recurrent IDA should be treated with IV iron. Children with concomitant inflammatory diseases should be treated with IV iron to overcome the restricting effects of hepcidin. All patients and families should be appropriately counseled on the indications, benefits, and risks of IV iron, and in the context of limited data, a shared decision-making approach is advised.

Conflict-of-interest disclosure

Clay T. Cohen: no competing financial interests to declare.
Jacquelyn M. Powers: discloses consultancy for Pharmacosmos, LLC.

Off-label drug use

Clay T. Cohen: Off-label use of IV iron in pediatrics is discussed.
Jacquelyn M. Powers: Off-label use of IV iron in pediatrics is discussed.

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EVIDENCE-BASED MINIREVIEW

Incidence, mechanism, and consequences of IV iron–induced hypophosphatemia

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LEARNING OBJECTIVES

- Define the incidence and risk factors for intravenous iron–induced hypophosphatemia, which is primarily caused by ferric carboxymaltose
- Explain the underlying pathophysiology of intravenous iron–induced hypophosphatemia and how elevations of FGF23 cause renal phosphate wasting
- Recognize signs and symptoms of severe hypophosphatemia to guide appropriate management

CLINICAL CASE

A 23-year-old woman presents with progressive fatigue and decreased exercise tolerance. Because of suspected iron deficiency from heavy menstrual bleeding, she was previously advised to take oral iron, but gastrointestinal distress limits her adherence. Laboratory testing reveals iron deficiency anemia: hemoglobin 9.8 g/dL and ferritin 4 ng/mL. She is referred for intravenous (IV) iron and gynecologic management of heavy menstrual bleeding. Based on the infusion clinic's formulary, she receives 2 weekly doses of ferric carboxymaltose (FCM), 750 mg each. One week later, she continues to experience fatigue and weakness, and she now describes new muscle aches and brain fog. Her physician initially attributes these symptoms to iron deficiency and counsels that they will soon resolve, but symptoms progress over the next day, leading her to present to the emergency department for further evaluation. Laboratory testing reveals improved hemoglobin, but a serum phosphate of 0.9 mg/dL (normal: 2.6–4.6 mg/dL), prompting inpatient admission.

Introduction

IV iron therapy is widely used to treat iron deficiency anemia because it is a more effective and faster means of repleting iron stores compared to oral iron.[1] Multiple IV iron formulations exist, but newer agents such as FCM, ferric derisomaltose (FDI), and ferumoxytol (FMX) enable safe administration of high doses of iron in 1-2 infusions. Despite these advances, hypophosphatemia following IV iron administration, which was once considered benign and self-limited, is now recognized as a serious adverse effect of certain IV iron formulations, most notably FCM.[2]

Several randomized trials and a comprehensive systematic review and meta-analysis reported a significantly higher incidence of hypophosphatemia, ranging from 47%-75%, among those treated with FCM versus <10% among patients treated with FDI,[2-4] FXM,[5] and low molecular weight iron dextran (LMWID).[6] Besides causing the highest rate of hypophosphatemia, FCM is also the only formulation associated with severe (<1.0 mg/dl) and prolonged hypophosphatemia that can persist for weeks to several months.[2]

Pathophysiology

FCM infusion causes acute 3- to 6-fold increases in circulating concentrations of fibroblast growth factor-23 (FGF23), which, along with 1,25-dihydroxyvitamin D and parathyroid hormone (PTH), is one of the main regulators of serum phosphate. The spike in FGF23 causes inappropriate urinary phosphate excretion due to reduced proximal tubular reabsorption of filtered phosphate.[2] As a result, phosphate levels typically reach their nadir approximately 2 weeks following the initial FCM infusion.[3,6,7] FGF23 also decreases circulating concentrations of 1,25-dihydroxyvitamin D, leading

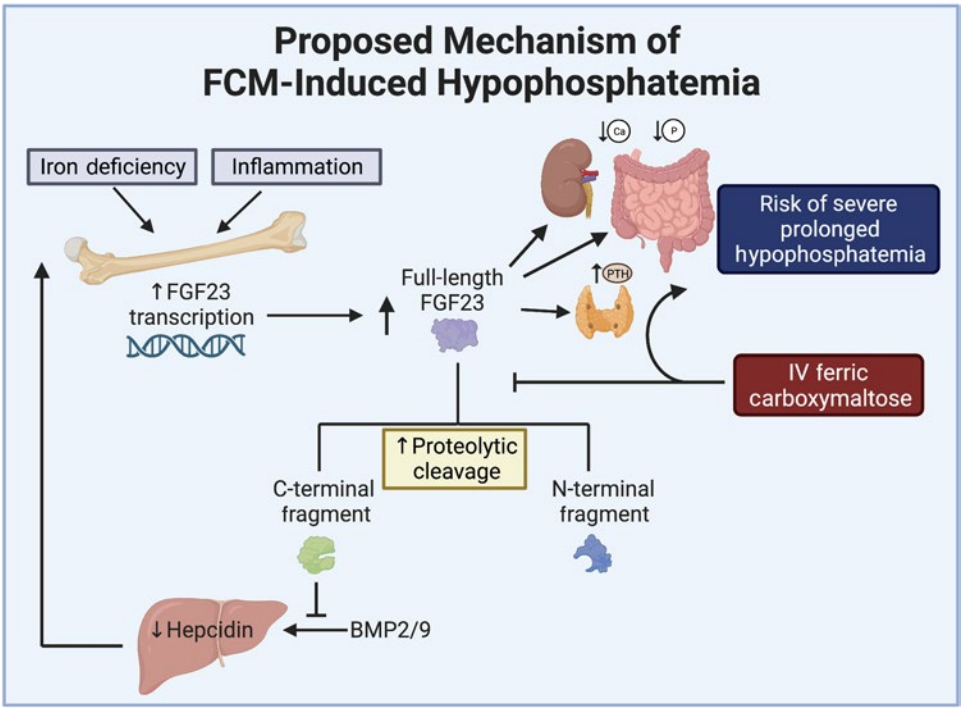

Figure 1. Proposed mechanism of ferric carboxymaltose (FCM)-induced hypophosphatemia. Iron deficiency increases FGF23 production, but this is balanced by increased cleavage of FGF23 into its C- and N-terminal fragments that do not affect phosphate homeostasis. Through unclear mechanisms, FCM appears to reduce FGF23 cleavage, resulting in higher levels of full-length FGF23 and, thus, hypophosphatemia. Independent of phosphate homeostasis, C-terminal FGF23 peptides appear to share a negative feedback loop with hepcidin to regulate iron homeostasis by inhibiting bone morphogenic protein (BMP) 2/9-mediated hepcidin production.

to decreased absorption of phosphate from the gut, decreased serum calcium, and secondary increases in PTH. Since PTH also increases renal phosphate excretion, FCM-induced hypophosphatemia is maintained beyond the initial period of FGF23 elevation by a second wave of PTH-mediated renal phosphate wasting.[8]

Growing evidence suggests intricate links between iron and phosphate homeostasis. Iron deficiency and inflammation increase FGF23 transcription and translation in bone, but do not cause hypophosphatemia because increased production of full-length FGF23 is coupled to an increase in intracellular proteolytic inactivation of FGF23 into C- and N-terminal fragments that do not affect phosphate homeostasis (Figure 1).[9] Though the exact mechanism remains unknown, it has been proposed that FCM uncouples FGF23 production and cleavage such that the excessive FGF23 production driven by iron deficiency culminates in higher full-length FGF23 levels upon FCM administration and, thus, hypophosphatemia.[6] The main difference between IV iron formulations—their carbohydrate moieties—may explain their differential effects on FGF23, but this warrants additional investigation.[6]

Independent of phosphate homeostasis that is regulated by full-length FGF23, C-terminal FGF23 fragments also regulate iron homeostasis. Recently, Courbon et al. reported that iron-deficient mice that are unable to augment production of C-terminal fragments had inappropriately increased hepcidin levels that further exacerbated iron deficiency.[10] Conversely, overexpression of C-terminal FGF23 fragments suppressed hepcidin levels by inhibiting bone morphogenic protein (BMP) 2/9 signaling. These novel findings suggest that osteocytes produce C-terminal FGF23 fragments in iron deficiency as part of a negative feedback loop to suppress hepcidin.

Risk factors and clinical manifestations of hypophosphatemia

In addition to the specific IV iron formulation, other risk factors for FCM-induced hypophosphatemia include normal kidney function, more severe iron deficiency, lower body weight, lower baseline serum phosphate, abnormal uterine bleeding as the etiology of iron deficiency, and repeated doses of IV iron.[2,5,9] Patients with kidney disease are partially protected from FCM-induced hypophosphatemia due to decreased glomerular filtration of phosphate, which limits urinary phosphate excretion.[9] Despite these findings, it is important to emphasize that even patients with no demonstrable risk factors can develop severe and prolonged hypophosphatemia after receiving FCM, and it remains challenging to accurately predict risk, severity, or duration of hypophosphatemia in individual patients.

Acute clinical manifestations of IV iron–induced hypophosphatemia include asthenia, fatigue, and myalgias, all of which reflect tissue-specific energy depletion due to decreased adenosine triphosphate (ATP) production. More severe cases can cause bone pain, myopathy, cardiac arrhythmia, respiratory failure, encephalopathy, and seizures.[9] Anecdotally, many patients report brain fog, perhaps as a consequence of ATP depletion in the brain. Symptoms of hypophosphatemia can mimic those of

iron deficiency anemia, which likely contributes to missed or delayed diagnoses in many affected patients. Chronic hypophosphatemia, particularly in those treated with repeat doses of FCM, can lead to musculoskeletal complications, including osteomalacia and fragility fractures.[7]

Treatment of IV iron–induced hypophosphatemia

Management of IV iron–induced hypophosphatemia is challenging. Oral and IV phosphate supplementation do not durably sustain normal serum phosphate because of the ongoing renal phosphate leak caused by elevated FGF23 and PTH.[9] Supplementing 1,25-dihydroxyvitamin D can help correct secondary hyperparathyroidism, but evidence to support its use in IV iron–induced hypophosphatemia is limited.[9] Furthermore, both phosphate and 1,25-dihydroxyvitamin D supplementation are known stimuli of FGF23, limiting their effectiveness. Therefore, primary prevention of hypophosphatemia by using iron formulations other than FCM is preferred. When formularies mandate use of FCM, we recommend serum phosphate testing at week 1, prior to administering the second infusion of FCM. If a patient

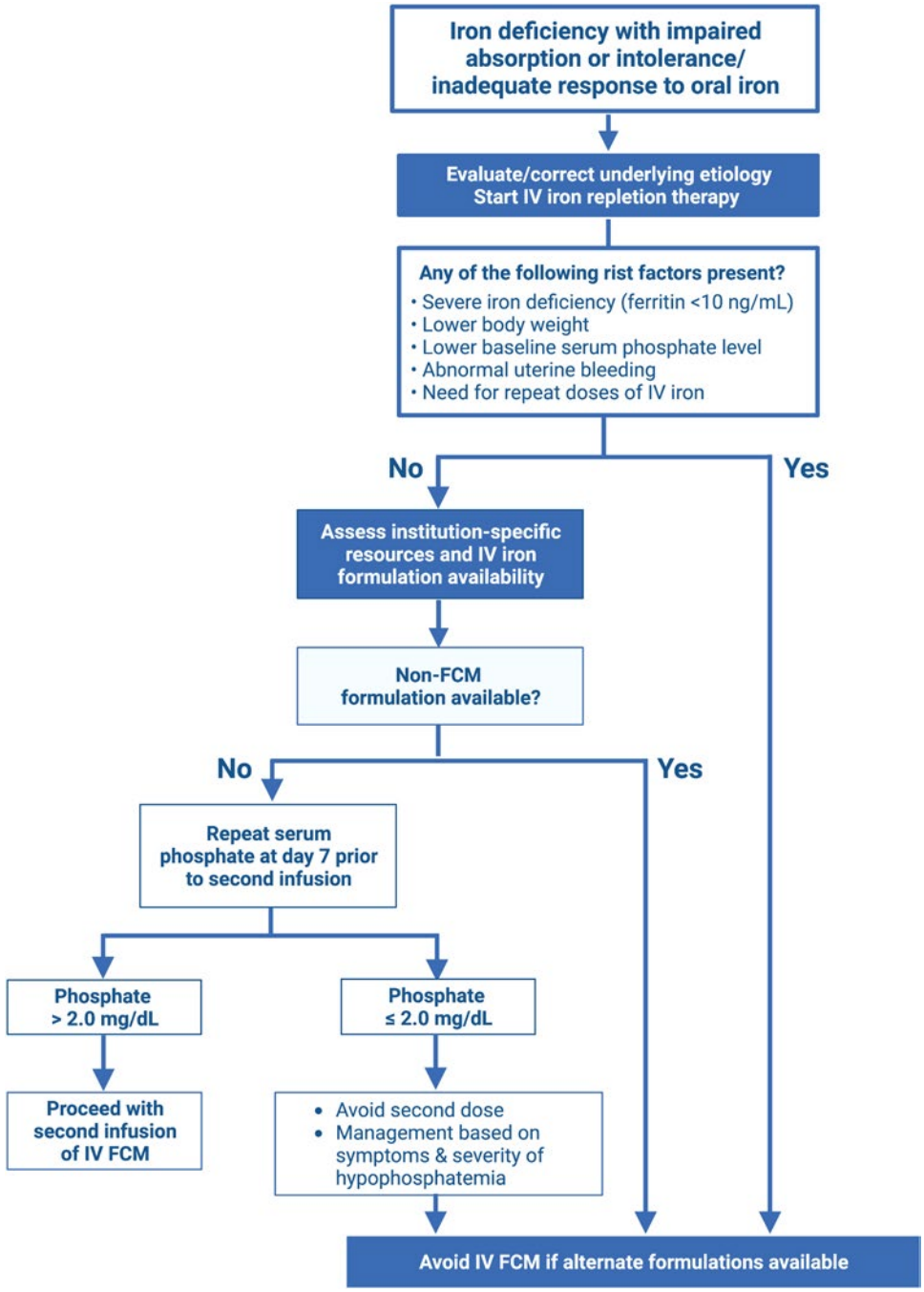

Figure 2. Algorithm for the selection and safe administration of intravenous (IV) ferric carboxymaltose (FCM) to avoid hypophosphatemia.

already manifests hypophosphatemia, the second dose should be withheld to prevent exacerbation of hypophosphatemia. Furthermore, we recommend that the risk of hypophosphatemia and its potential consequences be discussed with patients prior to any infusion.

Conclusions

Despite the high incidence of IV iron–induced hypophosphatemia, many physicians are still unaware and do not recognize acute and long-term complications of FCM. While considering the numerous constraints of modern-day practice, including infusion center availability, drug formulary limitations, and insurance coverage, it is imperative to avoid FCM in patients who require repeated doses (Figure 2). Future studies are needed to investigate why FCM, unlike other IV-iron formulations, disrupts the delicate balance between FGF23 production and cleavage in bone to cause severe hypophosphatemia. An improved understanding of the pathophysiology might help advance therapies to mitigate significant clinical consequences.

Recommendations

1. FCM should be avoided in the treatment of iron deficiency when alternate IV iron formulations are available, especially in patients who require repeat doses. (Grade 1B)
2. Serum phosphate should be tested at week 1, prior to administering the second infusion of FCM, and the second dose should be withheld in those with serum phosphate ≤2.0 mg/dL. (Grade 1B)
3. Symptoms of fatigue, weakness, myalgias, brain fog, and bone pain following IV FCM should prompt evaluation of serum phosphate, consideration of inpatient therapy if severe hypophosphatemia is confirmed, and avoidance of FCM in the future. (Grade 1A)

Conflict-of-interest disclosure

Kylee L. Martens: no competing financial interests to declare.
Myles Wolf: equity interests in Akebia, Unicycive and Walden; consultancy: Bayer, Enyo, Jnana, Launch, Pharmacosmos, and Reata.

Off-label drug use

Kylee L. Martens: Nothing to disclose.
Myles Wolf: Nothing to disclose.

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Managing pregnancy in patients with sickle cell disease from a transfusion perspective

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Advances in the management of sickle cell disease (SCD) have made it possible for most female patients (whether homozygous or compound heterozygous) to reach childbearing age and become pregnant. However, even in the less symptomatic forms of SCD a high risk of complications during pregnancy and the postpartum period can occur for both the mother (1% to 2% mortality) and the fetus. Coordinated care from the obstetrician and the sickle cell disease expert is essential, together with the active participation of the patient. Vaso-occlusive complications, such as vaso-occlusive crisis and acute chest syndrome, often increase in frequency when hydroxyurea treatment is interrupted. Obstetric complications, such as pre-eclampsia, fetal growth restriction, and preterm delivery, are more common in women with SCD. Recent meta-analysis–based studies support prophylactic transfusion. However, there have been no randomized trials assessing the benefits of prophylactic transfusion. Given the known risk of transfusion complications, including delayed hemolytic transfusion reaction and hyperhemolysis, transfusion is not systematically performed in pregnant women with SCD. We describe here a case-by-case approach to the management of pregnancy in women with SCD based on the medical and transfusion history of each patient.

LEARNING OBJECTIVES

- Understand maternal and fetal complications during pregnancy in SCD patients
- Evaluate the transfusion risk in pregnant women with SCD
- Learn how to manage treatment and transfusion in pregnant patients with SCD

Introduction

During pregnancy, sickle cell disease (SCD)–related complications, vaso-occlusive crises (VOCs), acute chest syndrome (ACS), and infections increase in frequency. The rates of obstetric complications, pre-eclampsia, prematurity, intrauterine growth restriction, and mortality are significantly higher in women with SCD than in other pregnant women.[1-4] Transfusion (TF)/exchange TF and hydroxyurea (HU) are the 2 most commonly prescribed preventatives and treatments for vaso-occlusive processes in patients with SCD.[2,5] HU is not recommended during pregnancy and may be used only in special cases.[6,7] There are not enough randomized trials assessing the benefits of prophylactic exchange TF in pregnancy to recommend its systematic use.[5,8-10] The risks of a delayed hemolytic TF reaction (DHTR) and alloimmunization must be considered because DHTR is associated with significant morbidity; TFs cannot, therefore, be systematically recommended.[11-13]

The American Society of Hematology (ASH) Expert Panel concluded that the number of robust randomized trials is insufficient to recommend a systematic prophylactic TF strategy for pregnant women with SCD. Women with a history of severe complications or comorbid conditions related to SCD (nephropathy) or a history of complicated pregnancies could undergo prophylactic TF at regular intervals early in pregnancy. However, no threshold percentage of sickle cell Hb (HbS) has been established as a target for TF.

DHTR is a severe TF complication diagnosed by the rapid disappearance of transfused red blood cells (RBCs). The diagnosis is made a few days after a TF with a drop in Hb and an increase in hemolysis markers, often accompanied by a vaso-occlusive complication. The diagnosis can also

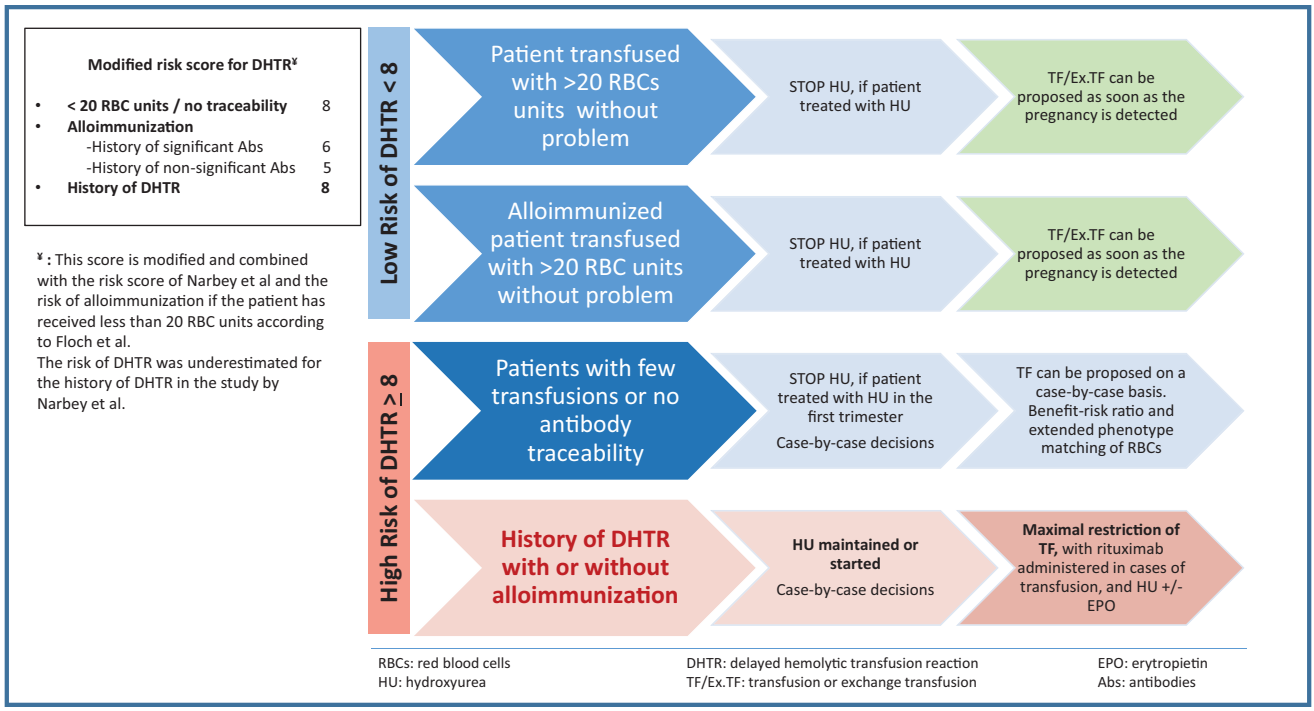

Figure 1. How to manage TF decisions in pregnant SCD patients. abs, antibodies.

be made by monitoring HbA, which represents the TF and disappears in 2 forms: "mild DHTR" and "DHTR or hyperhemolysis," according to the Mekontso nomogram (Visual Summary).[14]

Patients at risk of DHTR are identified using the DHTR risk score developed by Narbey et al.[15] This score is based on the number of RBCs received in the patient's lifetime, the presence of alloimmunization, and the patient's history of DHTR. Based on this risk, patients can be identified as "high responders" (patients with alloimmunization after a few TFs) and "low responders" (patients with no immunological reaction to TFs).[14,15] Patients with a history of DHTR have a risk score for DHTR greater than 8 and are considered high responders with a greater risk of DHTR recurrence, which is fatal in 6% of cases (Figure 1).[13] TFs should be limited as much as possible. Patients with a low DHTR risk score (<8) can undergo TF with no major risk, and patients who have received too few TFs or have undergone TF without antibody tracking are considered to be at risk because it cannot be determined whether they are high or low responders. Such patients are 8 times more likely to develop DHTR.[14,16]

Using clinical cases, we attempt to refine the decision-making process based on TF risk analysis and the algorithms published in the ASH guidelines (Figure 1).

CLINICAL CASE 1

A 26-year-old woman with homozygous SCD was followed for pregnancy. Her basal hemoglobin (Hb) level was 8 g/dL, with approximately 3 VOCs per year and a history of 2 intensive care unit (ICU) admissions for ACS requiring TF with RBCs. HU treatment was initiated after her first hospitalization for ACS. She had received 45 RBC units during her lifetime, without alloimmunization. The patient had no chronic lesions other than minimal microalbuminuria treated with an angiotensin converting enzyme inhibitor (this treatment was stopped during pregnancy). The patient's obstetric history included only 1 early spontaneous miscarriage. HU treatment was stopped when the pregnancy was detected, and the patient was placed on a 6-weekly erythrocytapheresis program. No episodes of VOC or DHTR occurred during the pregnancy. The hematologist and obstetrician visited the patient alternately, at 2-week intervals. Proteinuria did not increase (proteinuria/creatininuria <50 mg/mmol), and blood pressure remained normal. Fetal growth was monitored monthly, with biometrics between the 10th and 20th percentiles. It was decided to induce labor after cervical preparation with prostaglandins at 38 weeks and 1 day. Epidural anesthesia was induced before artificial rupture of the membranes and oxytocin infusion. The patient spontaneously delivered a boy weighing 2705 g. Postpartum blood loss was estimated at 300 mL. The patient decided not to breastfeed in order to resume treatment with HU. No VOCs occurred during the postpartum period, Hb levels remained under control at 7.4 g/dL, and the patient was discharged home after 1 week.

Questions raised by this case: why was the decision to transfuse taken so early? The patient was considered a low responder with a very low TF risk, (DHTR risk score <8). She had already received a large number of RBC units without becoming alloimmunized or suffering any other adverse event. It was therefore considered reasonable to initiate an effective treatment, such as erythrocytapheresis or partial exchange (depending on the patient's venous capital) as soon as possible after stopping HU treatment. The patient's microalbuminuria constituted an

additional risk factor for obstetric complications, further justifying the decision to transfuse.[2]

CLINICAL CASE 2

Mrs. B, a 28-year-old woman with homozygous SCD, was followed for pregnancy. Her baseline Hb level was 8 g/dL. She had 1 to 2 VOCs per year and had never been hospitalized for ACS. She had received only 6 RBC units during her lifetime and was alloimmunized, with anti-Jk^b antibody detected at the last TF. The patient had no cardiac or neurological history but had been treated for proliferative retinopathy 1 year before the pregnancy. The patient reported a pregnancy termination for medical reason. The pregnancy that followed was spontaneous. HU treatment was stopped as soon as the pregnancy was discovered. The patient was hospitalized for 1 day for a VOC at 12 weeks' gestation. A second hospitalization for a VOC occurred at 14 weeks' gestation, by which time the patient's Hb concentration had fallen to 5.1 g/dL. The patient received a TF of 2 RBC units in the emergency department in accordance with the standard phenotyping protocol for RBCs. After TF the patient had an HbA level of 36%. One week later, she was readmitted to the hospital for a third VOC with dark urine and high lactate dehydrogenase levels. The patient's HbA level decreased to 15%, and her Hb concentration was 6 g/dL; a diagnosis of DHTR was retained (Figure 2). The patient required no specific treatment for DHTR other than erythropoietin (EPO) and iron. Twenty days after the TF, the patient's HbA level had fallen to 0%, and a nonspecific antibody was detected.

At 19 weeks' gestation, the patient was hospitalized for a fourth VOC and admitted to the ICU for ACS without associated pulmonary embolism. HU treatment was initiated at 20 weeks' gestation. The pregnancy was also characterized by vascular growth retardation (third percentile) at 25 weeks' gestation, followed by pre-eclampsia diagnosed at 30 weeks and 1 day. The patient did not receive antenatal corticosteroids because of the risk of VOC and ACS. It was decided to perform a cesarean delivery at 34 weeks and 5 days due to abnormal fetal heart rate. The cesarean was performed under spinal anesthesia, and a boy weighing 1735 g was delivered and immediately transferred to the neonatal ICU. The patient had another VOC on postpartum day 2 but did not require TF. She chose not to breastfeed in order to continue treatment with HU.

Questions raised by this case: why was the decision to transfuse made so late? The patient was considered a high responder with a high risk of DHTR (few TFs and prior alloimmunization). It was therefore decided not to transfuse early. TF was performed when the patient was admitted to the emergency department for a VOC. The interruption of HU treatment led to a recurrence of VOC and hospitalization. We were forced to manage the recent DHTR in this patient without the possibility of TF. HU with or without EPO is a real alternative in such patients. For patients with a history of DHTR, discussions can be held in advance to determine the true feasibility of TF in an emergency situation (the availability of frozen or liquid-phase units or the unavailability of compatible RBC units) and the timing of immunotherapy, such as rituximab before TF (off-label).[16-19]

Background and current knowledge about pregnancy in SCD patients

Improvements in the effectiveness of care for women with SCD have made it possible for many of them to become mothers, whereas in previous decades pregnancy was not recommended or was associated with very high-risk mortality rates.[4] However, the risk of maternal death remains 50 times higher in these women than in the general population (Figure 3).[2,20] Obstetric risks include preterm delivery, which has been reported in 9% to 45% of pregnancies in women with SCD, depending on the

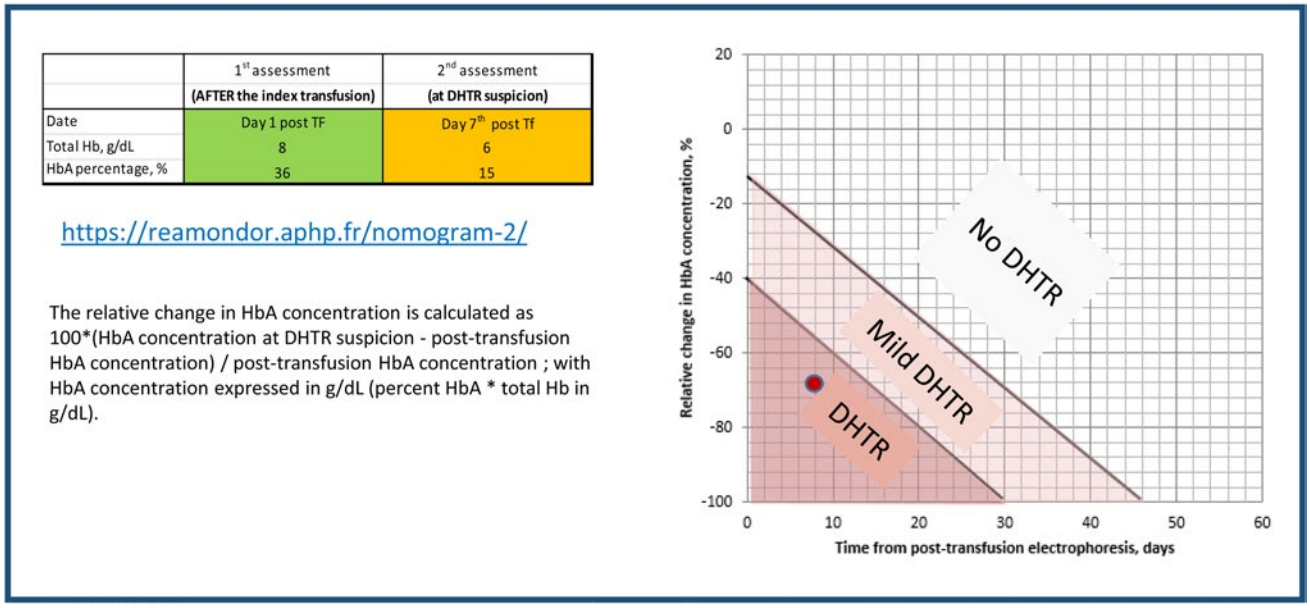

Figure 2. Mekontso-Dessap nomogram confirms the diagnosis of DHTR.

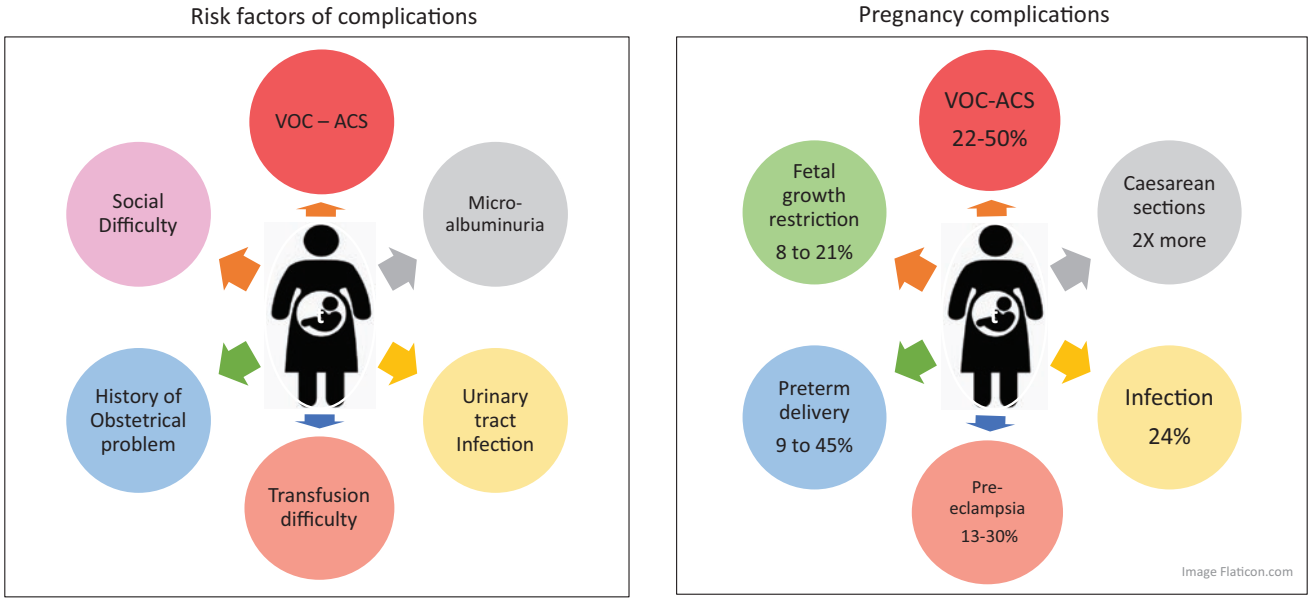

Figure 3. Risk factors of complications and complications rate.

series considered.[1,2,4,8,21] Several social and obstetric factors may account for the high risk of preterm delivery, including infections, particularly those affecting the urinary tract. It is important to ensure that patients receive appropriate doses of antibiotics relative to their glomerular filtration rate. Razazi et al. showed that plasma antibiotic levels were very low in patients with glomerular hyperfiltration and that young SCD patients often had high glomerular filtration rates.[22]

Pre-eclampsia is much more common in SCD patients than in the general population, occurring in 13% to 30% of pregnancies in women with SCD, vs only 2% to 5% of pregnancies in the general population.[3,23,24] Preexisting maternal endothelial dysfunction and early placentation abnormalities account for this high incidence.

The incidence of fetal growth retardation ranges from 8% to 21%, depending on the study, and increases the likelihood of induced preterm birth and the risk of fetal or neonatal death.[1,2] This growth retardation may be due to chronic fetal hypoxia and rheological abnormalities of maternal red blood cells in the placenta, as placental lesions are often found on pathology examination. Cordier et al recently showed that the placental villi of mothers with SCD were thinner than those of controls and that the rate of fibrinoid necrosis was higher in these mothers, who also had overabundant syncytial ganglia.[18] Mifsud reported patchy placental infarction and fetal growth restriction relative to controls.[25] The risk of fetal death is higher in this population than in the general population (1-4%). The causes of this higher risk remain unclear, but the risk appears to be highest during the third trimester.[23] Women with SCD undergo physiological changes during pregnancy, with increases in both anemia rates and cardiac output. Special attention must be paid to cardiac ultrasound findings for these patients.[2,26]

More than 50% of pregnancies in women with SCD end in cesarean delivery.[1,2,15] This high rate of cesarean delivery is due to a combination of several hematologic and obstetric risk factors, including a higher incidence of fetal growth restriction and pre-eclampsia and induced labor. The incidence of ACS increases in the postpartum period, and clinicians must be particularly attentive to the possibility of this complication.[1,27]

How should treatment be managed?

A large proportion of women with SCD are on HU treatment due to acute complications, such as VOC or ACS. Given the small number of publications on HU exposure during pregnancy, evidence on its safety is limited, and current recommendations state that HU treatment should be stopped 3 to 6 months before conception. HU is known to be teratogenic in animals, especially during the first trimester of pregnancy, but the doses used in animal studies are very different from those used in human treatment.[6] Kroner et al performed a retrospective multicenter study of 1788 pregnancies, 241 of which were exposed to HU.[7] Hydroxyurea increased the rate of spontaneous abortion and fetal death in utero when used just before and during early pregnancy, whereas the use of HU in the second and third trimesters appeared to improve pregnancy outcomes.[7] However, TFs and their effect were not considered in this study. The ESCORT-HU study, a European prospective multicenter study of a cohort of 1960 patients, found no evidence of teratogenicity in the 110 pregnancies most exposed in the first trimester to HU. Additionally, the rate of miscarriage was similar to that in the general population, and obstetrical complications were similar to published incidence of complications in SCD women. However, these studies do not provide sufficient evidence to recommend the systematic use of HU during pregnancy.[6]

Transfusion and its risks

Most SCD patients have undergone TF/exchange TF procedures during their lifetime, regardless of the region or country in which they live. Several groups have reported the use of TF or exchange TF during pregnancy but not in randomized trials.[7,15] A systematic review and meta-analysis suggested that prophylactic TF

strategies may reduce maternal and neonatal morbidity and mortality.[7] In addition, several groups have shown that TFs can effectively reduce the incidence of vaso-occlusive complications, such as VOC and ACS during pregnancy.[5,8,10,23] When clinicians are considering the possibility of TF, they must take into account the risk of DHTR and alloimmunization associated with TF.[13] The detection of placental abnormalities may lead to a decision to use TF in the early stages of pregnancy, during placental development, to improve blood circulation.[23,27] In most of the studies reporting no obstetric benefit of TF, exchanges were not initiated during the first trimester of pregnancy.[5,15] Knowledge of a patient's TF history is a key element to take into account when determining the risk/benefit ratio and deciding whether or not to proceed with TF. The indications for TF have changed significantly with improvements in our understanding of TF complications, including DHTR in particular. The immunohematological follow-up of patients remains an important issue due to the evanescence and secondary disappearance of antibodies detected at a given time.[8,10,21] RBCs must be compatible for Rh and Kell phenotypes, taking into account the patient's history of TF-related antibodies. TF protocols are continually updated according to the patient's immunohematological status.[14-16] In a series of 99 cases of DHTR, 31% of affected patients were pregnant women.[3] In this study performed between 2000 and 2013, patients were transfused systematically from the 22nd week of pregnancy, regardless of the patient's TF history or number of previous TFs.[15] Narbey et al attempted to identify risk factors for DHTR and found that patients who had previously received fewer than 12 RBC units were at risk because their low- or high-responder status could not be determined.[9] Other risk factors identified were a history of DHTR or alloimmunization.[9] Bauer et al reported an excess risk of alloimmunization in pregnant women.[11] Based on the recent study by Floch et al showing that the majority of alloimmunizations of interest occur before the TF of 20 RBC units and the latest guidelines for the prevention of alloimmunization, we recommend the approach described in Figure 1.[28]

TFs: who should receive them, when and how?

When assessing the risk of alloimmunization and hemolysis, patients can be classified into 4 groups (Figure 1:

1) **Patients who have received more than 20 RBC units without adverse effects:** These patients can be transfused according to the usual indications and recommendations. Patients treated with HU can be transfused as soon as HU is stopped, and patients without HU treatment can be transfused at the first sign of a clinical or biological problem or according to their history.[11]
2) **Alloimmunized patients with no history of DHTR** who have received more than 20 RBC units with no adverse event can undergo TF at the time of pregnancy detection if they were on HU treatment. TF can also be initiated for patients not on HU treatment at the time of pregnancy diagnosis or at the first sign of a clinical or biological problem.[15,16]
3) **Patients with a history of DHTR:** These patients are at high risk of DHTR if they undergo TF. If they are already on HU treatment, it is probably advisable to continue treatment, as vaso-occlusive complications may be more frequent if this treatment has already been prescribed. For patients not already on HU, this treatment should be initiated at the first clinical and laboratory signs of complications. EPO may also be used to treat anemia. The indications for TF in these patients are very limited. If an acute complication renders TF unavoidable, patients should receive prior immunotherapy (anti-CD20 antibody "off-label"), and TF-sparing measures (HU and high-dose EPO) can be implemented. The recommended TF protocol includes prophylactic expanded phenotype (FY, JK, MNS) matching for RBCs, regardless of the patient's alloimmunization status, and taking known antibodies into account. Outside the context of life-threatening emergencies, decisions about TF should be made in collaboration with experts and the TF center.[10–13,22]
4) **Patients who have received very few TFs (<20 RBC units) or who have been transfused without antibody traceability:** In these patients, the risk/benefit ratio of TF must be carefully evaluated due to the absence of information about responder status and the likelihood of TF reactions. In such cases, we recommend prophylactic extended phenotype (FY, JK, MNS) matching for RBCs, with weekly monitoring of HbA levels to monitor efficacy and the use of Mekontso nomograms to detect mild DHTR.[29] In case of complications, it should be borne in mind that DHTR remains a rare event and that TFs can be helpful. Finally, very complex situations may arise in patients with a rare blood phenotype who are also alloimmunized. In such cases the TF strategy must be discussed by a multidisciplinary team. Decisions depend on the resources available in the national rare blood bank, but alternatives to TF may be considered (HU, EPO). In all cases, TF performance should be monitored for up to 1 month by determining the percentage of HbA and through TF screening tests.[14,29]

Other actions: The National Institutes of Health recommends aspirin treatment, at a dose of 160mg per night, in pregnant women with SCD.[2,30] Ribeil et al showed, in a retrospective study, that home oxygen therapy at night may be safe and may reduce the need for TF.[12] The results of a randomized trial of oxygen therapy are currently being analyzed. Teratogenic treatments, such as iron chelators and enzyme conversion inhibitors, are generally discontinued during pregnancy in accordance with recommendations.

Conclusion

Pregnancy is a high-risk period for both mother and fetus, but TFs can provide protection if the risk of DHTR is low. The alternative to TF in patients with a history of DHTR is HU. In other cases, the ASH Expert Panel concluded that each case should be discussed individually to ensure the most appropriate decision is made. Collaboration between the hematologist, obstetrician, TF center, and anesthesiologist is critical in the management of pregnant women with SCD.

Conflict-of-interest disclosure

Anoosha Habibi: consultancy: GBT, Vertex, Novartis, addmedica.
Alexandra Benachi: no competing financial interests to declare.
Edouard Lecarpentier: no competing financial interests to declare.

Off-label drug use

Anoosha Habibi: Rituximab, erythropoietin, hydroxycarbamide. Alexandra Benachi: Rituximab, erythropoietin, hydroxycarbamide. Edouard Lecarpentier: Rituximab, erythropoietin, hydroxycarbamide.

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Logistics, risks, and benefits of automated red blood cell exchange for patients with sickle cell disease

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Red blood cell (RBC) transfusions treat and prevent severe complications of sickle cell disease (SCD) and can be delivered as a simple or exchange transfusion. During an exchange, some of the patient's abnormal hemoglobin (Hb) S (HbS) RBCs are removed. An apheresis device can accomplish an automated RBC exchange, simultaneously removing patient's RBCs while returning other blood components along with normal RBCs. Automated RBC exchange is therefore an isovolemic transfusion that can efficiently decrease HbS RBCs while limiting iron loading and hyperviscosity. However, specialized equipment, trained personnel, appropriate vascular access, and increased RBC exposure are required compared to simple or manual RBC exchange. Therefore, risks and benefits must be balanced to make individualized decisions for patients with SCD who require transfusion.

LEARNING OBJECTIVES

- Define appropriate venous access options for automated RBC exchange based on size of patient
- List 3 benefits of automated RBC exchange compared to simple transfusion

CLINICAL CASE

A 16-year-old Black male weighing 65 kg with homozygous SS sickle cell disease (SCD) developed an abnormal transcranial doppler at age 8 while treated with hydroxyurea (HU). He has been treated with monthly transfusions and HU for 8 years. His pretransfusion hemoglobin on HU is about 9–10 g/dL. He has received a mixture of simple and manual exchange transfusions (depending on pretransfusion hemoglobin). He is prescribed iron chelation but frequently misses doses and has evidence of iron overload. His peripheral access has been difficult, and some attempts at manual exchange have been aborted due to failed access, resulting in higher-than-target hemoglobin S (HbS) levels. Automated RBC exchange (aRCE) has been discussed, but patient has been resistant to surgery for placement of a central venous catheter (CVC). While he is trying to make his decision, he asks if he could stop chelation if he transitioned to aRCE and what changes in his HbS he could expect. This review will discuss the logistics, risks, and benefits of aRCE in SCD in order to address these and other clinical questions.

Introduction

Red blood cell transfusion was the first disease-modifying therapy for individuals with SCD and remains an integral component of the management of SCD today, both acutely to treat and prophylactically to prevent severe SCD complications. Transfusions provide benefit by increasing Hb and, therefore, oxygen delivery to tissues, but also by decreasing abnormal HbS RBCs that contribute to SCD vaso-occlusive pathophysiology. Transfusions can be administered either as a simple transfusion by infusing normal HbA RBCs to dilute the HbS cells or as an exchange transfusion to remove HbS RBCs and replace them with HbA RBCs. Exchange transfusions can be accomplished manually through alternating cycles of phlebotomy and transfusion or as an automated procedure using an apheresis device. Whole blood is drawn from the patient into the device where blood components are separated in a centrifuge according to density. The most dense RBCs will layer on the bottom, followed by white blood cells, platelets, and then plasma. The machine will divert a proportion of the RBC layer to a waste bag to remove RBCs while simultaneously returning most of the patient's white blood cells, platelets, and plasma along with donor RBCs to replace the

removed RBCs. There is some loss of other components in the waste bag, and transient decreases in white cells and platelets are expected.[1]

Logistics of performing an aRCE

Red blood cell volume to exchange

In order to calculate the total volume/number of RBC units required for aRCE, the patient's data and goals of an individual procedure can be entered into the apheresis device software (or an application downloaded to a smartphone[2]). The patient's height, weight, and sex are needed to calculate total blood volume (TBV); in addition, the patient's hematocrit as well as an estimated hematocrit of the RBC units that will be used for the exchange are entered. Citrate-phosphate-dextrose-adenine units typically have a hematocrit of ~75%, while units with additive solutions such as AS-1 usually have a hematocrit of ~55%; however, institution-specific averages should be obtained from local blood banks.[3] Next, the desired post-aRCE hematocrit as well as the pre- and target post-aRCE HbS levels are entered. With this information, the software can calculate the exact mL of RBCs required to achieve the desired final hematocrit and HbS. The machine cannot differentiate between HbS and HbA RBCs; therefore, the machine actually calculates the fraction of patient's cells remaining (FCR). Assuming the units are sickle cell negative with no HbS, HbS reflects the patient's RBCs and HbA reflects the transfused RBCs. Therefore, FCR is proportional to HbS:

- Pre-aRCE HbS = 75%
- Post-aRCE HbS = 30%
- FCR = 30/75 = 40%

If some data inputs are unknown or access to the software is not possible, the volume of RBC needed for an exchange can also be estimated by calculating 1.5 times the patient's RBC volume that would be removed/replaced. Over 1 RBC volume is used in the estimate because over the course of the exchange, HbA RBCs will be removed as well. At the beginning of the exchange, primarily HbS RBCs are removed. However, because the machine cannot differentiate HbS from HbA, HbA RBCs are removed as well, and as the exchange progresses, a larger proportion of the RBC volume removed will include the HbA RBCs transfused earlier in the procedure. Estimating 1.5 RBC volume to be removed/replaced accounts for this dynamic. For children, TBV can be estimated to be 70 mL/kg, with the exception of neonates/infants, who have larger blood volume per unit of weight, and obese patients, who have smaller blood volume per unit of weight. Formulas that include height and sex, such as the Nadler equation, can be used to calculate TBV for adults.[4]

An example calculation of 1.5 RBC volume for a 30 kg child with a hematocrit of 25% (therefore RBC volume is 25% of TBV) with no recent transfusion follows.

- TBV = 70 mL/kg × 30 kg = 2100 mL
- RBC volume = 2100 mL × 0.25 = 525 mL
- 1.5 × RBC volume = 787.5 mL
- RBC units have an average volume of approximately 300 mL. Assuming adsol units are available for this exchange, the RBC volume of available units would be approximately 165 mL (300 mL * hematocrit 55%).
- 1.5 × patient's RBC volume is estimated to remove the majority of patient's RBCs to decrease HbS from ~100% (no recent transfusion) to as low as possible. For a 30-kg patient on chronic aRCE with a lower pre-transfusion HbS, 3 units are likely sufficient.

Venous access

The success of aRCE depends on adequate intravenous (IV) access. Two lines are required: 1 draw line to remove whole blood from the patient and 1 return line. The draw line must be capable of supporting adequate flow rates without collapsing against the negative pressure created to pull blood into the device. The flow rate is dependent upon the patient's size. A rate of only 20-35 mL/min is likely sufficient for smaller children (~25-35 kg), whereas a larger adult (80 kg) might require ≥50 mL/min. Our policy is to require a CVC for all acute/urgent aRCE and to attempt peripheral access for all chronic/prophylactic aRCE. We prefer peripheral access for chronic aRCE to avoid complications related to CVCs. In order to optimize chances for success via peripheral IV (PIV) access, we educate all patients about adequate hydration in the 1-2 days prior to aRCE and ensure that patients are warm and calm prior to attempting PIV placement. In addition, apheresis nurses are trained to use ultrasound to guide PIV placement, allowing slightly deeper peripheral veins to be accessed and overall improving the rate of successful PIV placement.

For the draw line, a larger bore PIV capable of supporting the flow and negative pressure is required. A slightly smaller PIV can be placed for the return, but it must be sufficient to withstand the positive pressure of the returning blood. The exact size will depend on the patient size (Table 1). We are able to

Table 1. Appropriate PIV/CVC for aRCE according to patient weight

Weight of patient (kg)	Size of PIV for draw* (Gauge)	Size of PIV for return (Gauge)	Size of CVC (French)
<10**			5–6
10–30	22	22	6–7
30–40	18–20	20–22	7–9
40–50	18	18–20	8–10
>50	16–18	18	9–11.5

*A range of PIV sizes is suggested; however note that some patients may require smaller or larger PIVs based on their individual anatomy and the flow rates achieved.

**We have not performed apheresis procedures in patients <10 kg with PIVs.

establish peripheral access in approximately 60% of our chronic aRCE patients, which includes both children and adults. For patients with inadequate peripheral access, a variety of options for chronic aRCE are available. Most commonly, a double lumen implanted port is used. At the time of this writing, only 2 double lumen ports capable of apheresis are available in the US, the 9.5 Fr Bard Power Port Duo and the 11.4 Fr Angiodynamics Vortex, both of which can be used in adults or children whose weight >~45 kg (depending on expertise of surgeon) but not in smaller children. Other options include a single lumen port and PIV return, 2 single lumen implanted ports, a temporary CVC placed and removed on the day of an aRCE, and arterio-venous fistulas or grafts. No option has been demonstrated to be superior, and the choice depends on the patient's preference and anatomy as well as local resources and expertise of the multi-disciplinary team caring for the patient.

For patients who require urgent aRCE, we require a temporary CVC, as acutely ill patients may be dehydrated and thus peripheral access could be more difficult to obtain. The CVC must be an appropriate size for the patient (Table 1) and also rigid enough to withstand pressure and flow rates during the procedure. There are a variety of appropriate rigid lines specifically designed for apheresis or dialysis, typically called *power lines*. The package insert will confirm flow rates supported by a line to ensure appropriateness for aRCE.

One caveat to this discussion of 2 lines for aRCE is the recent introduction of software and tubing connectors to allow a single intravenous access line to function as both draw and return. Rather than simultaneously removing/returning blood, which occurs in the double-needle procedure, the single-needle (SN) procedure involves alternating cycles of first drawing whole blood and exchanging RBCs in the device and then returning blood to the patient through the same line. These discontinuous cycles repeat multiple times until the target parameters are met, resulting in a longer procedure. One group has reported that in their experience with SN procedures using a single lumen port, no difference was seen in pre- or post-HbS levels when compared to double-needle procedures. The group was able to increase flow rates to avoid longer procedure times.[5] In our experience using the SN procedure in an adult with 1 PIV, the procedure times are longer than those for the patient's historical double-needle aRCEs. Because SN procedures are not yet widely used, further experience is necessary to define outcomes.

Anticoagulation

An anticoagulant must be used during aRCE to prevent clotting of blood in the machine. Citrate is the most commonly used anticoagulant, as it primarily functions as an anticoagulant in the machine with minimal systemic effects. Citrate binds the calcium ions necessary for activation of calcium-dependent coagulation proteins and is added to the draw line so whole blood is anticoagulated as it enters the machine.[6] Upon return of blood to the patient, citrate is quickly metabolized by the liver, kidneys, and skeletal muscle.[6]

Benefits of aRCE

The benefits of aRCE are due to the removal of patient RBCs, rather than only dilution of patient RBCs, as well as the simultaneous return of blood with the removal.

Isovolemic transfusion

Blood viscosity increases with increasing hemoglobin and patients with SCD have increased viscosity compared to non-SCD controls even at the same hemoglobin level due to lower deformability of HbS RBCs.[7,8] Therefore transfusion to a hemoglobin level significantly higher than patient's baseline can result in symptoms of increased viscosity including hypertension, hemorrhagic stroke and even death.[9-14] The ability to remove blood (and decrease HbS) while transfusing RBCs allows an isovolemic transfusion and limits the risk of viscosity.[15,16]

Mitigation of iron loading

An RBC unit contains approximately 250mg of iron,[17] which accumulates in the liver and, to a lesser degree, in the heart, pituitary and pancreas over time.[18] Without chelation, excessive iron can result in liver or heart failure and contribute to mortality in SCD.[18-21] Removal of iron-containing RBCs with aRCE can mitigate iron loading, and multiple studies have shown less iron overload with aRCE than with simple transfusion or manual RCE.[22-25] Most of these studies were retrospective, and the ability to control for compliance with chelation medication was difficult or impossible. A secondary analysis of the Silent Cerebral Infarct Trial data was able to compare iron loading, as measured by rise in ferritin, that occurred prospectively in the first year of chronic transfusions before chelation was necessary.[26] This analysis clearly showed the ability of aRCE to attenuate rise in ferritin (Figure 1). It is important to note that iron balance depends on the difference between the pre- and post-aRCE hemoglobin/hematocrit. If the ordered post-aRCE hematocrit is significantly higher than the pre-aRCE hematocrit, the patient will remain in positive iron balance and chelation will likely be required.

HbS reduction

Exchange transfusion can decrease HbS to a significantly greater extent than can simple transfusion. An apheresis device can accomplish a larger volume exchange and therefore more efficiently lowers HbS than can manual RCE.[27] As described earlier in this article, a specific *posttransfusion* HbS level can be targeted with an individual aRCE procedure. For patients receiving an acute transfusion, the post-HbS level can be more efficiently and reliably decreased with aRCE. For patients treated with chronic transfusions, a specific *pretransfusion* HbS level is targeted, particularly for patients who undergo transfusion for stroke prevention. The Stroke Prevention Trial in Sickle Cell Anemia demonstrated transfusions that occurred approximately once a month to maintain a pretransfusion HbS of <30% were associated with a 92% reduction (P<0.001) in stroke risk compared to standard care, making 30% HbS the standard for stroke prevention.[28] While most case series have shown lower pretransfusion HbS with chronic aRCE compared to simple or manual RCE,[24,25,29,30] this association has not consistently been shown.[27] The pretransfusion HbS is harder to predict because it depends on (1) how low the post-HbS level was after the last transfusion, (2) the interval between transfusions, and (3) the patient's endogenous suppression/production of HbS. For an individual patient, the pretransfusion HbS must be monitored. If the HbS level is above target, consider 1 of the following modifications: (1) increase the number of RBC units during aRCE to decrease post-transfusion HbS levels, (2) decrease the transfusion interval, or (3) increase the post-aRCE hematocrit to suppress endogenous HbS

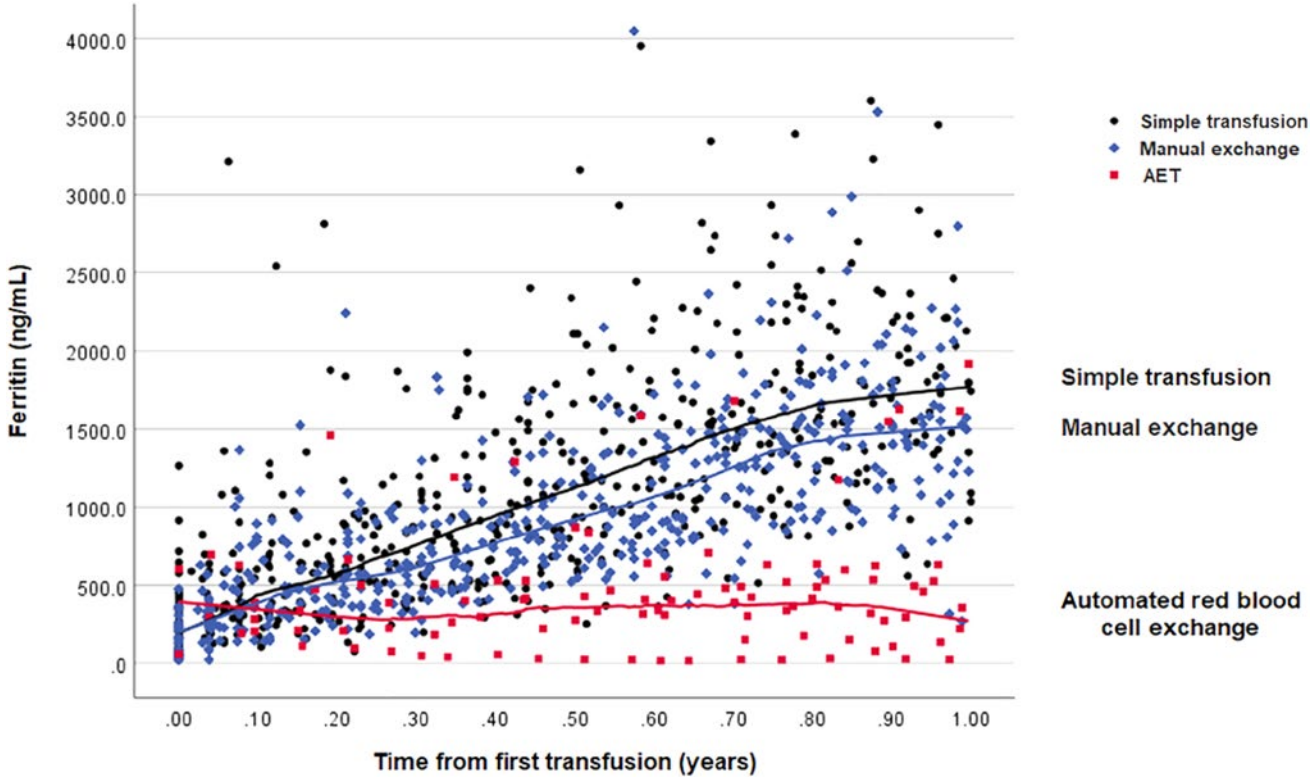

Figure 1. Automated exchange compared to manual and simple blood transfusion attenuates rise in ferritin level after 1 year of regular blood transfusion therapy in chronically transfused children with sickle cell disease.[26] Change in ferritin levels in participants of the Silent Cerebral Infarct Trial ($n = 83$) randomized to transfusions. The median (IQR) ferritin levels after 1 year of transfusion were as follows: 1800 ng/mL (IQR, 1426 to 2204 ng/mL) in simple transfusion participants, 1530 ng/mL (IQR, 1205 to 1805 ng/mL) in manual exchange participants, and 355 ng/mL (IQR, 179 to 579 ng/mL) in automated RBC exchange participants. Figure reprinted from Kelly et al.[26]

production. However, option 3 might not be possible for patients with higher baseline pretransfusion hemoglobin/hematocrit due to viscosity risk. In addition, significantly increasing hemoglobin levels after the procedure with respect to preprocedure levels will negate the potential mitigation of iron loading.

Risk of aRCE

In general, aRCE is well tolerated, even in the acute setting. Possible adverse affects are summarized in Table 2 and are more likely to be related to CVCs than the actual procedure. Because aRCE exposes patients to significantly more blood than simple transfusion, there has been concern of increased risk of transfusion-related adverse events, though aRCE has actually been associated with lower risk of alloimmunization and transfusion reactions.[31-33]

Indication for aRCE

There have been no randomized trials comparing simple transfusion to aRCE to define when one should be used instead of the other. Recommendations are based on case reports/series or expert opinion (Table 3). In general, in an acute setting, if the hemoglobin level is >2 g/dL lower than patient's baseline, a simple transfusion can be given to increase hemoglobin and improve tissue oxygenation. If the goal of the acute transfusion is to significantly decrease HbS, aRCE should be performed. Automated RBC exchange is most commonly indicated for acute stroke, severe acute chest syndrome, and multiorgan failure. Other indications should be evaluated on a case-by-case basis considering the risks and benefits in the individual patient. Automted RBC exchange should be considered in all patients treated with long term chronic transfusion therapy to mitigate iron loading.[34,35]

Cost of aRCE

The cost of an individual aRCE compared to simple or manual RCE is higher due to the cost of the kit, anticoagulation and tubing for the machine, increased blood units required, any central line–related costs, and skilled nursing time. Cost has been reported as a barrier to implementing aRCE programs.[36] However, studies that have balanced the cost savings created by decreased chelation, and SCD hospitalizations have actually shown chronic aRCE programs to be cost-effective.[24,37,38]

Resolution of clinical case and conclusions

The patient presented in the initial clinical case had a double lumen port placed and was transitioned to aRCE. His course demonstrates the potential benefit of aRCE, as both his HbS and ferritin levels significantly decreased (Figure 2). He did intermittently have issues related to his port, such as repeated need for local thrombolytic medication, highlighting potential adverse effects related to the required vascular access. These risks and

Table 2. Adverse effects of aRCE

Risk	Clinical comments and mitigation/treatment strategies
Catheter-related complications	
Thrombosis	Catheters should be locked with heparin or citrate after the procedure. Many institutions (including ours) also perform a 30–60 minute dwell of thrombolytic medication prior to all procedures. Resistance to draw or flush with frequent procedure alarms may suggest thrombosis, and radiologic evaluation of line such as fluoroscopy should be performed.
Infection	Blood cultures should be obtained in any febrile SCD patient with a CVC. High suspicion of catheter related bacteremia if signs of sepsis occur after flushing CVC, and prompt antibiotics are warranted.
Migration of line	Tip of line should be near superior vena cava/right atrial junction. Poor flow rates, frequent alarms, or resistance to draw or flush can suggest migration of line from central location and should be evaluated with chest radiograph.
Communication between 2 catheters of implanted ports creating recirculation	We have experienced this 3 times over the past 10 years and only suspected recirculation based on no change in HbS and platelets in post-aRCE labs compared to pre-aRCE labs because no alarms or other indications of difficulties occurred during procedure. Communication was diagnosed by fluoroscopy exam and necessitated line replacement.
Transfusion reactions	Febrile nonhemolytic transfusion reactions and allergic reactions are the most commonly seen transfusion reactions, though hemolytic and other transfusion reactions are also possible.
Hypocalcemia due to citrate toxicity	Citrate-induced hypocalcemia can cause paresthesias or nausea/vomiting though is typically asymptomatic when detected. Ionized calcium can be monitored during the procedure, and we elect to give calcium gluconate infusions through the return line to maintain normal ionized calcium.
Hypotension	Changes in blood pressure can occur, though rare, and are typically responsive to normal saline boluses.
Symptoms related to fluid shifts	Vasovagal symptoms, abdominal pain, nausea, and vomiting despite normal blood pressure and ionized calcium can be seen, though rare, and are presumed to be due to fluid shifts during the procedure. Normal saline boluses and/or antiemetics can be administered in future procedures for patients who experience these symptoms.
Alloimmunization	Prophylactic phenotypically matching RBCs at a minimum for Ce, Ee, and K antigens (in addition to ABO/D) is recommended for SCD.[34] Despite this, alloimmunization can occur and if multiple/rare RBC antibodies develop, and it can be difficult to maintain patients on chronic aRCE programs due to the need for rare blood. Note that lower alloimmunization rates with aRCE compared to chronic simple transfusion have been reported despite significantly increased exposure with aRCE.[31]

Table 3. Indications for aRCE

Indication*	Relevant literature or guidelines
Acute stroke	RBC exchange (manual or automated) during management of initial stroke was associated with a lower stroke recurrence (21% [8/38]) compared to simple transfusion (57% [8/14])[39] Category 1 recommendation by ASFA[35]**
Severe acute chest syndrome (ACS)	aRCE shown to reverse hypoxia within 24 hours in 5 patients.[40] Comparison of simple transfusion and aRCE in 81 children with ACS showed aRCE was given in more severe cases, yet similar length of stay/clinical course was achieved when compared to less severe cases treated with simple transfusion.[41] Suggested by ASH 2020 guidelines for SCD: transfusion support[34] and is a category 2 recommendation by ASFA[35]**
Multiorgan failure	No randomized trials, but small case series suggest improved outcomes with RBC exchange transfusion.[42,43] Of note, recent case reports describe improvement with plasma (rather than RBC) exchange.[44–46]
Prophylactic preoperative transfusion if hemoglobin >9 g/dL	Transfusion is indicated prior to surgery for patients with SCD due to high risk of perioperative complications in this population. A randomized trial demonstrated that a conservative regimen to achieve hemoglobin 10 g/dL was as effective as an aggressive regimen to decrease HbS <30%,[47] therefore aRCE should be used only in patients with high baseline hemoglobin who cannot receive simple transfusion. Suggested by ASH 2020 guidelines for SCD: transfusion support[34]
Long term chronic transfusion therapy	aRCE suggested by ASH 2020 guidelines for SCD: transfusion support[34] and category 1 recommendation by ASFA.[35]

ASH, American Society of Hematology.

*For all acute indications for aRCE, the hemoglobin upon acute presentation should be considered and if >2 g/dL below patient's baseline, a simple transfusion may be given first. In particular, this may be a temporizing measure to allow time for line placement and mobilization of apheresis service.

**The American Society for Apheresis (ASFA) provides evidenced based recommendation for apheresis procedures: category 1 (first line therapy), 2 (second line therapy), 3 (role of apheresis not established; decision-making should be individualized), 4 (ineffective or harmful).

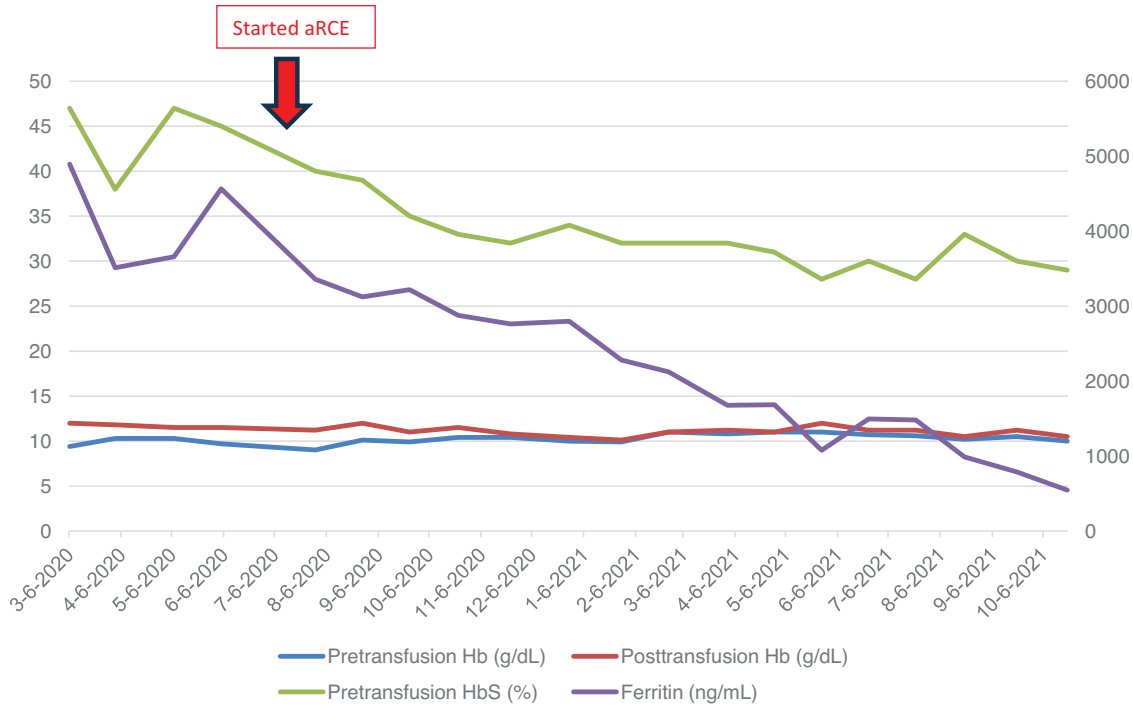

Figure 2. Laboratory values of patient reported in clinical case before and after aRCE. Pretransfusion and posttransfusion hemoglobin (Hb) and hemoglobin S (HbS) are shown on the left axis with units g/dL and percent, respectively. Units for ferritin shown on right axis with units ng/mL. A double lumen port was placed in July 2020, and patient started aRCE in August 2020 using 6 U for each aRCE. Note prior to this, posttransfusion hemoglobin shown in red was typically 11–12 g/dL. Over the next year on aRCE, his posthemoglobin level was closer to his pretransfusion hemoglobin level, typically 10 g/dL. On aRCE, his pretransfusion HbS level decreased and was ultimately maintained closer to his target 30%. His ferritin steadily declined, and he was able to stop chelation medication within 9 months.

benefits must be weighed to make patient-specific decisions for individuals with SCD who require chronic transfusions. Areas of future investigation should include further research regarding the SN procedure; comparing the effects of aRCE with chronic simple transfusion on clinical outcomes, such as prevention of stroke and other severe SCD complications; and examining mechanisms of decreased alloimmunization despite significantly increased RBC exposure with aRCE.

Conflict-of-interest disclosure

Shannon Kelly: no competing financial interests to declare.

Off-label drug use

Shannon Kelly: nothing to disclose.

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Alloimmunization and hyperhemolysis in sickle cell disease

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Alloimmunization against red blood cell antigens and delayed hemolytic transfusion reaction (DHTR) are major barriers to transfusion in sickle cell disease (SCD). In SCD, DHTR is a potentially life-threatening. Blood group polymorphism in SCD patients, who are of African ancestry and frequently exposed to antigens they do not carry; an inflammatory clinical state; and occasional transfusion in acute situations are risk factors for alloimmunization and DHTR. In patients at risk, the transfusion indication must be balanced against the risk of developing DHTR. However, when transfusion is absolutely necessary, protocols combining the prevention of exposure to immunogenic antigens with immunosuppressive treatments must be implemented, and patients should be carefully monitored during posttransfusion follow-up. This close monitoring makes it possible to diagnose hyperhemolysis as soon as possible; to avoid retransfusion, which can exacerbate hemolysis; and to administer specific treatments, such as anticomplement therapy, in severe cases. Finally, in patients with severe disease, hematopoietic stem cell transplantation may be indicated. However, transfusion is also required in this context, and its management is complex because these risks must be taken into account.

We discuss these issues here, based on a clinical case. With the exception of consensual prophylactic red cell antigen matching for ABO/RhD and Rh(C, E or C/c, E/e), the recommendation of these measures is conditional because certainty concerning the evidence of an effect is very low. This lack of evidence highlights the need for improving our understanding of transfusion complications in SCD and developing preventive strategies that take into account the mechanisms underlying hyperhemolysis syndrome.

LEARNING OBJECTIVES

- To know the risk factors of red blood cell alloimmunization and delayed DHTR in SCD
- To describe the options for managing complex transfusion situations in an SCD patient when transfusion and/or hematopoietic stem cell transplantation are absolutely indicated

Introduction

Sickle cell disease (SCD) is the most common inherited red blood cell (RBC) disorder in individuals of African descent. Transfusion remains a major treatment, though with a certain risk in SCD. Delayed hemolytic transfusion reaction (DHTR) occurs in about 4% of adult patients receiving occasional transfusions.[1] Delayed hemolytic transfusion reaction is an umbrella term encompassing posttransfusion hemolysis of all degrees of severity, with the potentially fatal development of hyperhemolysis occurring in the most severe cases.[2,3] Indeed, 5% of the 266 deaths analyzed in SCD adults were due to DHTR.[4]

The major cause of DHTR is RBC alloimmunization because of differences in blood groups between donors of Caucasian origin and recipients of African ancestry. Another risk factor is the inflammation status at the time of transfusion during acute complications.[3] However, many cases with no detectable antibodies are described, calling into question the underlying mechanism.[5-10]

In the most severe cases, the patient develops hyperhemolysis, with lower levels of hemoglobin (Hb) after transfusion than before, due to the destruction of transfused and autologous RBCs and, in some cases, profound reticulopenia.[11] The severity of the condition is due to major intravascular hemolysis with the physiological protection against free Hb (haptoglobin) and free heme (hemopexin, heme oxygenase) being overwhelmed. Accumulation of free heme generates oxidative stress, triggering symptoms of a vaso-occlusive crisis (VOC) and accentuating the damage to vascular endothelial cells. Whether occurring

through the classical or alternate pathways, complement activation, which results in membrane attack complex formation, is the predominant mechanism of hyperhemolysis. The patient's RBCs are destroyed because they are sensitive not only to oxidative stress but also to the nonspecific binding of activated complement fractions.[12,13]

Prevention of this accident is currently based on the prevention of alloimmunization but also involves immunosuppressive therapy. The treatment of this syndrome, particularly in severe cases, may require drugs acting on the complement activation cascade, such as eculizumab.[14-17]

We present here the clinical case of a patient with history of hyperhemolysis with antibody development for whom transfusion support continued to be indicated because of a severe symptomatic disease that could not be treated with hydroxycarbamide. The decision was made to perform hematopoietic stem cell transplantation (HSCT), which caused additional transfusion challenges.

THE CLINICAL CASE

A 15-year-old boy was referred to our department for highly symptomatic SCD refractory to hydroxycarbamide. In the last 2 years, he experienced 5 acute chest syndrome (ACS) and was hospitalized monthly for VOC. His RBC phenotype was B, D+ C-E- c+ e+, K-, Fya-Fyb-, Jka-Jkb+, M-N+ S- s. During a systematic antibody screening test without evidence of hemolysis, anti-Jka antibodies were detected after 3 transfusion episodes with prophylactic Rh- and K-matched units: one before cholecystectomy (3 RBC units) and two for treatment of ACS (2 RBC units each). After development of anti-Jka antibodies, a fourth transfusion to treat ACS was performed with 2 C-E-, K- and Jka- RBC units, with additional prophylactic matching for FY (Fya-Fyb-) and Ss (S-) leading to an immediate posttransfusion Hb level of 10.4 g/dL and 30% HbA. Ten days later, the patient developed joint pain, fever, jaundice, and hemoglobinuria. His Hb level fell to 6.4 g/dL (pretransfusion level: 8 g/dL), bilirubin rose from 18 (pretransfusion level) to 115 mmol/L and LDH level rose from 534 (pretransfusion level) to 1156 IU/L. The symptoms mimicked VOC, but the presence of hemoglobinuria and the clearance of HbA (15%) associated with the rapid rise in percentage of sickle cell Hb (HbS) led to a suspicion of hyperhemolysis. The patient received only supportive care (hydration, oxygenation, analgesic opioids), and the symptoms resolved. During the course of DHTR, antibody screening test revealed only the already known anti-Jka antibodies, but anti-M antibodies were detected 1 month later.

Given the severity of the disease, a transfusion program to prevent VOC recurrences[18,19] followed by haploidentical HSCT was considered during a national multidisciplinary pediatric care meeting. A decision was made on a transfusion program: C-E-, K-, Fya-Fyb-, Jka-, S- and M- units along with prophylactic immunosuppression. Rituximab was administered (375 mg/m^2 via IV) 10 days before the first transfusion. During the transfusion program, additional rituximab was administered to achieve continuous B-lymphocyte depletion. No adverse transfusion reaction occurred during the 6-month program.

The phenotype of the graft donor (the patient's mother) was O, Jka+, M+. The risk of an acute hemolytic reaction triggered by the donor's anti-B antibodies at the time of stem cell infusion was prevented by plasma reduction of the bone marrow graft. Recipient anti-Jka and anti-M antibodies were no longer detectable by the time of HSCT, but RBC reduction on the bone marrow graft was recommended to prevent restimulation by the donor's RBCs. HSCT was performed after a reduced-intensity conditioning regimen. During the preconditioning phase and after HSCT, the patient was transfused with O, C-E-, K-, Jka-, Fya-, S- and M- RBC units. The immunohematological work up remained negative. However, the patient experienced pure red-cell aplasia not of immunohematological origin and was dependent on RBC transfusion for 6 months after transplantation. He is now well and displays full engraftment with no acute or chronic graft versus host disease.

How to prevent posttransfusion hemolysis in patients with SCD

In our case, the patient initially developed antibodies after transfusion of 7 RBC units matched for Rh and K, reflecting his high-responder status. He presented only a delayed serologic reaction, as the development of the anti-Jka antibody was not associated with posttransfusion hemolysis. However, the patient was at risk of developing new antibodies and DHTR. The DHTR risk has been shown to increase with various factors: transfusion for an acute indication, history of immunization, history of DHTR, and transfusion with a smaller cumulative number of transfused units.[1,5] In the case described, the patient had all the known risk factors except prior DHTR. In accordance with current transfusion recommendations,[20,21] along with a Jka-negative protocol to take into account the preformed antibody, matching was extended to Fya and S antigens to prevent formation of new antibodies. In this case, the extended phenotype of the patient was known and previously performed with serologic techniques. As recommended, the patient had also benefited from a molecular work up to detect partial antigens and rare blood in the Rh blood group, which should also have been taken into account earlier. However, no variant was found. Despite this prophylaxis, the patient developed posttransfusion hemolysis with the appearance of anti-M antibodies. M-antigen matching is not included in the prevention of immunization at our center unless anti-M antibodies are already detectable or known in history. However, anti-M antibodies may be involved in DHTR, as shown by the most recent series of pediatric DHTR cases.[9,10] Then, considering the M antigen in already immunized patients can be an issue. Rituximab, which can be used to prevent alloimmunization in patients with history of severe DHTR, might have prevented the production of this new antibody.[22] This case highlights the complexity of decision-making in such cases and demonstrates that the risk of antibody development and the clinical significance of the antibodies associated with hyperhemolysis remain poorly understood.[23] Indeed, in the context of DHTR, when antibodies are detected, they may be of any type and have no correlation with severity.[7]

How to manage patients with history of hyperhemolysis

The complex patients in France are discussed at a multidisciplinary consulting meeting with a panel of clinicians and transfusion pathologists specializing in SCD (Figure 1). The first aspect discussed is always the risk/benefit ratio of continuing transfusion,

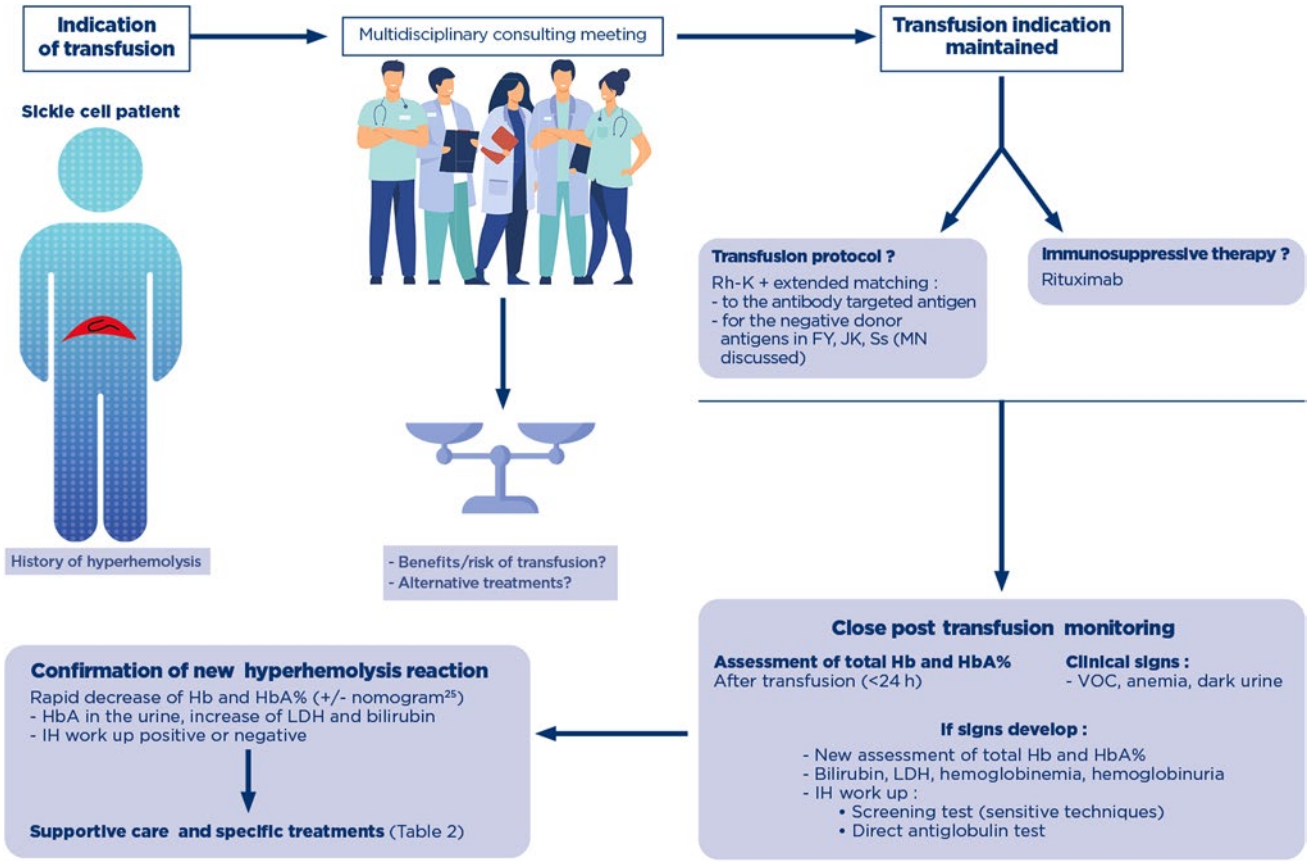

Figure 1. Decision making process for the indication of transfusion in a patient with history of hyperhemolysis. A patient with history of posttransfusion hyperhemolysis is at risk for recurrence of the syndrome, with or without detectable antibodies. When a transfusion is indicated, a shared decision-making process assesses the risk/benefit of transfusion, and if maintained, establishes the transfusion protocols in terms of RBC antigen matching and the use of immunosuppressive therapy. Close monitoring of the posttransfusion phase is absolutely necessary, and assessment of total Hb and HbA% immediately after transfusion will help diagnose posttransfusion hyperhemolysis with new assessment of these parameters. If clinical signs appear, evaluation of hemolytic biological parameters and an IH workup are performed. A negative IH workup does not rule out the diagnosis. After confirmation of hyperhemolysis, supportive care and specific treatments are considered. Hb, hemoglobin; IH, immunohematology; LDH, lactate dehydrogenase; RBC, red blood cell; VOC, vaso-occlusive crisis.

particularly for classical indications, such as the preoperative prevention of SCD symptoms. In the case described here, the clinical situation was reevaluated, and the continuation of programmed transfusions was considered the only appropriate treatment for this symptomatic patient before HSCT.

In a patient with a history of hyperhemolysis, when new transfusions cannot be avoided because of a severe disease, extended matching for RBCs for FY, JK and Ss is always indicated, and rituximab should always be provided, especially if the patient has developed hyperhemolysis with detectable antibodies, in accordance with a conditional recommendation of the ASH guidelines.[20] (Table 1). There is currently no other widely used preventive measure against alloimmunization and DHTR. A retrospective study suggests that rituximab can be safely used for preventing DHTR in patients with a previous history of DHTR and detected antibodies. However, despite rituximab prophylaxis, DHTR recurred in about 20% of the patients, albeit at moderate severity and without detectable antibodies, confirming the multifactorial origin of this syndrome.[23]

All patients with a history of hyperhemolysis undergoing transfusion again should be monitored closely to ensure that any new episode is detected as soon as possible so that ad hoc treatments can be rapidly initiated at specialist facilities. (Figure 1).

The diagnosis of posttransfusion hemolysis

Recurrence or appearance of VOC shortly after transfusion, dark urine, onset or worsening of anemia, or increase in LDH concentration should alert professionals to the likelihood of DHTR. This first step—recognition—is crucial to prevent further transfusions, which would exacerbate hemolysis, and for the initiation of treatment before irreversible multiple organ failure develops. Diagnosis is not dependent on immediate evidence of newly formed antibodies. In our case, the anti-M antibody was detected in the plasma 30 days after the trigger transfusion. However, no antibodies are ever detected in many cases, even some time after the DHTR. The HbA decrease relative to the values obtained immediately after transfusion is a key parameter for the confirmation of posttransfusion hemolysis. A nomogram can

Table 1. The guidelines of the American Society of Hematology (ASH) discussed in this review

	Recommendations or suggestions of ASH	Grading of recommendations	Remarks
Questions 3 and 4: Use of immunosuppressive therapy[20]	**ASH SUGGESTS:** Immunosuppressive therapy over no immunosuppressive therapy **for high risk of acute hemolytic transfusion reaction or severe history of DHTR**	**Conditional recommendation** based on very low certainty in the evidence about effects	Share decision-making process is critical to weigh the potential benefits and harms associated with transfusion versus the effect of ongoing SC symptoms
	ASH SUGGESTS: Immunosupressive therapy over no immunosuppressive therapy **in patients with DHTR and ongoing hyperhemolysis**	**Conditional recommendation** based on very low certainty in the evidence about effects	Share decision-making process Immunotherapy should be initiated promptly if ongoing hyperhemolysis with: - First line: IVIG and high dose steroids - Second line: Eculizumab - Rituximab only to prevent additional antibodies Precipitation of VOC with steroids should be considered

DHTR, delayed hemolytic transfusion reaction; IVIG, intra veinous immunoglobulin; SC, sickle cell; VOC, vaso-occlusive crisis.

facilitate diagnosis if assessments of total Hb and HbA% immediately after transfusion are available.[24] In a study on children performed at 1 institution, HbA clearance was also calculated based exclusively on the volume of RBCs transfused and the hematocrit of the units.[25] The appearance of HbA in the urine with a worsening of anemia may also indicate ongoing DHTR. Finally, a rapid decrease of 25% or more in total Hb levels with respect to pretransfusion levels should raise suspicions of DHTR. The diagnosis of DHTR and the elimination of other causes of acute anemia, such as splenic sequestration, autoimmune hemolytic anemia, acute hemolytic sickle cell crisis, and extensive bone marrow necrosis, are crucial because a history of DHTR affects treatment decisions, with patients subsequently receiving the smallest possible number of transfusions.

How to treat a patient with hyperhemolysis (Table 2)

In this case, the patient received only supportive care and did not require additional transfusions. In a recent pediatric series, 15% of patients received no treatment, whereas the others received EPO, rituximab, and/or eculizumab. Corticosteroids were also administered, but only for patients undergoing new transfusions due to life-threatening anemia.[9] In another series of 37 pediatric cases, 11 patients received supportive care only; the other patients received immunosuppressive therapy. Additional transfusion was required for 17 patients. In this report, treatment with a high dose (2 mg/kg) of corticosteroid in addition to transfusion made it possible to maintain Hb levels after transfusion.[10] Finally, in a series of cases in young adults,[26] 66% of patients received corticosteroids. By contrast, corticosteroid treatment is systematically avoided in the DHTR setting at some adult facilities due to the risk of hyperviscosity and new VOC.[27] However, in most of the retrospective case studies, the patients received different treatments simultaneously, making it difficult to determine which of the them was actually effective.[28]

There is still little consensus about DHTR management. It differs between adults and children and between the different centers treating patients; however, DHTR management also differs based on the treatments available, as some, such as eculizumab, are expensive and difficult to obtain at some facilities.

Aside from supportive care, current treatments are based on the putative mechanisms underlying DHTR and the consequences of the release of RBC content. RBC production has to be stimulated by EPO and the different RBC destruction pathways inhibited. IVIG treatment is considered against the macrophage-mediated destruction of RBCs induced by antibodies, and anti-complement treatment is considered against the complement activation through the classical and alternative pathways. Eculizumab is the only anticomplement agent used to date in the context of DHTR.[29] However, it must be administered at the very start of hyperhemolysis to prevent irreversible multiple organ failure. Another goal of treatment is to eliminate free heme and free hemoglobin released in the plasma, which can have deleterious effects on the endothelial cells in the vessels. Plasma exchange is a good option; however, extracorporeal volume must also be considered, as it may cause a further decrease in hemoglobin level.[30] Other drugs for removing free heme from the plasma, such as hemopexin, could be considered in the future. Other drugs have been used to treat hyperhemolysis, such as Tocilizumab, an anti–IL6 receptor agent that lowers the levels of inflammatory markers thought to be involved in the pathophysiology of severe DHTR.[31,32]

Finally, in some cases of profound anemia and organ hypoxia, further transfusions are inevitable. If transfusion is indicated, extended matching (Fy, Jk, Ss) is recommended in addition to rituximab prophylaxis, even if there are no detectable antibodies. Pediatric clinicians also consider administering a short course of corticosteroids to patients undergoing transfusion in this context because of this treatment's anti-inflammatory and immunosuppressive effects.[9,10]

The challenges of transfusion in the context of hematopoietic stem cell transplantation

Hematopoietic stem cell transplantation (HSCT), as in the case described, is the only curative treatment in patients with severe SCD-related complications, especially when transfusion difficulties are encountered due to the presence of multiple antibodies, a rare blood type, or a history of severe DHTR.[33] In SCD patients undergoing HSCT, transfusion support is required before the

Table 2. Current and potential treatments of posttransfusion hyperhemolysis based on putative mechanisms

Goals of treatments	Treatments currently used	Potentially useful drugs
Supportive care		
	Hydratation, analgesic, oxygenation	
Stimulation of erythropoiesis		
	Erythropoietin	
	Iron	
	Folates	
	Vitamin B12	
Inhibition of RBC destruction		
Macrophages/antibody-mediated		
	Intravenous immunoglobulin	
Complement cascade activation		
	Eculizumab	
		Other anti-C: C1 inhibitors[39]
Elimination of toxic molecules from the plasma		
Heme	Plasmapheresis	
		Hemopexin[40]
Hemoglobin	Plasmapheresis	
		Haptoglobin[40]
Antibodies	Plasmapheresis	
		IgG endopeptidase[41]
Activated fractions of complement, cytokines, other involved molecules	Plasmapheresis	
Anti-inflammatory action		
	Corticosteroids (balanced with vaso-occlusive risk)	
		Tocilizumab (case reports)
Safety of additional transfusion		
	Transfusion + rituximab	
	Transfusion + corticosteroids (balanced with vaso-occlusive risk)	
		Transfusion + Daratumumab (case report)[42]
		Transfusion + Bortezomib
Various goals		
		Bortezomib + Hemopure (case report)[43]

initiation of the conditioning regimen, with RBC exchange transfusion to decrease HbS% to about 30% to prevent vaso-occlusive complications and also to sustain the postconditioning aplasic phase. In nonmyeloablative HSCT transplantation, which is frequently sufficient to cure the disease, stable mixed chimerism is associated with a risk of immunohematological complications.[34] The coexistence of donor and recipient immune cells results in a risk of RBC antibody production by the immune cells of both donor and patient. In a series of 61 patients, 3 patients developed antibodies against the donor or recipient RBCs after HSCT. The complications observed ranged from nonsevere adverse effects to near-fatal hemolysis.[35] During HSCT for SCD, in addition to the usual management of ABO-mismatches, clinicians must also consider complicated transfusion situations, such as the production of multiple alloantibodies by the patient, rare blood groups, and the prevention of DHTR.[36] Thus, once the indication for HSCT has been validated, and the donor has been selected, the feasibility of transfusion support should be evaluated at 2 levels. First, the availability of extended phenotype-matched RBC or rare blood, compatible with both donor and recipient, should be checked. HSCT patients with a history of RBC alloantibody have been shown to receive significantly more RBC units during HSCT than

non-alloimmunized patients.[37] Secondly, prevention of DHTR by immunosuppressive therapy in patients with a history of DHTR should be discussed. In a retrospective series of 34 adolescents and young adults, 15% required prophylaxis for DHTR during preconditioning transfusion support.[34] With the increase in indications for HSCT in young and older patients,[38] we are likely to see a parallel increase in the frequency of complex transfusion situations.

Conclusion

In SCD, transfusion can induce reactions ranging from a delayed serologic reaction sometime after transfusion, with no evidence of hemolysis, to the development of posttransfusion hemolysis, with the most severe cases presenting a hyperhemolysis syndrome. The prevention of these reactions is currently based solely on avoiding exposure to immunogenic antigens, based on blood group stratification compatibility according to the patient's history, and the administration of immunosuppressive treatments.

However, even with such prophylaxis, some cases of DHTR occur and their pathophysiology remains unclear, as no RBC antibodies are detected in many cases.

The posttransfusion monitoring of patients with history of alloimmunization and/or posttransfusion hemolysis is highly important to ensure that specific treatment can be initiated rapidly if signs of hemolysis develop. Finally, when HSCT is indicated, transfusion protocols and the work-up on the bone marrow graft should take into account both the RBC phenotype of the graft donor and the complex transfusion situation of the patient. However, most of the measures described in this review are qualified by the American Society of Hematology (ASH) guidelines as conditional recommendations based on the very low certainty of the evidence for an effect.[19] (Table 1) This lack of evidence highlights the importance of improving our understanding of transfusion complications in SCD and of developing suitable methods for preventing hyperhemolysis based on the underlying mechanisms.

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Cytoreduction for ET and PV: who, what, when, and how?

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Thrombotic complications are the primary contributor to morbidity and mortality in essential thrombocythemia (ET) and polycythemia vera (PV). Cytoreductive therapy is the main tool for primary or tertiary thrombosis prevention in these diseases. In general, high-thrombotic-risk patients and those with symptoms that may be ameliorated from cytoreductive therapy are candidates for this treatment, although the decision is highly individualized. Approved options for cytoreduction in ET and PV include hydroxyurea, long-acting interferons, anagrelide in ET, and ruxolitinib in PV. Selecting the ideal agent requires careful consideration of the toxicity profiles and individual treatment goals. In this review the existing literature on cytoreductive decisions in ET and PV is summarized, with an emphasis on risk-stratification, highlighting the need for personalized care in order to maximize the benefit of these therapies while minimizing toxicities.

LEARNING OBJECTIVES

- Understand the current risk-stratification systems for ET and PV and their shortcomings
- Survey the currently available cytoreductive agents and review their clinical efficacy and adverse effects
- Develop a personalized method for determining when to initiate cytoreductive therapy in PV and ET and selecting an appropriate agent

CLINICAL CASE

A 61-year-old woman was incidentally noted to have a platelet count of 851×10^9/L. The rest of her complete blood count was normal. She reported feeling well, overall, and exercised regularly. Her examination was notable for a lack of splenomegaly. A further laboratory evaluation, including iron studies, was normal. A bone marrow (BM) biopsy was performed, demonstrating megakaryocytic hyperplasia with hyperlobulation and an absence of reticulin fibrosis consistent with a diagnosis of essential thrombocythemia (ET). Cytogenetic analysis revealed a 46,XX karyotype, and sequencing uncovered a type 1 *CALR* mutation at a variant allele frequency of 29%.

Introduction

ET and PV are myeloproliferative neoplasms (MPNs) characterized by the overproduction of platelets and red blood cells, respectively.[1] These proliferative MPNs are clinically and morphologically distinct, with PV associated with an expanded red cell mass and frequently leukocytosis and thrombocytosis, while thrombocytosis is typically the sole hematologic abnormality in ET. It is paramount to establish a correct diagnosis in order to guide risk-stratification and therapeutic intervention (Table 1). These diseases share a propensity toward thrombosis, which is the leading cause of morbidity and mortality.[2,3] The primary tools for reducing the burden of thrombotic events are medications to lower blood counts, termed *cytoreductive therapies*. While published guidelines by the NCCN and European LeukemiaNet (ELN) can be useful in determining the timing and choice of cytoreductive therapy, these judgments are nuanced and require the consideration of multiple factors.[4-6]

This review focuses on decisions regarding cytoreduction in ET and PV. Other management considerations, including therapeutic phlebotomy in PV, antiplatelet treatment, and anticoagulation in patients who have experienced a thrombosis, are essential but outside the scope of this monograph, as is novel therapeutic development.

Risk stratification in ET and PV

It is imperative to determine which patients are at higher thrombotic risk and may benefit from cytoreductive therapy. Conventional ELN risk-stratification for both ET and PV defines high-risk patients as those who are older than

Table 1. 2022 International Consensus Classification diagnostic criteria for PV and ET

Essential thrombocythemia	Polycythemia vera
Major criteria	
1. Platelet count ≥450×10⁹/L	1. Elevated hemoglobin concentration or elevated hematocrit or increased red blood cell mass[f]
2. BM biopsy showing proliferation mainly of the megakaryocytic lineage, with increased numbers of enlarged, mature megakaryocytes with hyperlobulated staghorn-like nuclei, infrequently dense clusters[a]; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; no relevant BM fibrosis[b]	2. Presence of *JAK2* V617F or *JAK2* exon 12 mutation[g]
3. Diagnostic criteria for BCR-ABL1-positive CML, PV, PMF, or other myeloid neoplasms are not met	3. BM biopsy showing age-adjusted hypercellularity with trilineage proliferation (panmyelosis), including prominent erythroid and granulocytic and increased pleomorphic, mature megakaryocytes without atypia
4. *JAK2*, *CALR*, or *MPL* mutation[c]	
Minor criterion	
Presence of a clonal marker[d] or absence of evidence of reactive thrombocytosis[e]	Subnormal serum erythropoietin level
The diagnosis of ET requires either all 4 major criteria or the first 3 major criteria plus the minor criterion	The diagnosis of PV requires either all 3 major criteria or the first 2 major criteria plus the minor criterion[h]

[a]Three or more megakaryocytes lying adjacent without other BM cells in between; in most of these rare clusters, 6 or fewer megakaryocytes may be observed. An increase in huge clusters (>6 cells) accompanied by granulocytic proliferation is a morphological hallmark of pre-PMF.

[b]Very rarely, a minor increase in reticulin fibers may occur at initial diagnosis (grade 1).

[c]It is recommended that highly sensitive assays be used for *JAK2* V617F (sensitivity level <1%) and *CALR* and *MPL* (sensitivity level 1% to 3%); in negative cases, consider a search for noncanonical *JAK2* and *MPL* mutations.

[d]Assessed by cytogenetics or sensitive next-generation sequencing techniques.

[e]Reactive causes of thrombocytosis include a variety of underlying conditions like iron deficiency, chronic infection, chronic inflammatory disease, medication, neoplasia, or history of splenectomy.

[f]Diagnostic thresholds: hemoglobin level above 16.5 g/dL in men and 16.0 g/dL in women; hematocrit above 49% in men and 48% in women; red blood cell mass 25% above mean normal predicted value.

[g]A BM biopsy may not be required in patients with sustained absolute erythrocytosis (hemoglobin concentrations above 18.5 g/dL in men or 16.5 g/dL in women and hematocrit values above 55.5% in men or 49.5% in women) and the presence of a *JAK2* V617F or *JAK2* exon 12 mutation.

[h]Highly sensitive assays for *JAK2* V617F (sensitivity level <1%) are recommended; in negative cases, consider searching for noncanonical or atypical *JAK2* mutations in exons 12 to 15.

CML, chronic myelogenous leukemia; PMF, primary myelofibrosis.

60 years of age and/or have experienced a prior thrombosis.[5,7,8] The latter is a strong predictor of subsequent thrombotic events,[9,10] but in the absence of a prior thrombotic event, advanced age has also shown to be a predictor of thrombosis. Specifically, patients with low-risk PV have approximately 2.5 thrombotic events per 100 persons per year compared to 10.9 events per 100 persons per year in high-risk disease.[9,11] However, these studies ignore functional status and comorbidities as key contributors toward "biological age" rather than "chronological age." Clearly, someone who is 60 years and 364 days old will not have a different risk profile the next day. Therefore, I emphasize a complete evaluation for thrombotic risk-stratification, including assessment of cardiovascular and functional status.

ET has several additional thrombosis risk models that incorporate risk factors, including cardiovascular comorbidities and the presence of the *JAK2* mutation.[12-14] These risk factors have been used to derive the International Prognostic Score of Thrombosis in World Health Organization—Essential Thrombocythemia (IPSET-thrombosis) score that was later revised (Figure 1).[10,15] IPSET-thrombosis low-, intermediate-, and high-risk patients have a thrombotic risk of 1.03%, 2.35%, and 3.56% per year.[10] Additional models have been developed to predict survival, including the MIPSS-ET and -PV scoring systems.[16,17] However, these models have not been calibrated to predict thrombosis and should not be used to determine appropriate patients for cytoreductive therapy.

I advocate the use of the revised IPSET-thrombosis score for ET and ELN risk-stratification for PV. In general, patients who are high risk should be treated with cytoreductive therapy. In terms of intermediate-risk ET patients (eg, age ≥60 with cardiovascular risk factors but without *JAK2* mutation or prior thrombosis), I aggressively manage modifiable risk factors and only provide cytoreductive therapy in the setting of vasomotor symptoms or acquired von Willebrand syndrome (aVWS), as described below.

The role of cytoreductive therapy in low-risk ET and PV

Some additional considerations for cytoreduction are outside thrombotic risk status. In ET, and occasionally in PV, patients with extreme thrombocytosis (ie, platelet count >1000×10⁹/L)

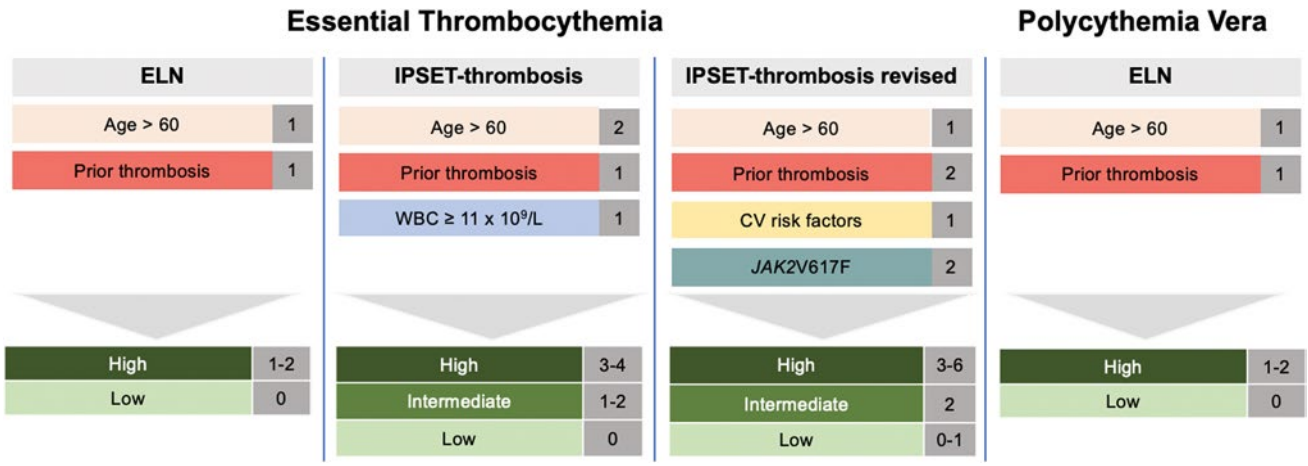

Figure 1. Risk-stratification for thrombosis in ET and PV. In ET there are 3 prognostic models to identify patients at low, intermediate, or high risk of thrombosis, while in PV there is only the European LeukemiaNet risk-stratification system.

are at risk of developing aVWS. In the case of bleeding from aVWS, cytoreductive therapy should be instituted to reduce the platelet number. In PV, cytoreduction can be utilized to reduce phlebotomy burden in patients who are intolerant of venesection or where it negatively impacts quality of life, although there is no specific number of phlebotomies to prompt the initiation of cytoreductive therapy in low-risk disease.[18] The recognition of symptoms as a result of iron deficiency, including fatigue and impaired concentration, is also emerging.[19] Other hematologic findings have also been proposed as indications for cytoreductive therapy, most notably leukocytosis in the case of PV. At present, I do not routinely cytoreduce for leukocytosis in the absence of other high-risk features, as an elevated white blood cell (WBC) has not consistently been demonstrated to be of thrombotic prognostic significance.[20-22]

An additional consideration for cytoreduction in low-risk patients is the presence of certain symptoms, particularly those that are microvascular.[23] Splenomegaly, although not frequently observed in ET and PV and typically mild,[16,24] can lead to early satiety, and the janus kinase (Jak) inhibitor ruxolitinib effectively ameliorate these symptoms.[25] Particularly in ET, where ruxolitinib is not approved, the development of splenomegaly should prompt a BM evaluation to exclude progression to myelofibrosis. Similarly, pruritus is particularly sensitive to treatment with Jak inhibitors.[25] Ropeginterferon-α-2b (ROPEG-IFN) has been explored in low-risk PV patients and has demonstrated a reduction in pruritus and night sweats, although with increased fatigue compared to standard treatment.[26] As such, I typically do not offer ROPEG-IFN to low-risk patients except to manage specific disease-related symptoms, although I do consider it in low-risk PV patients with phlebotomy intolerance, symptomatic splenomegaly, or progressive thrombocytosis or leukocytosis as recommended by the latest National Comprehensive Cancer Network (NCCN) guidelines.[4]

Importantly, noncytoreductive therapies for symptoms should be optimized (eg, aspirin for erythromelalgia) before initiating cytoreduction in a low-risk, symptomatic patient. Additionally, cytoreductive therapy for symptom control in ET and PV is most effective in patients with a high symptomatic burden, while minimally symptomatic patients may actually experience worsening symptoms, likely related to adverse events (AEs).[27]

CLINICAL CASE (continued)

Our patient was deemed to have low thrombotic risk by IPSET-thrombosis, and cytoreductive therapy was not recommended. After approximately 1 year, she developed worsening thrombocytosis to 1263×10⁹/L, associated with gum bleeding. She was diagnosed with aVWS, so cytoreductive therapy was reconsidered.

Cytoreductive therapies in ET

Currently available cytoreductive options for appropriate ET patients include hydroxyurea (HU), interferons, anagrelide, and busulfan. Starting with HU, its use is supported by randomized clinical trial data in high-risk patients.[28] HU has also been evaluated in lower-risk patients 40 to 59 years of age without a prior thrombosis and failed to show a significant reduction in thrombosis, bleeding, or death,[29] highlighting that cytoreductive therapy should only be offered in patients with a high risk of thrombosis.

Pegylated interferon (PEG-IFN) has been thoroughly evaluated for ET treatment both as an initial cytoreductive therapy and after HU intolerance/resistance. Data from the MPN-RC 111, which evaluated PEG-IFN after HU failure in both PV and ET, demonstrated an overall hematologic response rate of 69% among ET patients. Response rates were higher in *CALR*-mutated patients.[30] In the frontline setting in ET, the choice of HU vs PEG-IFN has been informed by several pivotal trials (Table 2). The first is the MPN-RC 112 trial, which randomized newly diagnosed high-risk PV and ET patients to HU or PEG-IFN. The primary end point of a complete response by ELN criteria at 12 months was similar in both treatment arms, as was spleen size reduction and WBC response. Similarly, there was no difference in symptom response between the HU (43%) and PEG-IFN (49%) groups. In the overall cohort (including PV), significantly more patients

Table 2. Summary of randomized trials of hydroxyurea and interferon in ET and PV

Trial	Patients	Arms	N	CHR at 24 months	Discontinuation rate because of AEs	Additional comments
MPN-RC 112[31]	High-risk ET (n=81) or PV (n=87)	HU	80	20%	11%	PEG-IFN led to greater reduction in *JAK2*V617F; HU had more histopathologic responses
		PEG-IFN	82	29%	15%	
DALIAH[33,44]	Newly diagnosed ET, PV, pre-PMF, or PMF	HU	38	21%	13%	Median *JAK2*V617F reduction was greater at 36 months in PEG-IFN arm compared to HU
		PEG-IFN[a]	164	26%	34%	
PROUD-PV/ CONTINUATION-PV[45]	High-risk PV untreated or <3 years of HU	HU	76	49%	4%	Molecular responses were higher in the ROPEG-IFN arm compared to standard therapy at 24 and 36 months
		ROPEG-IFN	95	71%	8%	

[a]PEG-IFN included both interferon-α-2a and interferon-α-2b.

PMF, primary myelofibrosis; PR, partial response.

had a histopathologic response in the HU arm. Reductions in the *JAK2* mutational allele burden regardless of treatment group were seen at 12 months, although there was a continued decrease with PEG-IFN, while the allele burden increased after 12 months in the HU arm. Notable toxicity differences included more grade 1 and 2 depression in PEG-IFN–treated patients compared with the HU arm. Other PEG-IFN–related AEs were common, including flu-like symptoms, injection-site reactions, and peripheral sensory neuropathy. Mucositis and anorexia were significantly more common in the HU arm.[31] Of note, the number of thrombotic and disease progression events were low, limiting the ability to detect differences between these outcomes.

The DALIAH trial randomized untreated MPN patients, including 73 ET patients, to either HU, PEG-IFN, or PEG-IFN-α-2b. Eligible patients had active disease as evidenced by the need for therapeutic phlebotomy, a WBC count above 10×10^9/L, a platelet count higher than 400×10^9/L, constitutional symptoms, pruritus, symptomatic splenomegaly, or previous thrombosis. At 36 months the overall hematologic response rate was higher in the HU group vs the combined interferon groups. In addition, treatment discontinuation because of AEs was significantly higher for interferon-treated patients. The median *JAK2* V617F allele burden reduction was higher in the interferon arms.[32] The *CALR* variant allele frequency did not significantly decline, and surprisingly, there were more treatment-emergent *DNMT3A* mutations in the interferon therapy arm compared with HU, suggesting that interferon does not prevent molecular evolution.[33]

Taken together, the MPN-RC 112 and DALIAH trials highlight that both HU and PEG-IFN are active treatments for ET and that either can be used as frontline cytoreductive therapy. In practice I advocate for a thorough discussion about toxicity profiles, about expected hematologic outcomes, and about the lack of substantial data on the differences in thrombosis or progression rates when discussing the difference between these agents.

In ET, another cytoreductive therapy option is anagrelide, an oral imidazoquinazoline derivative that represses megakaryocytic differentiation and that is dosed initially at 0.5 mg twice daily and up-titrated weekly by 0.5 mg based on hematologic response and tolerance.[34] Anagrelide has also been compared to HU as a first-line cytoreductive therapy. In a PT-1 study, patients with high-risk ET were randomized to either HU or anagrelide. During follow-up, anagrelide-treated patients were significantly more likely to experience thrombosis, hemorrhage, or death. Anagrelide treatment was associated with an increased rate of arterial thrombosis, hemorrhage, and transformation to myelofibrosis but a decreased rate of venous thrombosis.[35] Because the PT-1 trial used antiquated ET diagnostic criteria, the ANAHYDRET study was performed to assess whether anagrelide was noninferior to HU in untreated ET patients deemed to have high-risk factors, defined as aged 60 years or older, a platelet count equal to or greater than 1000×10^9/L, an increase in platelet count of more than 300×10^9/L within 3 months, cardiovascular risk factors including hypertension and diabetes, and/or a history of a thrombohemorrhagic event. There was no difference in the primary end point of thrombosis or bleeding, meeting its prespecified criteria for noninferiority. Cardiovascular side effects were more frequent in the anagrelide treatment arm, while leukopenia and minor infections were more common with HU.[36] Although an effective agent to lower platelet counts, dosing is often limited by toxicity, including palpitations/tachycardia, fluid retention, and diarrhea.[37]

Cytoreductive therapies in PV

The choice for first-line cytoreductive therapy in PV includes both HU and PEG-IFN, while ruxolitinib can be given in the setting of HU resistance or intolerance. HU use in high-risk PV is supported by retrospective data demonstrating a reduction in thrombosis,[38] although prospective randomized data demonstrating improvement in thrombotic burden with HU treatment are lacking. PEG-IFN has been explored in the treatment of PV in the MPN-RC 111 trial, which found an overall hematologic response rate at 12 months of 60%.[30] PEG-IFN and ROPEG-IFN are also an appropriate choice for frontline cytoreductive therapy as endorsed by NCCN guidelines.[4]

Ruxolitinib is approved for the treatment of PV in patients resistant or intolerant to HU based on the RESPONSE trial, which randomized PV patients with palpable splenomegaly to either ruxolitinib or best available therapy. Hematocrit control was significantly improved in ruxolitinib-treated patients. Importantly, ruxolitinib led to significant improvements in symptoms, with particularly striking reductions in pruritus, night sweats, and early satiety.[25] These findings were also echoed in the PV population without splenomegaly in a subsequent trial.[39] In the recently published MAJIC-PV study, which randomized HU-intolerant or -resistant PV patients to either best available

therapy or ruxolitinib, there was increased duration of response, improvement in event-free survival (thrombosis, hemorrhage, transformation, and death), and a significant increase in molecular responses observed with ruxolitinib therapy.[40] Ruxolitinib is a particularly attractive cytoreductive agent for patients with considerable symptom burden, particularly pruritus, and may reduce thrombotic burden.[40,41]

Another cytoreductive agent for both ET and PV is the alkylating agent busulfan, which is given at a starting dose of 2 mg/d. This agent can induce hematologic responses and can be given intermittently when counts rise.[42] However, it has been associated with an increased risk of leukemia, and therefore its use should be limited to elderly patients who have failed or are intolerant to other cytoreductive agents.[43]

Similar to ET, multiple investigations have compared first-line cytoreductive therapies in PV (Table 2). The previously discussed MPN-RC 112 trial showed that the overall response rate (ORR) at 12 months was not statistically different in the PV patient population.[31] The DALIAH trial also included a cohort of PV patients. There was no significant difference in overall response rates (ORRs) between the HU and interferon groups; however, maintenance of complete hematologic response was longer, and a reduction in the *JAK2* V617F allele was greater with interferon compared to HU.[44]

A monopegylated formulation of interferon-α-2b, ROPEG-IFN, has been developed that allows for every-other-week dosing. It is approved for the treatment of PV based on the PROUD-PV/CONTINUATION-PV study, which enrolled patients who were treatment naive or previously treated for less than 3 years with HU. High-risk patients were defined as aged older than 60 years or having a prior thrombotic event, phlebotomy intolerance, progressive splenomegaly, a platelet count above 1000×10^9/L, or a leukocyte count higher than 10×10^9/L. Importantly, these inclusion criteria are different than the MPN-RC 112 trial, most notably the allowable duration of HU exposure before enrollment (3 years vs 3 months), limiting comparisons. There was no significant difference in complete hematologic response (CHR) at 12 months between ROPEG-IFN and HU. At 36 months, the end point of CHR plus improved disease burden (defined as resolution or clinical improvement of disease-related splenomegaly, microvascular disturbances, pruritus, or headache) was higher in the ROPEG-IFN arm. Molecular responses were significantly higher with HU at 12 months but became higher at 24 and 36 months in the ROPEG-IFN arm. There was no significant difference in thrombosis rates between the two arms.[45] Although it is tempting to correlate the reduction in *JAK2* mutational burden with a decreased rate of progression, there is insufficient evidence at present to support this claim. Of note, retrospective evidence suggests that interferon-based therapies are associated with a decreased myelofibrosis-free and overall survival.[46] Although provocative, given the inherent limitations with this study design, these findings need to be confirmed in larger, ideally prospective investigations before they can be incorporated into clinical practice.

The decision to use ROPEG-IFN vs HU for cytoreduction in PV is largely redundant with PEG-IFN vs HU. If an interferon-based approach is determined, I will employ ROPEG-IFN given its dosing schedule and full regulatory approval for the treatment of PV.

Assessing response (focus on hematologic parameters)

Once a cytoreductive strategy has been chosen, how do you determine a response? ELN criteria for both ET and PV dictate the normalization of blood counts, in addition to a lack of thrombosis, bleeding, or disease progression to myelofibrosis.[47] However, these response criteria have not been validated, and in fact several lines of retrospective evidence suggest that obtaining an ELN response in ET and PV is not associated with a decreased risk of thrombosis or survival (Table 3).[48-51] As the primary function of these response criteria is for uniform reporting of clinical trial results, increasing the dose of cytoreductive therapy or changing treatment to achieve a response is not recommended. In ET the optimal platelet goal is not established, and the fact that there is no clear relationship between platelet count and thrombosis risk is well recognized.[52,53]

I typically aim to control platelet count to a near-normal range without compromising tolerability. In particular, if the normalization of platelet count results in symptomatic anemia or moderate neutropenia, then the dose of a cytoreductive agent should be reduced. In PV patients being treated with cytoreductive therapy who require therapeutic phlebotomy, I titrate the dose to limit phlebotomies to less than 3 annually, as this has been associated with a decreased risk of thrombotic complications (albeit only in the context of HU treatment).[54] Importantly, interferon-based therapies may take longer to achieve a response.[45] I typically increase the dose monthly to optimize hematologic control and other treatment goals. Most importantly, it is key to continually assess changes in symptoms, both in terms of improvements as well as the emergence of therapy-related AEs.

Special circumstances

In selected circumstances there is a clear preference for one cytoreductive therapy over another. In the setting of pregnancy and in patients trying to conceive, PEG-IFN has demonstrated safety based on retrospective series,[55,56] although available data are not robust enough to exclude safety concerns as they relate

Table 3. Studies assessing the association of ELN response and thrombosis or survival

Study	Disease	N	Treatment	Association with ELN complete response	
				Thrombosis	Survival
Hernandez-Boluda et al[48]	ET	134	HU	No	No
Hernandez-Boluda et al[49]	ET	154	Anagrelide	No	No
Alvarez-Larrán et al[50]	PV	261	HU	No	No
Tremblay et al[51]	PV	398	HU PEG-IFN Ruxolitinib	No	No

to fetal development. In contrast, HU is a known teratogen. Therefore, in younger patients who are considering conception I recommend interferon-based therapies. There has been a historic concern that HU is leukemogenic; however, contemporary analysis has not substantiated this association.[57] Interferons should not be given in the setting of severe depression, anxiety, or other psychiatric conditions or in patients with acute/untreated autoimmune disease.[58] Anagrelide should be used with caution in patients with congestive heart failure or arrhythmias.

Conclusions

As described above, the choices around who, what, when, and how to deliver cytoreductive therapy in ET and PV are highly individualized and should involve the consideration of multiple factors, including patient preference. To date, there is insufficient evidence to suggest that there are clinically significant efficacy advantages to one cytoreductive therapy over another. Ideally, prospective clinical trials would be able to discriminate thrombosis rates, time to disease progression, and ultimately survival. However, these events occur over the course of decades at relatively low rates, requiring large and long clinical trials that are not feasible. Targeting high-risk subjects to increase the expected event rate should be considered when designing studies with thrombotic end points, in particular.[50] Biomarkers and additional clinical features may also serve as surrogate end points in the future, although data to identify and validate these metrics are lacking. In the future, with the development of novel therapies for ET and PV that can not only reduce thrombotic burden but also modify disease progression and survival, the use of risk scores that predict survival (eg, MIPSS-ET and -PV) may be relevant for deciding when and how to initiate cytoreductive therapy. Increased recognition and attention toward the identification/validation of surrogate end points for thrombosis and the development of novel therapeutics will be required to advance ET and PV clinical research and identify the optimal cytoreductive strategy in these MPNs.

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Off-label drug use

Douglas Tremblay: pegylated interferon

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Evolving landscape of JAK inhibition in myelofibrosis: monotherapy and combinations

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Myeloproliferative neoplasms (MPNs) are characterized by clonal myeloproliferation in 1 or more of the hematopoietic stem cell lineages. Primary myelofibrosis (MF), post–polycythemia vera MF, and post–essential thrombocythemia MF have the worst prognosis and are characterized by the presence of cytokine-mediated symptom complex, splenomegaly, progressive marrow failure, and clonal instability, leading to leukemic transformation. The key therapeutic aims encompass the management of symptoms, splenomegaly, and anemia and the improvement of survivals. These therapeutic aims have evolved with the availability of Jak inhibitors and novel agents, making disease modification potentially achievable. Novel agents may potentially target MPN stem cells, epigenetic alterations, signaling pathways, and apoptotic pathways. In this case-based review, we outline our approach to the management of MF and discuss the therapeutic landscape of MF, highlighting the utility of Jak inhibitors and novel Jak inhibitor–based combinations.

LEARNING OBJECTIVES

- Cite the efficacies of Jak inhibitors and novel agents in the first- and second-line settings
- Discuss the therapeutic approach to MF, taking into consideration patient characteristics and clinical needs

CLINICAL CASE

A 71-year-old woman diagnosed with polycythemia vera (PV) in 1999 had received regular venesections, aspirin, and hydroxyurea. Since 2015 the number of venesections and the dosage of hydroxyurea required for optimal hemoglobin (Hb) control had gradually decreased. From September 2022 onward, she had developed progressive weight loss. Physical examination showed mild pallor and an 8-cm splenomegaly. A complete blood count showed an Hb level of 8.5 g/dL, a leukocyte count of 8.2×10^9/L, and a platelet count of 226×10^9/L. There was a leukoerythroblastic blood picture with 1% blasts and teardrop poikilocytes. The bone marrow (BM) aspirate was a "dry tap." The BM trephine biopsy showed a diffuse increase in reticulin (MF-3) and collagen fibrosis (grade 2). Blasts were not obviously increased. Features were consistent with post-PV myelofibrosis (MF). Next-generation sequencing showed *JAK2* V617F with a variant allele frequency (VAF) of 0.89, a *DNMT3A* p.R749L missense variant (VAF, 0.45), and a *TET2* splicing variant (VAF, 0.43). Karyotyping showed t(6;11)(q23;q23) in 4 of 20 metaphases. According to the Myelofibrosis Secondary to PV and ET-Prognostic Model, the risk was intermediate-2. At the latest follow-up, her Myelofibrosis Symptom Assessment from (MFSAF) Version 4.0 Total Symptom Score (TSS) was 27.

Introduction and treatment needs in MF

The classical Philadelphia chromosome–negative myeloproliferative neoplasms (MPNs) comprise PV, essential thrombocythemia (ET), and primary MF (PMF), which are associated with mutations of the driver genes *JAK2*, *CALR*, and *MPL*. PMF is subclassified into prefibrotic/early PMF and overt PMF. Overt PMF is characterized by marrow fibrosis, cytokine-mediated systemic symptoms, anemia, hepatosplenomegaly, and a propensity for progression to acute myeloid leukemia. PV and ET may progress to post-PV and post-ET—MF with symptoms resembling those of overt PMF. The key treatment needs in MF include managing symptoms deriving from constitutional problems, insufficient quality of life, and anemia; controlling splenomegaly; and improving survival via disease modification (the 3 *S*s) (Figure 1). Given the current understanding of the disease's biology, disease modification in MF is defined as therapy resulting in a clinically meaningful impact on survival outcome and/or the restoration of normal hematopoiesis with improvement in marrow fibrosis and durable

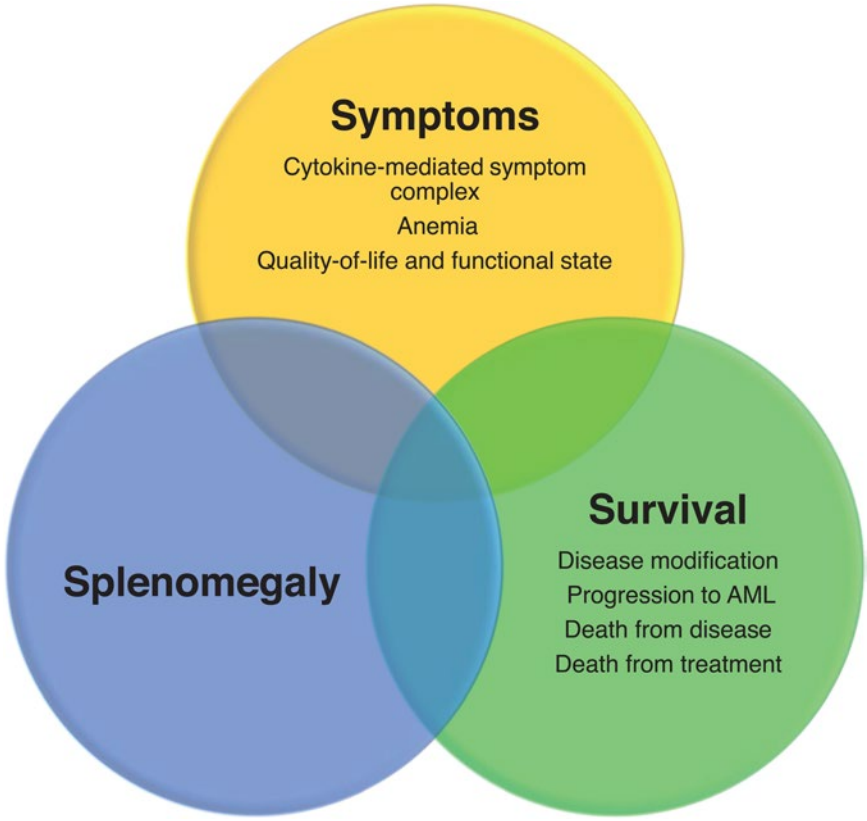

Figure 1. Treatment needs in patients with MF. AML, acute myeloid leukemia.

reduction in the clonal burden.[1] Allogeneic hematopoietic stem cell transplantation is currently the only potentially curative therapy, although applicability is limited by significant mortality and morbidity. Ruxolitinib, a first-in-class Jak inhibitor, is the most widely used, effectively improving symptoms and splenomegaly and possibly modestly improving survival.[2,3] However, ruxolitinib may not adequately address the underlying disease biology, showing generally modest effects on mutant allele burdens, BM fibrosis (BMF), and the prevention of leukemic transformation. Three other Jak2 inhibitors, fedratinib, pacritinib, and momelotinib, have also been developed, but they, too, do not address all the unmet needs in MF, especially in the second-line setting and disease modification.[4] To address these problems, multiple "non–Jak inhibitor" molecules have been developed and are being tested in phase 2 and 3 studies, either as monotherapy or in combination with Jak2 inhibitors (Table 1) (Table 2).

CLINICAL CASE (continued)

The patient was started on ruxolitinib at 20 mg twice per day. She responded well with an improvement of MFSAF-TSS to 4 after 8 weeks of therapy. The splenomegaly decreased to 5 cm. Her Hb level remained stable, between 8 to 9 g/dL. Twenty-eight months later, her symptoms worsened, with the MFSAF-TSS increasing to 25 and the splenomegaly, to 12 cm. The latest complete blood count showed a Hb level of 7.1 g/dL, a leukocyte count of 12.5×10^9/L, and a platelet count of 120×10^9/L. The ruxolitinib was reduced to 15 mg twice a day in view of the worsening anemia and thrombocytopenia.

Jak inhibitors as monotherapy

Ruxolitinib—what have we learned so far?

Ruxolitinib is a nonselective Jak1/2 inhibitor that controls cellular proliferation and splenomegaly by inhibiting constitutively activated Jak/STAT signaling and constitutional symptoms by reducing the production of pro-inflammatory cytokines. Pooled analysis and long-term follow-up of the COMFORT-I and COMFORT-II studies showed that ruxolitinib treatment significantly prolonged overall survival (OS).[2] Patients receiving ruxolitinib for 12 months or more from diagnosis had better spleen responses, longer OS, and fewer hematologic toxicities.[2]

Approximately 50% of patients discontinue ruxolitinib after 3 years, mostly due to disease progression, suboptimal response, or cytopenia. Definitions of "ruxolitinib failure" vary and are largely based on studies in the second-line setting, generally including disease progression to accelerated or blast phase, suboptimal response of spleen or constitutional symptoms, increases in splenomegaly or constitutional symptoms after initial response, and the development of transfusion-dependent (TD) anemia or grade 3/4 thrombocytopenia or hemorrhagic events while on ruxolitinib.[5] Outcome after ruxolitinib discontinuation is generally poor, with a median OS of approximately 14 months. Patients with 3 or more nondriver gene mutations generally have a shorter time to discontinuation. A clinical prognostic model was developed

Table 1. Results of selected studies of Jak inhibitors as monotherapy in MF

Jak inhibitor/ targets	Study/phase	Population	Treatment/ sample size	Control/ sample size	TSS50 at wk 24	SVR35 at wk 24	Anemia response	Molecular responses/BMF reduction	Relevant toxicities
Ruxolitinib Jak1, Jak2	COMFORT-1 phase 3	Int-2/high-risk MF Platelets ≥100×10⁹/L Intolerant/resistant to available therapy	15 mg twice a day for platelets 100–200×10⁹/L; 20 mg twice a day for platelets >200×10⁹/L N = 155	Placebo N = 154	45.9%	41.9%	TI: 41% (14/34 TI patients)	*JAK2* V617F VAF: 8% reduction at 24 wk; 17% at 48 wk BMF reduction: NR	Anemia, thrombocytopenia, headache, opportunistic infections
	COMFORT-2 phase 3	Int-2/high-risk MF Platelets ≥100×10⁹/L	Same as COMFORT-1 N = 146	BAT N = 73	NR	32%	NR	*JAK2* V617F VAF: 38% with >20% reduction at 168 wk BMF reduction: 16% after median of 26 mo	
Fedratinib Jak1, Jak2, Jak3, TYK3	JAKARTA phase 3	Int-2/high-risk MF Platelets ≥50×10⁹/L Jak inhibitor naive	400 mg or 500 mg/d N=193	Placebo N = 96	36% with 400 mg/d; 34% with 500 mg/d	36% with 400 mg/d; 40% with 500 mg/d	TI: 88% (7/8 TD patients)	*JAK2* V617F VAF: 0.4% increase at 24 wk/ BMF: NR	Anemia, thrombocytopenia, gastrointestinal toxicity, transaminitis, raised amylase and lipase, Wernicke's encephalopathy (black box warning)
	JAKARTA-2 phase 2	Int-1 MF with symptoms Int-2/high-risk MF Platelets ≥50×10⁹/L Ruxolitinib failure/ intolerance	400 mg/d N = 97	NA	26%	55%	NR	NR	
Pacritinib Jak2, FLT3, IRAK1, CSF1R, ACVR1	PERSIST-1 phase 3	Int-1/int-2/high-risk MF Any platelet count Jak inhibitor naive	400 mg/d N = 220	BAT N = 107 (excluded ruxolitinib)	19%	19%	TI: 25% (9/36 TD patients)	*JAK2* V617F VAF: 15.8% reduction at 24 wk	Thrombocytopenia, anemia, diarrhea and gastrointestinal toxicity, fluid retention, heart failure, squamous cell skin cancer
	PERSIST-2 phase 3	Int-1/int-2/high-risk MF Platelets <100×10⁹/L Jak inhibitor exposed or naive	400 mg/d or 200 mg twice a day N = 211	BAT N = 100 (45% on ruxolitinib)	25%	18%	TI ≥8 wk or ≥2 g/dL increase in Hb: 25% (11/44 with Hb <10 g/dL)	NR	
	PAC203 phase 2	Int-1/int-2/high-risk MF Any platelet count Ruxolitinib failure/ intolerance	100mg/d or 100mg twice a day or 200 mg twice a day N = 165	NA	7.5%	9.3% for 200 mg twice a day; 1.8% for 100 mg twice a day; 0% for 100 mg/d	≥1 g/dL increase in Hb: 10% (4/42 with Hb <10 g/dL)	NR	

Table 1 Results of selected studies of Jak inhibitors as monotherapy in MF (Continued)

Jak inhibitor/ targets	Study/phase	Population	Treatment/ sample size	Control/ sample size	TSS50 at wk 24	SVR35 at wk 24	Anemia response	Molecular responses/BMF reduction	Relevant toxicities
Momelotinib Jak1, Jak2, ACVR1	SIMPLIFY-1 phase 3	Int-1 MF with symptoms Int-2/high-risk MF Platelets ≥50×10⁹/L Jak inhibitor naive	200 mg/d N = 215	Ruxolitinib N = 217	28.4%	26.5%	TI at 24 wk: 66.5%	NR	Anemia, thrombocytopenia, neutropenia, transaminitis, raised amylase/lipase, peripheral neuropathy, first-dose effect (transient hypotension, flushing, dizziness, and nausea)
	SIMPLIFY-2 phase 3	Int-1 MF with symptoms Int-2/high-risk MF Any platelet count Suboptimal response/intolerance to ruxolitinib	200 mg/d N = 104	BAT N = 52 (89% ruxolitinib)	26.2%	7%	TI at 24 wk: 43%	NR	
	MOMENTUM phase 3	Int-1/Int-2/high-risk MF with symptoms Platelets ≥25×10⁹/L Hb <10 g/dL Jak inhibitor exposed	200 mg/d N = 130	Danazol N = 65	25%	23%	TI at 24 wk: 31%	NR	
Jaktinib Jak1, Jak2, ACVR1, TYK2	NCT03886415 phase 2	Int-1/Int-2/high-risk MF with symptoms Platelets ≥75×10⁹/L	100 mg twice a day or 200mg/d N = 118	NA	69.6% with 100 mg twice a day; 57.5% with 200 mg/d	54.8% with 100mg twice a day; 31.3% with 200 mg/d	Hb increase: 36% (in patients with Hb <10 g/dL)	NR	Anemia, thrombocytopenia

CSF1R, colony stimulating factor 1 receptor; Int-1, intermediate-1 risk by Dynamic International Prognostic Scoring System; Int-2, intermediate-2 risk by Dynamic International Prognostic Scoring System; IRAK1, interleukin 1 receptor associated kinase 1; NA, not available; NR, not reported.

Data compiled from Verstovsek et al[2]; Mascarenhas et al[4]; Pardanani et al[8]; Harrison et al[9]; Mesa et al[12]; Harrison et al[13]; Verstovsek et al[14]; Zhang et al.[32]

Table 2. Results of selected Jak inhibitor-based combinations for MF

Agent	Setting	Regimen	Phase	Study population/sample size	Clinical responses	Molecular responses	BMF reduction
Targeting hematopoietic stem cells							
INF-α	First-line	Ruxolitinib + PEG-IFN-α2A	1/2	Int-1, int-2, and high-risk MF Jak inhibitor naive/ N = 37 (phase 1, N =18; phase 2, N = 19)	≥50% reduction in spleen length at 24 wk: 70%	*JAK2* V617F VAF decreased from a median of 84% (range, 23%-96%) at baseline to 65% (range, 16–95) and 53% (range, 16–92) after 6 and 12 mo	NR
Targeting epigenetic regulators							
HMAs	First-line	Ruxolitinib + azacitidine 25–75 mg/m^2/d days 1–5 every 4 wk	2	Int-1, int-2, and high-risk MF Jak inhibitor naive/ N = 46	>50% reduction in spleen length at 24 wk: 62% (21/34); best TSS50: 54% (25/46); TI: 20% (1/5)	81% (13/16) had reduction in *JAK2* V617F VAF at 24 wk	57% (8/14) had BM reticulin fibrosis reduction at 24 wk
BET inhibition	First-line	Ruxolitinib + pelabresib	2	Int-1, int-2, and high-risk MF Jak inhibitor naive/ N = 84	SVR35 at 24 wk: 68%; TSS50 at 24 wk: 56%; ≥1.5g/dL over 12 wk: 24%	NR	28% evaluable patients had reduction in BMF at 24 wk
	Second-line	Ruxolitinib + pelabresib	2	Int-1, int-2, and high-risk MF Jak inhibitor naive/ N = 86	SVR35 at 24 wk: 20% (16/81); TSS50 at 24 wk: 37% (30/81)	NR	26% evaluable patients had reduction in BM reticulin fibrosis at 24 wk
Targeting apoptotic pathways							
BCLXL/BCL2 inhibition	First-line	Ruxolitinib + navitoclax	2	Int-1, int-2, and high-risk MF Jak inhibitor naive/ N = 32	SVR35 at 24 wk: 52%; TSS50 at 24 wk: 31%; TI: 55%	50% and 36% of patients had >20% reduction in *JAK2* V617F VAF	35% had reduction in BM reticulin fibrosis at any time
	Second-line	Ruxolitinib + navitoclax	2	Int-1, int-2, and high-risk MF Suboptimal response to ruxolitinib N = 34	SVR35 at 24 wk: 26.5%; TSS50 at 24 wk: 30%; TI: 64%	46% had >10% reduction in VAF of driver gene mutations	33% had reduction in BM reticulin fibrosis at any time
Selective inhibition of nuclear export	First-line	Ruxolitinib + selinexor	1/2	Int-1, int-2, and high-risk MF N = 22	SVR35 at 24 wk: 64% overall; 79% (11/14) in the 60-mg group and 38% (3/8) in the 40-mg group, respectively; TSS50 at 24 wk: 45% overall; 58% (7/12) in the 60-mg group and 25% (2/8) in the 40-mg group;	50% (4/8) had >10% reduction in VAF, and 25% (2/8) had >20% reduction in VAF in the 60-mg group	NR
Targeting bone marrow microenvironment							
Activin receptor IIB ligand trap	Second-line ("add-on")	Ruxolitinib + luspatercept	2	Patients on ruxolitinib ≥16 wk prior to enrollment and TD; N = 38	31.6% achieved TI for ≥12 wk over entire treatment period; 50% had ≥50% reduction in transfusions (≥4 units) over 12 wk	NR	NR

Int-1, intermediate-1 risk by Dynamic International Prognostic Scoring System; Int-2, intermediate-2 risk by Dynamic International Prognostic Scoring System; NR, not reported; PI3K, phosphatidylinositol 3-kinase; RBC, red blood cell.

Data compiled from Kiladjian et al[15]; Masarova et al[18]; Mascarenhas et al[21]; Harrison et al[22]; Passamonti et al[25]; Ali et al[29]; Gerds et al[31]; Harrison et al[33]; Passamonti et al[34]; Passamonti et al[35]; Pemmaraju et al.[36]

to determine survival after 6 months of treatment with ruxolitinib in patients with MF.[6] Risk factors for worse OS after 6 months included a ruxolitinib dose less than 20mg twice a day at baseline, 3 months, and 6 months; a palpable spleen length reduction from baseline of 30% or less at 3 and 6 months; and transfusion need at 3 and/or 6 months or at any time point. The "response to ruxolitinib after 6 months" model effectively stratified patients into 3 prognostic risk groups (low risk: median OS not reached; intermediate risk: median OS equal to 61 months, and high-risk: median OS equal to 33 months).[6]

Fedratinib, pacritinib, and momelotinib—what can they offer?

Fedratinib is a potent Jak2–fms-like tyrosine kinase 3 (FLT3)–bromodomain 4 (BRD4) inhibitor approved for intermediate-2 and high-risk MF irrespective of prior ruxolitinib use. In the JAKARTA studies,[7,8] fedratinib effectively reduced splenomegaly and symptom burden in patients naive or exposed to Jak inhibitor. In Jak inhibitor–naive patients, a 35% reduction in spleen volume (SVR35) and a 50% reduction in MF TSS (TSS50) was achieved in 47% and 40% of patients during week 24.[8] Fedratinib-treated patients also achieved clinically meaningful improvement in health-related QOL.[9]

Pacritinib is a Jak2/FLT3 inhibitor approved for intermediate-2 or high-risk MF with platelet counts equal to or less than 50×10^9/L. In the pooled analysis of the PERSIST-1 and PERSIST-2 studies comprising 189 patients with platelet counts of equal to or less than 50×10^9/L (median platelet count, 28×10^9/L; 63.5% with a Hb level less than 10 g/dL; 35% with prior Jak inhibitor treatment), SVR35 and modified TSS50 was achieved in 23.1% and 25% of cases at week 24.

Momelotinib is a Jak1/Jak2 inhibitor that has additional inhibitory effects against activin A receptor type 1 (ACVR1).[10] ACVR1 is an important mediator of SMAD2/3 signaling that upregulates hepcidin production and results in iron-restricted erythropoiesis. SMAD2/3 signaling is particularly implicated in the inhibition of terminal erythroid maturation and ineffective erythropoiesis. In the SIMPLIFY-1 study,[11] which uniquely compared head-to-head momelotinib with ruxolitinib, the rate of SVR35 was similar between the 2 arms at week 24 (momelotinib, 26.5%; ruxolitinib, 29%), while symptom score reduction at week 24 was higher in the ruxolitinib arm (momelotinib, 28.4; ruxolitinib, 42.2%). However, the rate of red cell transfusion independence (TI) at week 24 was remarkably different (momelotinib, 66.5%; ruxolitinib, 49.3%).[11] The achievement of TI with momelotinib was associated with superior 3-year OS at 77.2%.[11,12] In the SIMPLIFY-2 study, momelotinib was evaluated in patients with a suboptimal response or intolerance to ruxolitinib.[13] At week 24, SVR35 was achieved in 7% in the momelotinib arm compared with 6% in patients on best available therapy (BAT, with 89% receiving ruxolitinib).[13] TI was achieved in 49.3% in the momelotinib arm and 21% in the BAT arm.[13] The phase 3 MOMENTUM study evaluated Jak inhibitor–exposed patients with intermediate- or high-risk MF with an Hb level lower than 10 g/dL, a symptom score of 10 of above, and a platelet count of 25×10^9/L or higher.[14] SVR35 and symptom score response rates at week 24 were achieved in 23% and 24.6% in the momelotinib arm and 3% and 9.2% in the danazol arm.[14] The rates of TI at week 24 were 31% for momelotinib and 20% for danazol.[14]

CLINICAL CASE (continued)

The patient was concerned about her long-term survival and risk of leukemic progression. After counseling, the patient was referred to a clinical trial involving ruxolitinib in combination with a novel agent.

Promising "partners" of Jak inhibitors

Targeting hematopoietic stem cells

Interferon alfa (IFN-α)

In a phase 1/2 study of ruxolitinib in combination with pegylated IFN-α2A (PEG-IFN-α2A) in 37 patients with MF, 70% of patients achieved a reduction of 50% or better in palpable spleen length with significant reductions of *JAK2* V617F VAF.[15] ROPEG-IFN-α2b, with more potent activity against MPN hematopoietic stem cells (HSCs), is being evaluated as a single agent in early/prefibrotic and low- and intermediate-1 risk MF, with significant clinical and molecular responses achieved.[16] In an interim analysis of 56 patients, 36 (92%) of 39 *JAK2* V617F–mutated patients had stable or improved *JAK2* V617F VAF by droplet digital polymerase reaction.[16]

Telomerase inhibition

Human telomerase reverse transcriptase (hTERT) activity is overexpressed in MPN HSCs, leading to uncontrolled myeloproliferation.

Imetelstat is a first-in-class telomerase inhibitor that binds to the RNA component of telomerase in MPN HSCs, suppressing telomerase activity. In a randomized phase 2 study, 107 patients with Jak inhibitor relapsed/refractory MF were treated with imetelstat at 9.4 mg/kg or 4.7 mg/kg given intravenously every 3 weeks.[17] SVR35 and TSS50 were achieved in 10.2% and 32.2% in the 9.4 mg/kg arm and 0% and 6.3% in the 4.7 mg/kg arm. In patients treated with imetelstat at 9.4 mg/kg, BMF reduction was observed in 40.5% of patients, with a median OS of 29.9 months.[17] A confirmatory phase 3 study is ongoing (NCT04576156).

Targeting epigenetic regulators

Hypomethylating agents (HMAs)

In a phase 2 study of azacitidine in combination with ruxolitinib as the first-line treatment of 46 patients with intermediate-risk and high-risk MF, 62% and 54% achieved a reduction of 50% or higher in palpable spleen length and TSS50 at week 24.[18] A reduction in *JAK2* V617F VAF was observed in 81% of patients at 24 weeks, with 54% of evaluable patients showing a reduction in marrow fibrosis. Ruxolitinib in combination with decitabine achieved an overall response rate of 42.9% (9/21) in patients with accelerated or blast phase MPNs.[19]

BRD and extraterminal (BET) protein inhibition

BET proteins (BRD2, BRD3, BRD4) are a family of chromatin-reader proteins binding to acetylated lysine residues on histones to initiate oncogene transcription and proinflammatory NF-κB activation.[20] The BET inhibitor pelabresib in combination with ruxolitinib in the frontline resulted in SVR35 and TSS50 of 68% and 56% at week 24 in 84 patients with intermediate- and high-risk MF.[21] In addition, 36% of patients showed improvement in Hb level. A reduction in BMF and a reduction greater than 25% in *JAK2* V617F VAF was achieved in 28% and 29.5% of patients. In 86

patients with relapsed/refractory MF on ruxolitinib, pelabresib as an "add-on" therapy achieved SVR35 of 20% and TSS50 of 37% at 24 weeks.[22] Beyond 24 weeks, a BMF reduction of 1 grade or more was observed in 26% of evaluable patients.[22]

Lysine-specific demethylase-1 (LSD1) inhibition

In MPNs, LSD1 is overexpressed and maintains self-renewal of MPN HSCs. The LSD1 inhibitor bomedemstat (IMG-7289) was evaluated in 89 patients with MF failing prior to janus kinase inhibitor (Jak) and achieved SVR35 and TSS50 in 37% and 6% of patients with prior ruxolitinib exposure.[23] In TI patients (N = 41), 90% had stable or improved Hb levels. In TD patients, 14% became TI. In 59 evaluable patients, 85% experienced stable or improved BM reticulin fibrosis by at least 1 grade or more.[23] Fifty-three percent of patients with improved BMF also demonstrated improvement in Hb levels or a reduced transfusion requirement.[23] In 43 patients with follow-up genomic data, the VAF in one or more alleles was reduced in 42%. *ASXL1* mutations were the most commonly reduced, with most consistent declines in truncating *ASXL1* mutations clustered around codon 642.[23] A phase 2 study of ruxolitinib in combination with bomedemstat in the frontline and second-line setting is ongoing (NCT05569538).

Targeting apoptotic pathways

B-cell lymphoma extra-large (BCLXL) inhibition

BCLXL is a member of the B-cell lymphoma-2 (BCL2) family protein that is highly expressed in MF regardless of *JAK2* mutational status. Navitoclax is a novel BCL2/BCLXL inhibitor being evaluated in combination with ruxolitinib. In the phase 2 REFINE study, navitoclax as an "add-on" treatment in 34 patients with suboptimal responses to ruxolitinib achieved SVR35 and TSS50 in 26.5% and 30% of patients, respectively, at week 24.[24] In addition, 64% of TD patients achieved TI.[24] Forty-six percent of patients had a decrease of more than 10% in the VAF of driver gene mutations, and 21% had improvement in marrow fibrosis at 24 weeks.[24] In the frontline setting evaluating 32 patients with MF, navitoclax in combination with ruxolitinib achieved SVR35 in 52% at week 24, with benefits seen across all risk groups.[25] A reduction in *JAK2* V617F VAF greater than 20% from baseline was demonstrated in 50% (14/28) of patients at week 12 or 24.

Human double-minute 2 (HDM2) inhibition

HDM2 negatively regulates *TP53* by ubiquitination and is overexpressed in MF HSCs. HDM2 can be targeted to restore *TP53* activity.[26] Following earlier studies, the MDM2 inhibitor navtemadlin (KRT-232) was evaluated in a proof-of-concept phase 2 study as

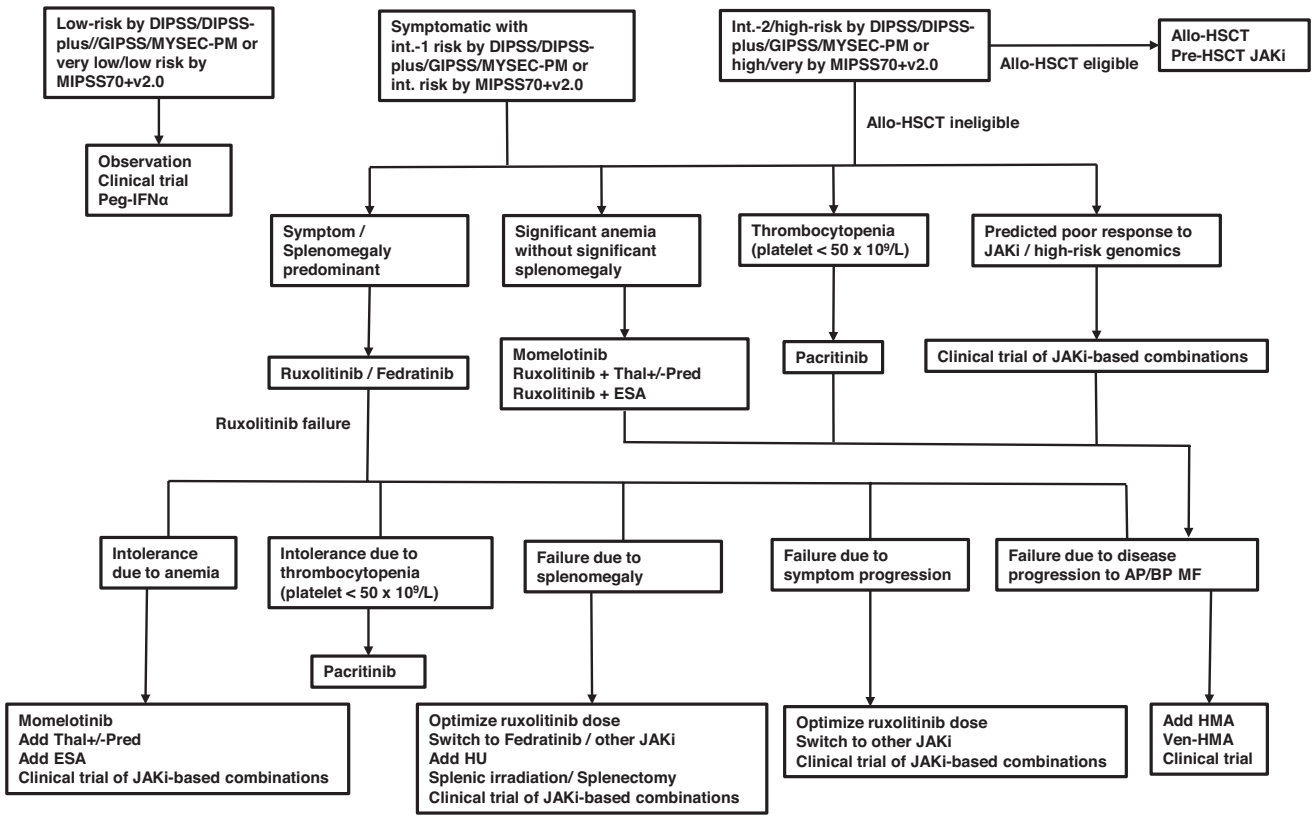

Figure 2. Treatment algorithm in patients with MF. alloHSCT, allogeneic hematopoietic stem cell transplantation; AP/BP, accelerated phase/blast phase; DIPSS, Dynamic International Prognostic Scoring System; ESA, erythropoiesis-stimulating agents; GIPSS, Genetically Inspired Prognostic Scoring System for Primary Myelofibrosis; HU, hydroxyurea; MIPSS70+v2.0, Mutation-Enhanced International Prognostic Scoring System 70 plus version 2.0; MYSEC-PM, Myelofibrosis Secondary to PV and ET Prognostic Model; Pred, prednisolone; Thal, thalidomide; Ven, venetoclax.

a single agent in patients refractory or resistant to Jak inhibitors. In 113 patients treated with navtemadlin, SVR35 and TSS50 were achieved in 16% and 30% of patients at week 24.[27] Thirty-four percent experienced a reduction of 20% or more in VAF of driver or high-molecular-risk gene mutations, and 27% achieved improvement in marrow fibrosis.[27] Based on these findings, the phase 3 BOREAS study has been initiated to evaluate the efficacy and safety of navtemadlin vs BAT for patients with MF refractory or resistant to Jak inhibitors (NCT03662126).

Selecting inhibition of nuclear export

The inhibition of nuclear-cytoplasmic transport by selective inhibitors of nuclear export leads to the nuclear accumulation of p53 and induces apoptosis of *JAK2* V617F+ cell lines resistant to Jak inhibitors.[28] In addition, selective inhibitors of nuclear export act synergistically with ruxolitinib in vitro and in vivo.[28] In a phase 1/2 study of ruxolitinib in combination with selinexor (40mg or 60mg/wk) in the frontline setting, 64% (14/22) overall achieved SVR35 at week 24 (79% in the 60-mg group vs 38% in the 40-mg group).[29] At week 24, TSS50 was achieved in 45% (9/20) overall (58% in the 60-mg group and 25% in the 40-mg group.[29] In the 60-mg group, the reduction of *JAK2* V617F VAF at week 24 was greater than 10% in 50% (4/8) and greater than 20% in 25% (2/8).[29]

Targeting of the BM microenvironment

Depleting cytokine production via transforming growth factor β (TGF-β) inhibition

The activin receptor IIA ligand trap luspatercept binds to TGF-β superfamily ligands to stimulate terminal erythroid maturation and improve anemia.[30] Luspatercept was evaluated in patients with MF in a phase 2 study.[31] In a subgroup of 38 patients on ruxolitinib who were TD, 26.3% achieved TI during the primary treatment period.[31] Nineteen patients (50.0%) experienced a 50% or more reduction in transfusion burden during the primary treatment period, with 8 (21.1%) achieving a mean increase in Hb level of 1.5 g/dL or greater from baseline throughout the entire treatment period.[31]

Conclusions and future perspectives

The availability of multiple Jak inhibitors and novel agents has made feasible an individualized therapeutic approach to MF based on unique patient characteristics and treatment needs (Figure 2). Furthermore, the application of novel agents has realized the possibility of disease modification even in hematopoietic stem cell transplantation–ineligible patients. Results of ongoing phase 2 and 3 studies of novel agents and Jak inhibitor–based combinations are eagerly awaited and will add to the evolving management algorithm of MF.

Conflict-of-interest disclosure

Harinder Gill: consultancy: Bristol Myers Squibb, GSK, Novartis, Pfizer, PharmaEssentia; advisory board: Bristol Myers Squibb, GSK, Novartis, Pfizer, PharmaEssentia; investigator-initiated study collaboration: Imago Biosciences, Novartis, PharmaEssentia.

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Off-label drug use

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Are transplant indications changing for myelofibrosis?

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Myelofibrosis is a devastating myeloid malignancy characterized by dysregulation of the JAK-STAT pathway, resulting in splenomegaly, constitutional symptoms, anemia, thrombocytopenia, leukocytosis, and an increased likelihood of progression to acute leukemia. The only curative option is allogeneic stem cell transplantation. The numbers of transplants have been increasing every year, and although there have been improvements in survival, there remain many unanswered questions. In this review, we will evaluate patient selection and appropriate timing for transplantation. We will cover the current prognostic scoring systems, which can aid in the decision of when to move forward with transplant. We will also review the different donor options, as well as the conditioning regimens. The peritransplant management of splenomegaly will be reviewed. We will discuss management of posttransplant complications such as loss of donor chimerism or disease relapse. Finally, we will review what is known about the outlook of patients who have undergone allogeneic stem cell transplant with regards to quality of life and long-term survival.

LEARNING OBJECTIVES

- Compare the different prognostic scoring systems for patients who have myelofibrosis when considering allogeneic stem cell transplant
- Evaluate different donor sources and conditioning regimens
- Compare different methods of management of splenomegaly in the peritransplant setting
- Apply concepts to management of posttransplant issues such as disease relapse, as well as long-term outlook

CLINICAL CASE

The patient is a 57-year-old woman with primary myelofibrosis (PMF) diagnosed when she presented with anemia and leukocytosis in 2018. At the time of diagnosis, she had a hemoglobin of 12 g/dL and white blood cell (WBC) count of 15×10^9/L with leukoerythroblastosis but no peripheral blasts. Her spleen was palpable at 3 cm below the left costal margin. She had mild night sweats and weight loss. Bone marrow biopsy specimen showed 3/3 reticulin fibrosis and no increase in blasts. She had a *JAK2* mutation (variant allele frequency [VAF] 35%) and *TET2* mutation (VAF 26%). She was started on ruxolitinib with improvement of her spleen, as well as symptoms. After 3 years, she started to have worsening (hemoglobin 10 g/dL), and WBC count increased to 29×10^9/L. She had 2% blasts in her peripheral blood. Her spleen was palpable at 7cm below the left costal margin. Bone marrow biopsy specimen showed ongoing fibrosis and no increase in blasts. Cytogenetics showed a new trisomy 8, and next-generation sequencing (NGS) showed *JAK2* mutation (VAF 43%), *TET2* (VAF 40%) mutation, and a new *ASXL1* (VAF 45%) mutation. She was referred to a bone marrow transplant specialist.

Allogeneic stem cell transplant (ASCT) is the only curative treatment for patients with myelofibrosis (MF). Although the benefit of transplant is well established, there are still many questions as to timing of transplant. Myelofibrosis is a disease that can span over many years; therefore, identifying the correct time in the disease course is critical.

When approaching a patient who has MF, there are 2 critical aspects to review with the patient: first, whether they desire a transplant once they understand the risks and benefits of a transplant and, second, what their disease risk is. The patient's perception of transplant for MF can vary significantly and in many cases can be a barrier to transplantation. In an Internet-based survey, of 129 patients, only 41 patients were referred for transplant, and of those, only 16 patients intended on proceeding with transplant.[1] In another study of 116 transplant-eligible patients, only 102

decided to proceed with human leukocyte antigen typing, 41 patients went to upfront transplant, and of those who did not, only 15 went to a salvage transplant.[2] For this reason, I feel it is important that patients are referred to transplantation early in the disease course, to allow for time to process the information and make an educated decision.

Once it is determined that the patient is interested in transplant, it is important to perform an optimal disease risk assessment. Many prognostic scoring systems can be applied, including a dynamic international prognostic scoring system (DIPSS), DIPSS plus, a molecularly annotated international prognostic scoring system 70 (MIPSS70), MIPSS70 plus v2.0, and MYelofibrosis SECondary to PV and ET–Prognostic Model (MYSEC-PM) (see Table 1). The most commonly used one is the DIPSS, a prognostic scoring system for patients with PMF, which takes into account laboratory factors such as hemoglobin less than 10 g/dL, WBC count greater than 25×10^9/L, and peripheral blasts ≥2%, as well as clinical factors such as age and constitutional symptoms. Each factor gives a score of 1 except anemia, which conveys a higher risk, so it has a score of 2 points. The DIPSS stratifies patients into low, intermediate 1 (Int-1), intermediate 2 (Int-2), and high-risk categories, corresponding to a median overall survival (OS) of not reached, 14.2 years, 4.0 years, and 1.5 years, respectively.[3] Generally speaking, transplant is reserved for Int-2 and high-risk disease.[4] The use of DIPSS Int-2 risk disease as a cutoff was validated in a retrospective study that compared patients who had ASCT vs retrospective comparators from a 14-center registry between 2000 and 2014. There were 551 patients who underwent transplant and 1377 who underwent nontransplant management. The median time for follow-up was 72 months (3-193) for the transplanted patients and 63 months (<1-208) for the nontransplanted patients. It is notable that only 10% of the patients who underwent transplant were exposed to ruxolitinib as compared to 30% who were not exposed to ruxolitinib.[5] There was a survival advantage appreciated in Int-1, Int-2, and high-risk patients, although in the Int-1 risk patients, that benefit was not observed for a number of years, and there was a high rate of early mortality.[5] More refined scoring systems are needed to determine which patients with Int-1 risk disease will benefit from transplant. It is important to acknowledge that the DIPSS and DIPSS plus are validated in PMF but not secondary MF. MYSEC-PM, a score designed for secondary MF, is more predictive for outcomes following transplant for MF but still does not perform well.[6] Further, the same comparative studies evaluating survival with and without transplant have not been done with the MYSEC-PM.

Over the past decade, the genetic landscape of patients with MF has been under intense study. With regard to the driver mutations associated with MF, patients with type 1 *CALR* mutations have the most favorable prognosis, and patients who do not have a driver mutation, also known as triple negative, have the worst prognosis.[7] Additionally, several other mutations have been associated with a higher-risk prognosis, including *ASXL1*, *IDH1/2*, *EZH2*, *SRSF2*, and *U2AF1*.[7] To account for the impact of the somatic mutations, a novel scoring system, the mutation enhanced international prognostic scoring system, was created: MIPSS 70 and MIPSS70 plus 2.0. These scoring systems take into account similar data as the DIPSS but also include driver mutation, grade fibrosis, NGS, and cytogenetics (in MIPSS70 plus 2.0). In the MIPSS70, there are 3 risk categories, including low risk, intermediate risk, and high risk; the MIPSS70 plus 2.0 creates 4 risk categories: low, intermediate, high, and very high.[7] These scoring systems place heavy consideration on both the mutational landscape as well as the cytogenetics. It is critical to acknowledge that patients may stay in a given risk category for years and, based on laboratory variations, may fluctuate between risk levels. It is important to

Table 1. Prognostic scoring systems for myelofibrosis

	DIPSS[3]	DIPSS plus[38]	MIPSS70[7]	MIPSS70 plus	MYSEC[39]
Age	☑	☑	☑	☑	☑
Anemia (hemoglobin <10)	☑	☑	☑	☑	☑
WBC >25	☑	☑	☑		
Blast %	☑	☑	☑	☑	☑
Constitutional symptoms	☑	☑	☑	☑	☑
Platelets <100		☑	☑		☑
Red cell transfusion dependent		☑			
Unfavorable karyotype*		☑		☑	
Bone marrow fibrosis grade			☑		
High-risk molecular mutations (1)			☑	☑	
High-risk molecular mutations (≥2)			☑	☑	
Type 1 *CALR* absence			☑		☑
Scoring	Low risk: 0	Low: 0	Low: 0–1	Low: 0–2	Low: <11
	Int-1: 1–2	Int-1: 1	Intermediate: 2–4	Intermediate: 3	Int-1: 11–13
	Int-2: 3–4	Int-2: 2–3	High: ≥5	High: 4–6	Int-2: 14–16
	High risk: 5–6	High risk: 4–6		Very high: ≥7	High: >16

*Unfavorable karyotype: Complex karyotype or one or two abnormalities that include +8, –7/7q–, i(17q), –5/5q–, 12p–, inv(3), or 11q23. High-Risk molecular mutations: *ASXL1, IDH1/2, EZH2, SRSF2*, and *U2AF1*.

monitor the trend of labs and have ongoing discussions with the patient regarding their disease risk.

In the patient described above, when initially seen, she would have been considered Int-1 risk, only scoring a point for constitutional symptoms. It would be appropriate to have her meet with a transplant specialist at the initial time point, as starting discussions with the transplant physician earlier can be beneficial for the patient. However, based on current recommendations, she would not be considered a transplant candidate at that point. When she started to progress through ruxolitinib, she would be still be considered Int-1 risk based on DIPSS but would be considered high risk based on MIPSS70 and MIPSS70 plus 2.0. Additionally, she has progressed through ruxolitinib, which is associated with a median survival of about 14 months.[8] Therefore, she is at a time when it would be very appropriate to consider ASCT.

Other factors that should be accounted for when considering transplant include age and hematopoietic cell transplant comorbidity index. Patients older than 55 years have a increased risk of mortality in the setting of transplant for MF.[4,9] There has not been a direct comparison in recent years of patients older vs younger 65 years of age, but in a recent analysis of patients older than 65 years, the 5-year survival was 40%, as compared to 50% in a recent analysis including all age groups.[10] The upper age limit has not been established. The European LeukemiaNet (ELN)–European Society for Blood and Marrow Transplantation (EBMT) recommendations from 2015 suggest 70 years is the upper age limit for transplant,[4] but many centers have higher upper age limit cutoffs, and the aforementioned study included patients up to 75 years of age. In unadjusted multivariate analysis, age less than or greater than 68 did not impact outcome.[10] Another predictive model can be employed at this time, the Myelofibrosis Transplant Scoring System, at this time point. This scoring system takes into account factors such as patient age, mutation status (non-*CAL-R/MPL* driver mutation and presence of *ASXL1*), platelets, WBC count, patient performance status, and donor status (matched related/unrelated vs mismatched unrelated donor).[11] This scoring system accounts for variables at the time of transplant and is applicable to both PMF and secondary MF.[11]

Patient has no siblings but does have 2 adult children. An unrelated donor search was done, which showed one 10/10 matched unrelated donor (MUD) and several 9/10 mismatched unrelated donors (MMUDs). Her adult children are both haploidentical, and she does not have any donor-specific antibodies. Choice of donor for ASCT has evolved over the past 10 years. Over time, the donor pool has expanded in that MMUD and haploidentical donors can be considered. In the setting of transplant for MF, there appears to be increased intolerance of mismatches, particularly in the unrelated donor setting, where a 9/10 donor is thought to be associated with inferior outcomes as compared to a MUD or a matched related donor (MRD), with 5-year survival around 38% to 48% (see Table 2).[12-14] Until recently, there were very little data evaluating the use of a haploidentical donor in the setting of MF, but recently, Kunte et al[14] published a multicenter retrospective analysis of haploidentical transplant for MF and found 3-year survival of 72% (95% CI, 59%-81%). Another retrospective study done using the EBMT registry of patients undergoing haploidentical transplant showed a 2-year survival of 57%.[15] It is important to note, in the United States, any patient who has Medicare must participate in the Myelofibrosis Medicare Study. In this study, the donor must have a fully matched related or unrelated donor. If

Table 2. Donor source and outcomes

Reference	N	TRM	OS
Kröger et al. 2009[13]	**103 PMF, post-ET MF, post-PV MF** HLA matched: 82 HLA mismatched: 21	**At 1 y** MRD: 10% MUD: 13% MMUD: 38%	**At 5 y** MRD/MUD: 74% MMUD: 38% *P*=.03
Rondelli et al. 2014[40]	**66 PMF, post-ET MF, post-PV MF** MRD: 30 Haplo: 2 MUD: 25 MMUD: 9	MRD: 22% MUD: 59%	MRD: 75% MUD: 32%
Gupta et al. 2014[41]	**233 PMF** MRD: 79 MUD: 104 MMUD: 50	**At 1 y: 18%** **At 5 y: 24%** MUD: 3.92 MMUD: 9.37 (*P*<.0001)	**Adjusted OS at 5 y** MRD: 56% MUD: 48% MMUD: 34% (*P*=.002)
Raj et al. 2019[15]	**PMF 42, secondary MF 14** 56 MMRD	**35% (95% CI, 22%-48%) @ 1 y**	**61% (95% CI, 48%-74%) @ 1 y**
McLornan et al. 2021[42]	**4142 patients** **PMF 3239, post-ET MF 494, post-PV MF 409** MRD: 1430 MUD: 1554 MMRD: 226 MMUD: 537 CB: 31	**NRM hazard ratio:** MRD: 0 MUD: 1.3 (1.11–1.54), *P*=.001 MMRD: 1.76 (1.32–2.36) *P*≤.001 MMUD: 1.9 (1.56–2.31), *P*≤.001	**OS hazard ratio** MRD: 0 MUD: 1.21 (1.06–1.38), *P*=.005 MMRD: 1.51 (1.16–1.97), *P*=.002 MMUD: 1.67 (1.41–1.97), *P*≤.001
Kunte et al. 2022[14]	**69 patients** **PMF 35, post-ET MF 19, post-PV MF 15**	**@ 1 y: 21% (95% CI, 12%-32%)** **@ 3 y: 23% (95% CI, 14%-34%)**	**@ 1 y: 74% (95% CI, 61%-83%)** **@ 3 y: 72% (95% CI, 59%-81%)**

CB, cord blood; ET, essential thrombocythemia; HLA, human leukocyte antigen; MMRD, mismatched related donor; NRM, non-relapse mortality; PV, polycythemia vera; TRM, treatment-related mortality.

neither of them are available, a haploidentical donor may be used (Medicare Clinical Trials, cibmtr.org). Therefore, in this case, if the MUD did not work out, I would likely consider a haploidentical donor over a 9/10 donor, using a posttransplant cyclophosphamide-based graft-versus-host disease prophylaxis.

Conditioning regimen has also been an area of debate in the field of transplantation. In the situations where a myeloablative regimen is preferred, busulfan-based regimens can be considered, pairing it with fludarabine or cyclophosphamide.[16] Reduced intensity conditioning (RIC) regimens, such as fludarabine/melphalan, or fludarabine/8 to 10 mg/kg busulfan have been used.[17] A recent publication evaluating over 800 patients with MF who underwent ASCT suggest that both myeloablative and RIC regimens including busulfan and fludarabine appear to have improved outcomes as compared to busulfan/cyclophosphamide and fludarabine/melphalan regimens, respectively.[18] In those patients who can tolerate a myeloablative regimen, it is unclear whether an ablative regimen is superior to an RIC regimen. A retrospective analysis done by EBMT did not see a significant difference between those transplanted with RIC or myeloablative conditioning regimens, but there was a trend toward decreased risk of relapse in patients who underwent MA transplant.[19]

Another factor that must be considered prior to ASCT is management of an enlarged spleen. Several studies have suggested outcomes of transplant in patients with a spleen >15 cm are associated with worse outcomes.[20,21] There are increasing data that use of ruxolitinib prior to transplant is not only safe but also may provide improved outcomes (see Table 2).[22,23] JAK inhibition can be continued until the time of the conditioning regimen,[24] but some studies have used it up until engraftment,[25] day 30,[22,25] or even up to 1 year.[22] In this case, where there has been clear progression through ruxolitinib, one of the newer generation of JAK inhibitors such as fedratinib and pacritinib can be considered,[26,27] although there are limited data of their use in this setting. In the setting where JAK inhibition is contraindicated or not tolerated, both splenic radiation and splenectomy can be considered. Splenic irradiation has been studied in the pretransplant setting and appears to be safe, but whether it provides an advantage over splenectomy or no treatment is not clear.[28,29] In an analysis by the EBMT, they found that in patients whose spleen was greater than 15 cm below the left costal margin (LCM), improved survival was appreciated in patients who underwent splenectomy.[21] However, substantial morbidity and mortality are associated with splenectomy,[30] and this analysis only included patients who survived the splenectomy to make it to transplant, so it is possible that patients who would otherwise be transplant candidates may be unable to receive a transplant once they have undergone splenectomy (Table 3).

CLINICAL CASE (continued)

Patient undergoes ASCT from a 10/10 MUD. Her transplant course is unremarkable. At her day 100 analysis, she was noted to have 10% JAK2 positivity in her peripheral blood; CD33 sorted chimerism showed 90% donor, 10% recipient, but otherwise no evidence of MF.

Relapse remains a problem in transplant for MF; between 15% and 45% of patients will experience a relapse.[4] Definition of relapse may also be a challenge following transplant. Determination cannot be made by the presence of fibrosis of the bone marrow as it may take up to 2 years for fibrosis to resolve.[31] Molecular relapse includes persistence of the driver mutation at 3 to 6 months and loss of donor chimerism.[32] Overt relapse is characterized by increasing fibrosis or cellularity, as well as reappearance of megakaryocyte atypia.[33] Although the literature is fairly consistent in the increased risk of relapse in loss of myeloid chimerism,[32] persistence of JAK2 mutation following transplant is not as clearly associated with relapse.[32,34] In a large retrospective review done by EBMT, different approaches to relapse were compared, and there was no significant difference between donor lymphocyte infusion (DLI) alone, chemotherapy + DLI, or DLI and second transplant.[35] In this case, I would proceed with a DLI.

CLINICAL CASE (continued)

Patient was given a DLI and cleared the JAK2, and CD33 sorted chimerism returned to 100% donor. She wants to better understand her long-term outlook.

Although there is a high rate of treatment-related mortality with transplant, the long-term outlook is encouraging. With regards to quality of life, 61% of patients report feeling better at 1 year posttransplant as compared to pretransplant.[36] Long-

Table 3. JAK inhibitor prior to allogeneic stem cell transplantation

Reference (first author)	Prospective/ retrospective	Conditioning	N	Spleen response	Ruxolitinib discontinuation	Graft failure	GVHD II-IV	TRM	OS
Kröger 2018[24]	Pros	RIC	12	50%	Day +28	0%	8%	0% 17 mo	100% @ 17 mo
Kadir 2018[43]	Retro	RIC	46	39%	Varied	4%	37%	23% @ 2 y	72.7 @ 2 y
Gupta 2019[44]	Pros	RIC	21	45%	Prior to conditioning	16%	47%	28% @ 2 y	66% @ 2 y
Salit 2020[23]	Pros	RIC/MAC	28	NR	Prior to conditioning	0	78%	23% @ 1 y	86% @ 2 y
Kröger 2021[45]	Retro	RIC/MAC	277	56%	NR	NR	29%	26% @ 1 y	66% @ 1 y
Robin 2021[46]	Pros	RIC	59	46%	Prior to transplant—varied	3%	66%	42% @ 1 y	68% @ 1 y
Ali 2022[25]	Pros	RIC	18	NR	Day +30	0%	45%	23% @ 1 y	77% @ 1 y

GVHD, graft-versus-host disease; MAC, myeloablative conditioning; NR, not reported; Pros, prospective; Retro, retrospective.

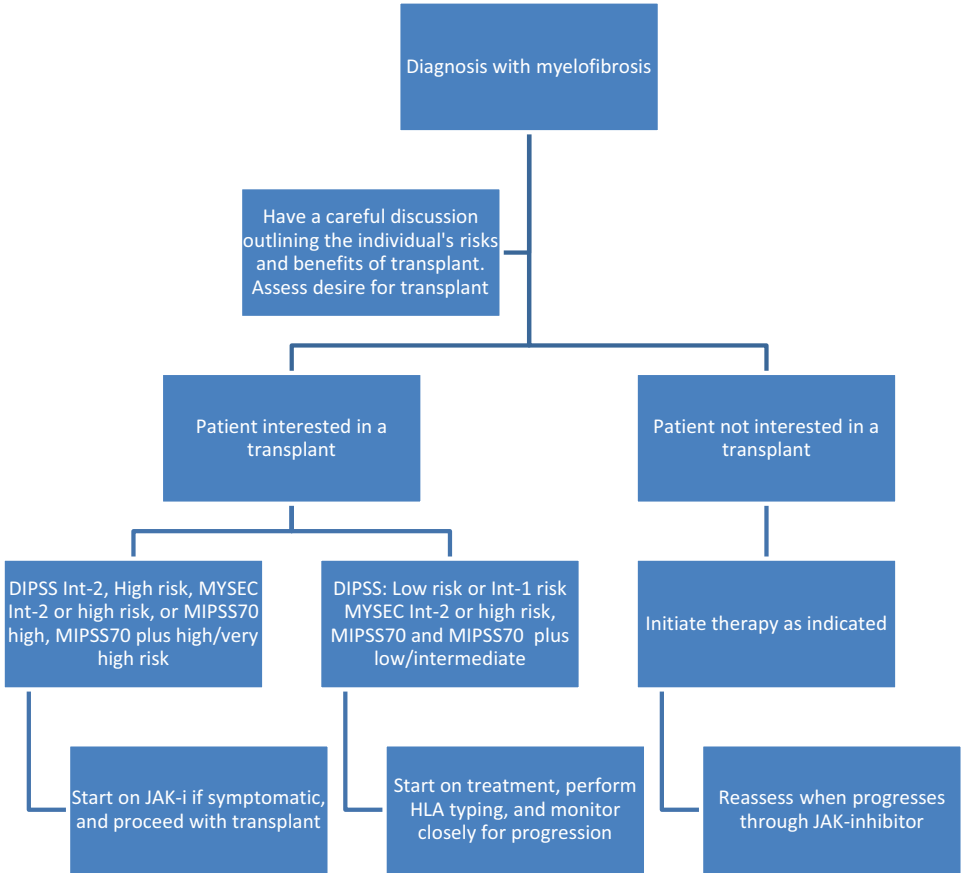

Figure 1. Approach to myelofibrosis. This table outlines an approach for patients with myelofibrosis when considering transplant. It is important to highlight the importance of the initial discussion needed prior to assessing interest in proceeding with transplant.

term survival was assessed in an EBMT registry study doing a landmark analysis on patients who were alive at 2 years following transplant. Of 2,459 patients who received first allo transplant between 1995 and 2014, 1,055 were alive at 2 years, and 10-year OS and disease-free survival for 2-year survivors were 74% (71%-78%) and 64% (60%-68%), respectively.[37] Factors that are associated with a higher risk of late mortality include older age, secondary MF, male sex, and no graft-versus-host disease prior to the landmark date.[37]

In summary, ASCT is a curable therapy for patients with MF. It is important to be methodical when approaching a patient with MF (see Figure 1). Decision on timing of transplant is a careful evaluation of age, patient preference, and careful risk assessment with the available risk calculators. Donor choice should be MRD > MUD > haploidentical donor > MMUD. If there is splenomegaly, ideally the patient should be treated with a JAK inhibitor prior to transplant, but whether the JAK inhibitor should be continued following transplant is not clear. Splenectomy or splenic irradiation may be an option for the appropriately selected patient. Following transplant, it is important to monitor chimerism and driver mutation as a means of determining relapse and consider DLI. Looking forward, once a patient has survived the first year or 2 following transplant, long-term outlook is far more optimistic.

Conflict-of-interest disclosure

Jeanne Palmer: no competing financial interests to declare.

Off-label drug use

Jeanne Palmer: not applicable.

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Understanding differential technologies for detection of MRD and how to incorporate into clinical practice

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Patient- and leukemia-specific factors assessed at diagnosis classify patients with acute myeloid leukemia (AML) in risk categories that are prognostic for outcome. The induction phase with intensive chemotherapy in fit patients aims to reach a complete remission (CR) of less than 5% blasts in bone marrow by morphology. To deepen and sustain the response, induction is followed by consolidation treatment. This postremission treatment of patients with AML is graduated in intensity based on this favorable, intermediate, or adverse risk group classification as defined in the European Leukemia Network (ELN) 2022 recommendations. The increment of evidence that measurable residual disease (MRD) after induction can be superimposed on risk group at diagnosis is instrumental in tailoring further treatment accordingly. Several techniques are applied to detect MRD such as multiparameter flow cytometry (MFC), quantitative (digital) polymerase chain reaction (PCR), and next-generation sequencing. The clinical implementation of MRD and the technique used differ among institutes, leading to the accumulation of a wide range of data, and therefore harmonization is warranted. Currently, evidence for MRD guidance is limited to the time point after induction using MFC or quantitative PCR for *NPM1* and core binding factor abnormalities in intermediate-risk patients. The role of MRD in targeted or nonintensive therapies needs to be clarified, although some data show improved survival in patients achieving CR-MRD negativity. Potential application of MRD for selection of conditioning before stem cell transplantation, monitoring after consolidation, and use as an intermediate end point in clinical trials need further evaluation.

LEARNING OBJECTIVES

- Recognize that measurable residual disease (MRD) assessment is recommended as prognostic factor in intensively treated patients as measured after induction chemotherapy
- Understand that molecular MRD monitoring at the end of treatment and during follow-up is recommended for selected patients
- Appreciate that clinical relevance of MRD at other time points and different therapies (targeted/nonintensive) needs further evaluation
- Realize that standardization of MRD measurements is essential for in vitro diagnostic regulations and use of MRD as an intermediate end point

CLINICAL CASE

A female patient of 65 years of age was referred to our hospital. Bone marrow (BM) aspiration showed a high proportion of myeloblasts by morphology and multiparameter flow cytometry (MFC), which led to the diagnosis of acute myeloid leukemia (AML). The disease was classified as de novo AML, not otherwise specified.[1] The patient was stratified as European Leukemia Network (ELN) 2022 intermediate risk[2] based on normal karyotype, an *FLT3*/ITD mutation (at low allelic ratio, although ELN 2022 does not require allelic ratio), and a *DNMT3A* mutation. At diagnosis, the leukemia was characterized by MFC and revealed a leukemia-associated phenotype (LAIP) with the combination of cluster of differentiation markers (CD) of $CD34^{+}CD13^{+}CD7^{+}$.

The patient's age, medical history, and overall health indicated her eligibility for intensive 7+3 chemotherapy (cytarabine continuously for 7 days, along with short infusions of an anthracycline on each of the first 3 days). She was enrolled in a randomized trial comparing standard

7+3 chemotherapy with midostaurin to 7+3 chemotherapy including a different (more targeted) tyrosine kinase inhibitor. After the first induction cycle, the patient reached morphologic complete remission (CR; <5% blasts in BM by microscopy). MFC–measurable residual disease (MRD) showed 0.15% LAIP cells of the total white blood cells. Considering the applied MFC-MRD assay, this was deemed MRD positive (≥0.1%). Retrospective analysis using next-generation sequencing (NGS)–MRD confirmed MRD positivity for *FLT3*/ITD at 0.36%. A donor search was initiated, and she received the second chemotherapy cycle. After this second cycle, MFC-MRD showed MRD negativity of 0.04% (MRD negative), while the post hoc analysis identified the patient MRD positive for *FLT3*/ITD with a variant allele frequency (VAF) of 0.09%. The patient received myeloablative conditioning and peripheral blood (PB) stem cells from a matched unrelated donor for allogeneic stem cell transplantation (alloSCT), and the patient achieved CR_{MRD} negative by both MRD techniques and resumed the FLT3 inhibitor after blood count recovery.

Disease monitoring was performed at 1 and 3 months, at which time MFC and NGS identified MRD relapse by MRD conversion from negative to positive. In the BM, the MFC-MRD was 0.2% of a new LAIP ($CD34^+CD13^+CD56^+$), while the original LAIP was absent, and *FLT3*/ITD was detectable with a VAF of 1.6%. Another BM after 2 weeks confirmed MRD relapse (MFC 0.3%, NGS 2.6%). This coincided with a mixed donor/patient T-cell chimerism detected in PB.[3] The immunosuppression was decreased and donor lymphocyte infusions were started.[4,5] This resulted in a decrease in the LAIP cells, and MRD was cleared entirely after 3 months. After 2 years, the MRD monitoring was stopped, and the patient was followed up by clinical visits and standard laboratory evaluation.

The ongoing trial the patient was enrolled in is designed to evaluate MRD as a secondary end point for surrogacy of the clinical end-point event-free survival (EFS). In case the primary end-point EFS is improved by the new tyrosine kinase inhibitor and MRD is indeed significantly lower after a defined period of treatment, it is ideally suited to establish surrogacy of MRD for faster and more efficient evaluation of new treatment strategies.

In this review, we discuss the pros and cons of different MRD technologies, MRD results, and their clinical implications based on the available data.

Introduction

AML is a heterogeneous disease in both biology and clinical outcome. Main reasons for poor outcome are refractory disease (no CR) or relapse. To tailor therapy, estimating the risk of poor response to treatment is now imperative. Therefore, after the initial assessment of AML, the diagnostic workup needs to be more detailed to determine the prognostic risk group. This is mainly based on cytogenetic and molecular aberrations, leading to 3 ELN risk groups (favorable, intermediate, and adverse).[2] First-line treatment of patients with AML aims at reaching CR by eradicating the majority of leukemia cells. This commonly consists of 1 or 2 cycles of intensive induction chemotherapy. For *FLT3*/ITD-mutated patients, addition of the multitargeted kinase inhibitor midostaurin significantly increased EFS and overall survival (OS)[6] and is now standard of care. The induction phase is then followed by consolidation treatment to extend the response, and the intensity is commonly selected based on risk group. First-line treatment options with increasing intensity are (1) up to 3 cycles of chemotherapy (commonly intermediate-dose AraC), (2) myeloablative chemotherapy rescued by autologous stem cell transplantation, and (3) allogenic stem cell transplantation (alloSCT). Increasing treatment intensity will improve antileukemia efficacy but comes with adverse side effects and increased nonrelapse mortality (Figure 1). According to ELN, alloSCT is the preferred consolidation treatment for patients with an estimated relapse risk exceeding 35% to 40%.[2,7] It needs to be emphasized that intensity of treatment is not solely based on laboratory testing but on patient-related factors as well, such as older age or comorbidities.

The ELN-AML guideline[2] recommends refinement of morphologic response assessment by more sensitive MRD assessment, and the ELN-MRD guidelines[8] provide the tools for standardized MRD evaluation (Table 1). Flow cytometry is commonly used for AML diagnosis, and hence the technique is widely available. MFC-MRD testing still needs expertise and strict criteria due to the rather extensive and subjective gating strategies. In addition, MFC may not be sensitive enough (10^{-3}-10^{-4}) to detect minimal numbers of residual cells. Molecular techniques such as quantitative polymerase chain reaction (PCR) or digital PCR are more sensitive (10^{-6}) but only suitable for patients with specific molecular aberrations (*NPM1* mutation or core binding factor [CBF] translocations). This may be resolved when several mutations can be followed simultaneously using NGS.[9] *FLT3*/ITD has been a difficult target to monitor MRD due to the variable length of the insertions and the duplicity of sequences.[10] However, NGS techniques have been applied[11] and the getITD analysis algorithm is increasingly used for MRD monitoring.[12]

MRD at diagnosis

For accurate risk classification,[2] the leukemic cells are molecularly characterized by cytogenetic analysis and DNA sequencing. When BM is available (or, if not, PB), MFC-MRD can be performed to characterize the patient-specific LAIP at diagnosis, thereby allowing to follow the LAIP in the MRD setting. A LAIP is defined as CD marker expression combinations present on >10% of blasts that are not found in BM from healthy individuals. A LAIP can be identified in about 90% of patients.[13] Measurement of MFC-MRD is recommended when patients do not harbor *NPM1* mutations or CBF translocations. Otherwise, patients should be monitored by quantitative PCR or digital PCR for *NPM1* mutations or CBF translocations.[8] Based on the recent data on *FLT3*/ITD monitoring by NGS, we expect a rapid uptake of this approach to refine or replace MFC-MRD monitoring as in our patient.[14,15] Furthermore, mutations such as *DNMT3A*, *TET2*, and *ASXL1* (DTA) mutations may reflect clonal hematopoiesis of indeterminate potential and can be often present with a relatively high VAF in nonmalignant clones and therefore cannot be used for MRD assessment.[14,16]

MRD after the first cycle of chemotherapy

For accurate detection of MFC-MRD after chemotherapy, BM material is required and patients should be in morphologic CR. For the MFC-MRD measurement, the LAIP approach would require only measuring the flow cytometry panel tube that contains the LAIP found at diagnosis. However, during ther-

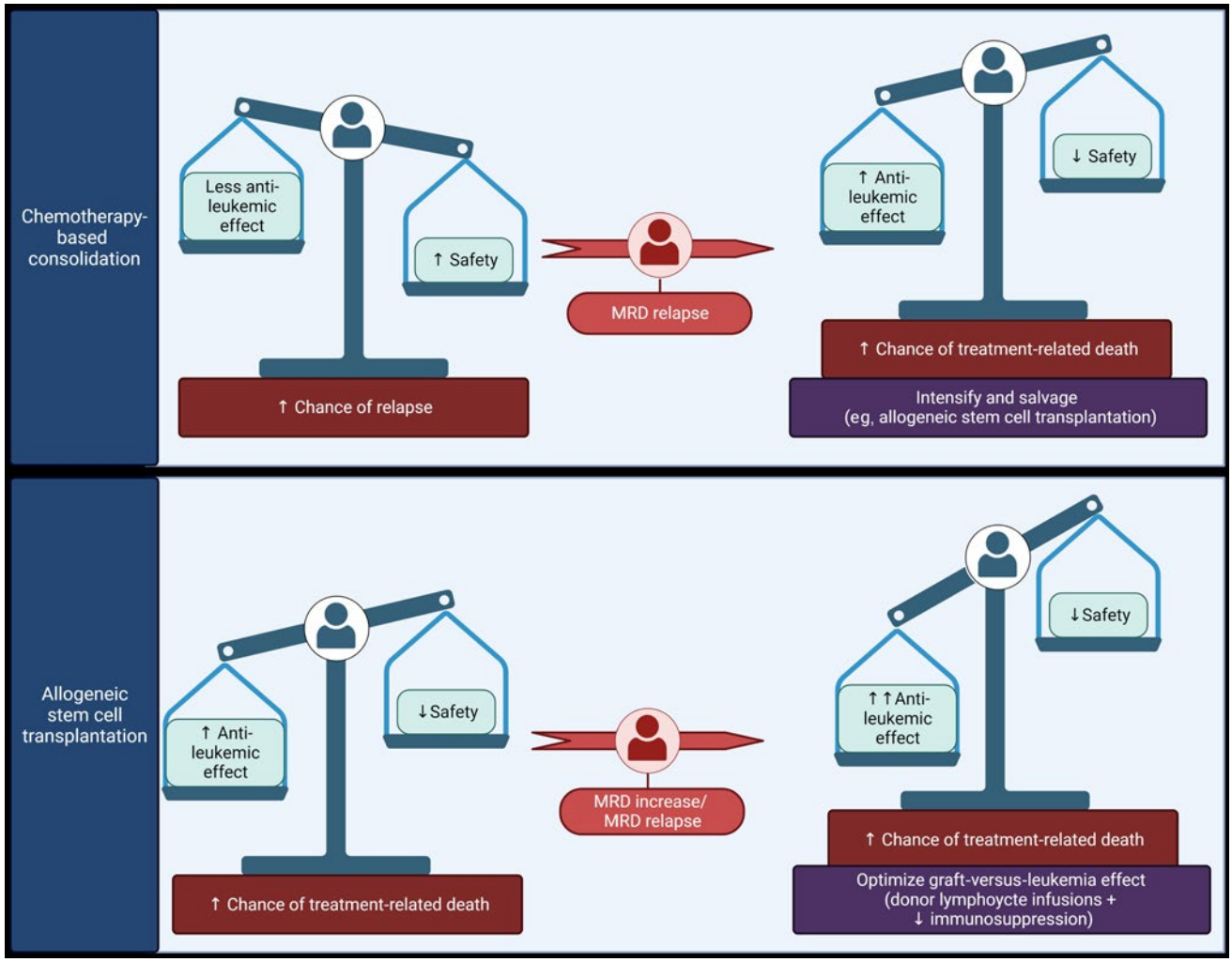

Figure 1. Considerations for postremission clinical decision-making. For patients with relatively low (<40%) risk of relapse, postremission consolidation may be deintensified to non-alloSCT (another cycle of chemotherapy or autologous stem cell transplantation). Several studies indicate this for favorable-risk patients and MRD-negative intermediate-risk patients.[19–21] AlloSCT has a stronger antileukemia effect but comes with more side effects and is therefore recommended for MRD-positive intermediate-risk patients and adverse risk patients, and some studies also showed benefit for ***FLT3***/ITD-positive patients in first remission.[28,29] By MRD monitoring, early return of disease can be detected, which for non-alloSCT can be salvaged by alloSCT,[22] and for alloSCT, an increase in MRD levels can be an indication for further stimulating the antileukemia effect by donor lymphocyte infusions and a decrease of immunosuppression.[4,5] Created by BioRender.

apy, small cell populations that were undetectable at diagnosis may be selected and proliferate, causing an immune phenotypic shift that the LAIP approach can miss. Another MFC-MRD approach can detect these emerging cell populations and is referred to as different-from-normal, which can also be used when no diagnostic sample is available and is essential to recognize clonal evolution.[17] ELN recommendations advocate the combined use by measuring all antibody panels at follow-up to assess MRD as LAIP-based different-from-normal,[8] allowing to monitor the dominant LAIP cell population and the possible emergence of a new LAIP.

Often, the search for a donor for alloSCT starts at diagnosis, while some argue the time point after the first cycle of chemotherapy would suffice, since very few patients who are MRD negative become MRD positive after the second cycle and the effort of searching a donor could be spared for nonresponding and MRD-positive patients.[18] For adverse risk patients, it is debated whether patients with MRD-positive CR should receive a second cycle of chemo or immediately undergo alloSCT.[19]

MRD after the second cycle of chemotherapy (postremission therapy)

MFC-MRD with a cutoff of 0.1% after induction therapy is now established as important prognostic factor for outcome.[8] The clinical implementation of MRD to guide further postremission strategies varies widely among treatment centers. Recently, several studies indicated that alloSCT could safely be omitted[19-21] for many intermediate-risk patients, and those who relapsed could be salvaged effectively with alloSCT.[22] The data of Zhang and collaegues[19] suggest that alloSCT in favor-

Table 1. Different techniques for measuring residual disease

Technique	Methods	Material	Cutoff	Advantage	Disadvantage
Multiparameter flow cytometry					
LAIP[13]	Identify and follow a specific LAIP	BM	0.1%*	LAIPs found in 90% of patients	Misses clonal evolution
Different from normal[17]	Identify leukemia cells in the empty spaces of normal BM flow plots	BM	Any MRD	No need for a diagnostic sample	Needs ample experience on regenerating normal BM
LAIP-based different from normal[48]	Identify a LAIP at diagnosis but measure the whole panel to detect emerging cell populations	BM	0.1%	Usable for almost all patients	Still needs experience on background LAIP levels
Targeted molecular[9]					
qPCR	*NPM1*	BM/PB	2% copies per ABL copies	Highly sensitive, rapid, standardizable	Can be low-level present with no clinical consequences
qPCR	Translocations†	BM/PB	2% copies per ABL copies or 3–4 log reduction	Highly sensitive, rapid, standardizable	BM cannot be as measured frequently as PB
Next-generation sequencing					
NGS[24,25]	*NPM1*	BM/PB	0.01%-0.1%	Can be well standardized, reproducible, sensitive, flexible	Not yet standardized, not comparable to qPCR yet, expensive, time consuming
NGS[15,23,26]	*FLT3*/ITD	BM/PB	0.01%	Can be well standardized, reproducible, sensitive, flexible	Expensive and clinical application needs to be validated

qPCR, quantitative polymerase chain reaction.

*LAIP cells/CD45-expressing cells.

†*RUNX1-RUNX1T1, CBFB-MYH11, PML-RARA, KMT2A-MLLT3, DEK-NUP214, BCR-ABL*.

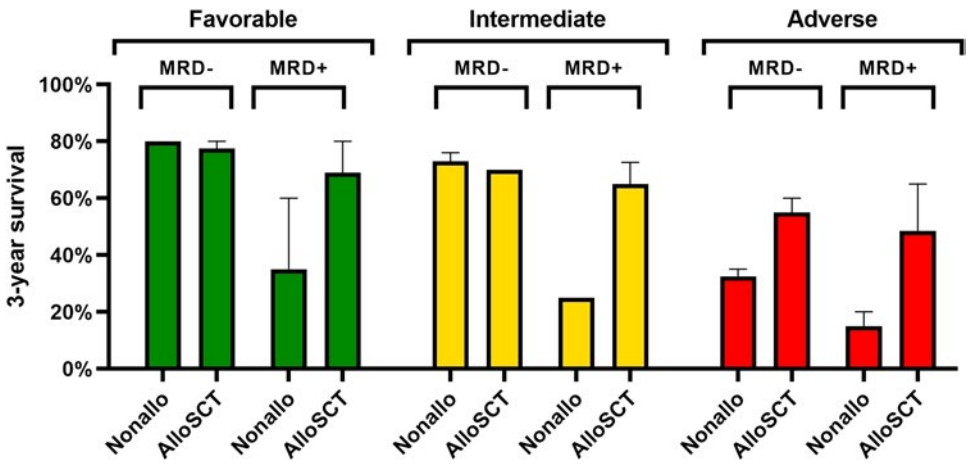

Figure 2. Prognostic effect of consolidation treatment per ELN risk group and MRD status. In favorable- and intermediate-risk patients, non-alloSCT treatment does not negatively affect survival compared to alloSCT in MRD-negative patients. In adverse risk patients, the alloSCT is beneficial independent of MRD. Combined data (means and standard error of the mean) calculated from Zhang et al,[19] Venditti et al (intermediate risk-patients),[20] and Tettero et al.[22]

able- and intermediate-risk patients may only benefit MRD-positive patients. Published data of the 3 trials were highly comparable, as shown in Figure 2. To gather further evidence that MRD-negative intermediate-risk patients can be safely treated with non-alloSCT treatment, a randomized trial would be required showing noninferiority of the non-alloSCT treatment approach compared to alloSCT.

Although some studies recently showed its potential prognostic relevance,[15,23] *FLT3*/ITD MRD is not as well clinically validated as *NPM1*.[24,25] Current data show that *FLT3*/ITD is a

reliable MRD marker before alloSCT at a ≥0.01% VAF cutoff (Table 1).[15,23,26] This also applied to patients treated with an FLT3 inhibitor, as shown in the Quantum first trial, which confirmed the 0.01% cutoff as an ideal cutpoint.[27] Residual *FLT3*/ITD MRD should be considered an indication for alloSCT, while it is unclear how the unfavorable prognostic effect of MRD before alloSCT may be mitigated.

Should every *FLT3*/ITD-positive patient undergo alloSCT? While this is common practice in the United States,[28,29] there are data that question this approach. A post hoc analysis of the Ratify trial identified 318 patients with available data comparing allogeneic hematopoietic cell transplantation and non–allogeneic hematopoietic cell transplantation treatment approaches.[30] Patients were grouped according to ELN 2017 risk groups based on *NPM1* mutation status and *FLT3*/ITD allelic ratio. Patients with favorable risk (*NPM1* mutated and *FLT3*/ITD low allelic ratio) had long-term survival at 75% when treated with midostaurin independent of alloSCT. Although the survival of the intermediate-risk group was worse with approximately 50% long-term survival, there was no OS difference with or without alloSCT in the midostaurin group. Only the ELN adverse risk group had a significant benefit from alloSCT, especially in the midostaurin-treated group. The ELN 2022 classification has abandoned the *FLT3*/ITD allelic ratio and groups all *FLT3*/ITD-positive patients in the intermediate-risk group. The recent studies evaluating *FLT3*/ITD NGS-MRD show a long-term survival for MRD-negative patients of 50% to 75%.[15,23,26] It is thus conceivable that the mutation profile at diagnosis (eg, *NPM1* mutated, *FLT3*/ITD low allelic ratio), the *FLT3*/ITD MRD status after 2 cycles of chemotherapy, or a combination of both can identify *FLT3*/ITD-mutated patients who will not benefit from alloSCT. Recently, the results of the BMT-CTN 1506 (MORPHO) study were presented, which evaluated gilteritinib maintenance after alloSCT in *FLT3*/ITD-mutated patients.[31] Gilteritinib treatment was associated with an improved relapse-free survival (hazard ratio, 0.515; 95% CI, 0.316-0.838) for the 50.5% of patients with detectable MRD pre- or post-alloSCT, compared to those without detectable MRD. The effect of gilteritinib on OS in patients with detectable MRD has not been reported yet. More research is needed to assess the clinical application of MRD in this subgroup of patients.

MRD monitoring after consolidation completion

The current evidence for accurate MRD monitoring at follow-up is only strong enough for *NPM1* and CBF molecular aberrations.[8] To further establish the role of MFC-MRD for disease monitoring, this is often still measured every 3 months for the first 2 years after consolidation or alloSCT, or when there are clinical indications of disease recurrence. Collecting these data in clinical trials is warranted as MFC-MRD monitoring after consolidation is not yet common clinical practice but may be beneficial for early intervention options and early relapse detection (Table 2). There are several considerations for using MRD for monitoring. First, the type of patient material matters. For MFC-MRD, PB is not well investigated, and MFC-MRD studies suggested that MRD in PB has higher specificity but 10-fold less sensitivity than BM.[32,33] Molecular MRD can be very sensitive, so for this technique, PB is considered suitable. Second, the threshold for MFC-MRD positivity at follow-up and subsequent potential clinical intervention still needs to be established. The cutoff of 0.1% may clearly indicate a high risk for relapse, but lower levels may also already indicate recurrent disease. It is also suggested that kinetics, measured as a certain level of increase in MRD, point to disease recurrence. For molecular techniques, a 1-log increase is considered a useful parameter,[8] but for MFC-MRD, sufficient data are lacking. Third, the duration of monitoring needs to be clarified. Previous reports suggest that most relapses occur within 2 years. PB-MRD can be analyzed more frequently (every 4-6 weeks) than BM (every 3 months). Because some relapses occur very fast when a small clone has gained a growth advantage, MRD monitoring would preferably be done in PB. Our patient case showed early positivity after alloSCT, which has been described in 7.5% of patients monitored by MFC-MRD shortly after alloSCT.[34]

MRD relapse

MRD relapse is defined by the ELN recommendations independent of the technique that is used: "MRD relapse is defined as either (1) conversion of MRD negativity to MRD positivity independent of the MRD technique or (2) increase of MRD ≥1 log10 between any 2 positive samples measured in the same tissue (PB or BM) in patients with low-level MRD. Conversion from negative to positive MRD in PB or BM should be confirmed within 4 weeks, in a second consecutive sample, preferably with a BM sample."[8] After MRD relapse, alloSCT can be beneficial for curation.[22] The most suitable technique, best time points, duration of MRD monitoring after alloSCT, and for which subsets of patients are still under investigation. An early indication that NGS-MRD is also prognostic after relapse was recently published for patients treated with fludarabine, high dose cytarabine (Ara-C), idarubicin and granulocyte-colony stimulating factor (G-CSF) and venetoclax, where MRD-negative patients had an excellent survival.[35]

Intermediate end point

Since many new drugs are currently available, MRD as intermediate end point would be worthwhile to fasten the process of drug approval.[36] The association between MRD and prognostic value has been very well established for intensively treated patients.[37] However, limited positive trials have included the measurement of MRD to show that the treatment effects are associated with a corresponding decline in MRD or the number of MRD-negative patients.[38] Thus, for the regulatory agencies to accept MRD as intermediate end point, additional data are required.[39]

Discussion

MRD monitoring in AML is quickly evolving from risk stratification to clinical decision-making to guide therapy, monitor the disease, detect MRD relapse early, and improve the drug development process by using MRD as an intermediate end point for clinical trials. For any MRD-including trial design, it is instrumental to have the exact information on when and what clinical decisions are made on MRD. Some clinical aspects may change the treatment decision, and the prognostic value of MRD is lost when treatment is guided based on MRD.[21] In addition, it still needs to be assessed whether quality of life improves with omitting or delaying alloSCT, since these data are currently lacking.

With the new technologies and insights that are currently under investigation, we may improve patient management in the future. For MFC-MRD, one of the advancements would be detecting the leukemia stem cell (LSC) load, measured as a per-

Table 2. Clinical studies using MRD in adult AML

Trial/study group	Patients	MRD technique	Key finding
MRD guidance after induction in complete remission in intermediate-risk patients			
GIMEMA 1310, risk-adapted, MRD-directed therapy for young adults with newly diagnosed AML[20]	N=429 ≤60 years	MFC	Similar 2-year survival in MRD-negative intermediate-risk patients receiving autoSCT compared to MRD-positive patients receiving alloSCT.
HOVON-SAKK 132: Addition of lenalidomide to intensive treatment in younger and middle-aged adults with newly diagnosed AML[21]	N=780 AML/high-risk MDS ≤65 years	MFC and *NPM1*	No difference in outcome between MRD-negative and MRD-positive intermediate-risk patients, while MRD-negative patients were considered eligible for non-alloSCT treatment.
ChiCTR-TRC-10001202 and 10001209 NCT03021330: Prognostic effect and clinical application of early MRD by flow cytometry on de novo AML[19]	N=769 <60 years	MFC	Overall survival of MRD-negative patients in favorable and intermediate-risk groups was comparable between transplant and nontransplant patients.
Post-remission measurable residual disease directs treatment choice and improves outcomes for patients with intermediate-risk acute myeloid leukemia in CR1[49]	N=235 Intermediate risk 14–60 years	MFC	Retrospective analysis of real-world data postremission MRD directs treatment choice and improves outcomes for patients with intermediate-risk AML in CR1.
Current retrospective studies showing relevance of *FLT3*/ITD as MRD marker			
DNA sequencing to detect residual disease in adults with AML prior to hematopoietic cell transplant[15]	N=1075 AML in CR1 before transplant ≥18 years	NGS	Persistence of *FLT3*-ITD or *NPM1* variants in blood at an allele fraction of 0.01% or higher was associated with increased relapse and worse survival compared with those without variants detected.
Prognostic value of *FLT3*-ITD residual disease in AML[23]	N=161 *FLT3*-ITD$^+$ AML in CR1 after induction ≥18 years	NGS	*FLT3*-ITD MRD is prognostic and better identifies patients at risk for relapse compared to MFC or NGS-based *NMP1* MRD alone.
Pretransplant FLT3-ITD MRD assessed by high-sensitivity PCR-NGS determines posttransplant clinical outcome[26]	N=104 Pretransplant AML 17–68 years	NGS	Pre-HCT detection of FLT3-ITD MRD is related to poor prognosis and can be an indication for future MRD-directed therapeutic strategies.
Allogenic stem cell transplantation conditioning/donor selection			
Impact of conditioning intensity of allogeneic transplantation for acute myeloid leukemia with genomic evidence of residual disease (CIBMTR)[50]	N=190 ≥18 years in first CR	NGS: *FLT3*, *NPM1*, *IDH1*, *IDH2*, and/or *KIT* variants	MAC rather than RIC in patients with AML with genomic evidence of MRD before alloHCT can result in improved survival.
Impact of pre-transplant induction and consolidation cycles on AML allogeneic transplant outcomes: a CIBMTR analysis in 3113 AML patients[51]	N=3113 ≥18 years	MFC, cytogenetics and molecular	Detectable MRD at the time of MAC alloHCT did not impact outcomes while detectable MRD preceding RIC alloHCT was associated with an increased risk of relapse.
Measurable residual disease, conditioning regimen intensity, and age predict outcome of allogeneic hematopoietic cell transplantation for acute myeloid leukemia in first remission[52]	N=2292 (EBMT) ≥18 years	MFC and molecular	Patients aged <50y with AML CR1 MRD-positive status should preferentially be offered MAC alloHCT. Prospective studies are needed to address whether patients who are AML CR1 MRD negative may be spared the toxicity of MAC regimens.
Haploidentical allograft is superior to matched sibling donor allograft in eradicating pre-transplantation MRD of AML patients as determined by MFC: a retrospective and prospective analysis[53]	N=339 ≤60 years	MFC	For MRD-positive patients, haploSCT was associated with lower incidence of relapse and better survival, suggesting a stronger antileukemia effect compared to matched sibling donor transplantation.
Monitoring during maintenance			
Prospective phase II (NCT00801489): Common kinase mutations do not impact optimal molecular responses in CBF AML treated with fludarabine, cytarabine, and G-CSF based regimens[54]	N=174 ≥18 years	Optimal PCR response *FLT3*, *RAS*, and *KIT*	Attainment of PCR <0.01% during/after consolidation improved RFS and was more important than achieving early optimal PCR response (post C1 PCR <0.1%).
Observational cohort: MRD status and FLT3 inhibitor therapy in patients with *FLT3*/ITD mutated AML following allogeneic hematopoietic cell transplantation[55]	N=34 ≥18 years	NGS: *FLT3/ITD*	Prognostic significance of NGS-based MRD monitoring for *FLT3*/ITD and the ability of post-alloSCT maintenance to prevent relapse and death.

Table 2. Clinical studies using MRD in adult AML (*Continued*)

Trial/study group	Patients	MRD technique	Key finding
Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial[56]	N=53 ≥18 years CR-MRD$^+$	qPCR: *NPM1*, *DEK-NUP214*, *RUNX1-RUNX1T1*, *CBFb-MYH11*	MRD-guided treatment with azacitidine prevented or substantially delayed hematologic relapse with an acceptable safety.
Monitoring after treatment			
Posttransplant MRD and T-cell chimerism status predict outcomes in patients allografted with AML/MDS (FIGARO)[3]	N=187 ≥18 years Peri-alloSCT	MFC and T-cell chimerism	Post-alloSCT MRD is an important predictor of outcome and is most informative when combined with T-cell chimerism.
Bone marrow CD34+ molecular chimerism as an early predictor of relapse after alloSCT in patients with AML (PROMISE)[57]	N=168 <75 years After alloSCT	Molecular *WT1* and CD34$^+$ chimerism	Molecular chimerism and *WT1* after alloSCT (first and third months) are useful MRD markers. When considered together at third month, CD34$^+$ molecular chimerism could represent an earlier predictor of relapse compared to *WT1*.
MRD in nonintensively treated patients			
Undetectable measurable residual disease is associated with improved outcomes in AML irrespective of treatment intensity. Retrospective analysis of real world data[58]	N=635 (250 nonintensively treated) ≥18 years	MFC: excluding APL and CBF AML	Achievement of MRD negativity should be the key objective of AML therapy in both high- and low-intensity treatment regimens.
Measurable residual disease response and prognosis in treatment-naïve acute myeloid leukemia with venetoclax and azacitidine (VIALE-A)[46]	N=190 (164 in CRc for MRD assessment) ≥18 years	MFC	Patients who achieved CRc and MRD negativity at any time point during treatment with venetoclax and azacitidine had longer duration of response, EFS, and OS than responding MRD-positive patients
Ibrutinib added to 10-day decitabine for older patients with AML and higher risk MDS (HOVON-SAKK 135)[59]	N=144 Unfit AML/high-risk MDS >60 years	MFC	After 3 cycles of treatment, 28 (49%) of 57 patients were MRD negative. In this limited number of cases, MRD revealed no apparent impact on outcome.
MRD characterization for targeted therapy			
ALLG AMLM26 phase 1B/2 study investigating novel therapies to target early relapse and clonal evolution as pre-emptive therapy in AML (INTERCEPT): a multi-arm, precision-based, recursive, platform trial[60]	Patients with MRD-relapse ≥18 years	MFC and molecular	Ongoing study: The primary end point is MRD response (≥1 $\log_{10}$ reduction in molecular MRD or flow MRD <0.1%) within 100 days of the first dose of study drug.
Intermediate end point			
Early assessment of clofarabine effectiveness based on measurable residual disease, including AML stem cells (HOVON-SAKK 102)[38]	N=291 ≤65 years	MFC including leukemia stem cells and *NPM1*	Lower levels of MRD were found in clofarabine-treated patients than in patients treated without clofarabine in the intermediate-I risk group.
Umbrella trial in myeloid malignancies: the MyeloMATCH National Clinical Trials Network Precision Medicine Initiative[61]	Older adults, MDS and young adults	MFC and NGS	Ongoing study: Assignment of treatment to patients based on biomarkers related to the disease such as mutations and MRD.

alloHCT, allogeneic hematopoietic cell transplantation; APL, acute promyelocytic leukemia; autoSCT, autologous stem cell transplantation; CRc, complete remission rate; EBMT, European Society for Blood and Marrow Transplantation; haploSCT, haploidentical stem cell transplantation; HCT, hematopoietic cell transplantation; MAC, myeloablative conditioning; MDS, myelodysplastic syndrome; RFS, relapse free survival; RIC, reduced intensity conditioning.

centage of CD34$^+$/CD38$^-$/LSCmarker$^+$ cells per CD45-expressing cells.[40] Several studies have shown that a high LSC load was associated with a relatively poor outcome at diagnosis and after induction.[41-44] Although promising, more evidence is needed to incorporate LSC load into treatment decisions.

The NGS-based MRD detection is promising but requires standardization. The clinical utility of NGS has been reported for *FLT3*/ITD and *NPM*1 mutations. To move this field further, consensus should be accomplished in various aspects such as selection of the most relevant molecular markers, sequencing approaches, sampling tissue (BM or PB), cutoffs, and timing of sampling.

In conclusion, MRD is an important prognostic factor to inform consolidation treatment after CR in intermediate-risk patients. Based on recent MRD data from clinical trials and real-world clinical practice, more evidence is being gathered for the broader

use of MRD for the management of AML (Table 2). Still, clearly more data are necessary to determine how MRD (cutoffs, kinetics, patient material, risk group, etc) can best be implemented in clinical practice. The relevance of MRD using current MRD techniques also needs additional studies when novel (targeted) or nonintensive treatments are used.[45-47] Improved standardization of MRD measurements should enable the combination of data from different trials and is essential in the light of the in vitro diagnostics regulation. When harmonized MRD data become available, additional MRD applications may be implemented in newly updated guidelines in the coming years.

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Conflict-of-interest disclosure

Jacqueline Cloos serves an advisory role for Novartis; has received research grants for her institution from Novartis, Merus, Takeda, Genentech, and BD Biosciences; and has received a royalty/license from Navigate and BD Biosciences.

Lok Lam Ngai: no competing financial interests to declare.

Michael Heuser serves an advisory role for Abbvie, BMS, Glycostem, Servier, PinotBio, Amgen, Pfizer, and LabDelbert; has received honoraria from Certara, Jazz Pharmaceuticals, Janssen, Novartis, Pfizer, and Sobi; and has received research funding to his institution from Abbive, Agios, Astellas, BergenBio, BMS, Glycostem, Jazz Pharmaceuticals, Karyopharm, Loxo Oncology, and PinotBio.

Off-label drug use

Jacqueline Cloos: nothing to disclose.

Lok Lam Ngai: nothing to disclose.

Michael Heuser: Use of venetoclax in relapsed/refractory AML patients and in combination with FLAG-IDA is neither approved by FDA nor EMA. Giltertinib maintenance after alloSCT in FLT3/ITD-mutated patients is neither approved by FDA nor EMA.

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Novel immunotherapies in the treatment of AML: is there hope?

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The success of allogeneic stem cell transplantation has demonstrated the potential for immunotherapy to treat acute myeloid leukemia (AML). Although alternative T-cell-based immunotherapies have shown efficacy, they also pose the risk of on-target off-leukemia hematotoxicity. So far, adoptive autologous or allogeneic chimeric antigen receptor (CAR) T/natural killer cell therapy is almost exclusively employed as a bridge-to-transplant strategy in the context of clinical trials. For now, clinical trials predominantly target lineage-restricted antigens, but emerging approaches focus on leukemia-associated/specific intracellular target antigens, including dual and split targeting strategies. Adapter CAR T cells and T-cell-recruiting bispecific antibodies offer transient exposure with enhanced safety and multitargeting potential against antigen-escape variants. However, these have yet to demonstrate sustained responses and should be used earlier to treat low leukemia burden, preferably if measurable residual disease is present. To address immune dysregulation and enhance T-cell fitness, novel CAR T and bispecific designs, along with combinatorial strategies, might prove essential. Furthermore, genetic associations with inflammatory bone marrow signatures suggest the need for tailored platforms in defined AML subtypes. The eagerly anticipated results of trials investigating magrolimab, an anti-CD47 antibody targeting the "do not eat me" signal in p53-mutated AML, should shed further light on the potential of these evolving immunotherapeutic approaches.

LEARNING OBJECTIVES

- Learn about the different categories of AML antigens for immunotherapeutic targeting and recognize their relevance for the unique challenges confronted in AML
- Review the current landscape of immunotherapy platforms for AML in adults
- Understand the barriers to the successful development of AML immunotherapy

CLINICAL CASE

A 64-year-old male patient with European Leukemia Net (ELN) intermediate risk, fms like tyrosine kinase 3 (FLT3) and nucleophosmin 1 (NPM1) gene wildtype (wt), *de novo* acute myeloid leukemia (AML) received "7 + 3" intensive induction therapy and achieved complete remission. The patient received 1 cycle of high-dose cytarabine consolidation therapy. A measurable residual disease (MRD) assessment by multiparameter flow cytometry revealed persistent disease at 0.2%. The patient was evaluated as eligible for transplantation from 2 suitable haploidentical children. The patient received a Reduced-intensity conditioning (RIC) regimen, followed by a haploidentical stem cell transplant (T-cell replete/post-transplantation cyclophosphamide [PTCy]), and went into MRD-negative complete remission with full donor chimerism. Unfortunately, the patient relapsed 6 months later and was enrolled in a phase 1 clinical trial of autologous CD33-redirected chimeric antigen receptor (CAR) T-cell immunotherapy with a backup graft from the initial stem cell donor. He successfully underwent T-cell apheresis and manufacturing, and entered complete response with incomplete cell recovery (CRi) 30 days after CD33 CAR T-cell infusion with antecedent lymphodepletion.

Introduction

Patients with relapsed or refractory (R/R) AML have poor prognoses, and their treatment remains challenging. In the majority of patients, allogeneic hematopoietic stem cell transplantation (allo-SCT) represents the only curative

approach, albeit with limited long-term benefit (<15% 3-year overall survival [OS]).[1,2] Even in patients in first remission, the risk of relapse after allo-SCT is markedly increased in those with detectable MRD prior to allo-SCT (eg, 2-year cumulative incidence of relapse 41% in MRD-positive vs 20% in MRD-negative patients), although reported results vary.[1,3-5] Importantly, disease biology is associated with MRD status and significantly impacts outcome after allo-SCT: for example, patients with an adverse genetic risk profile, such as TP53 mutation/deletion, do poorly after allo-SCT (2-year OS: 28%).[6] Hence, for patients with an increased risk of relapse, either determined by genetics upon initial diagnosis or MRD positivity throughout the course of the disease, as demonstrated by our clinical case, there is a high medical need for further treatment strategies prior to or after allo-SCT. In addition, despite the feasibility of allo-SCT in an increasing number of older patients, the majority of patients with AML are ineligible for allo-SCT (of an estimated 20 380 new cases of AML in 2020, only 3373 patients (16%) received a stem cell transplant).[7] Importantly, non-Hispanic Black patients have the lowest allo-SCT rate compared to Hispanic patients and non-Hispanic White patients highlighting disparities in access to allo-SCT.[8]

Alternative nontransplant immunotherapy approaches are therefore needed and aim to elicit immune responses (often T-cell mediated) against AML cells. This encompasses, on the one hand, therapies that either reactivate (immune checkpoint inhibition) or transfer (donor lymphocyte infusions [DLI] after allo-SCT) existing immune responses. On the other hand, many novel immunotherapeutic approaches, such as T-cell-engaging antibodies or CAR T cells, are often directed against self-antigen targets that are not immunogenic *per se* due to their expression and/or function in healthy tissues. Furthermore, and in contrast to the successful immunotherapy platforms implemented in B-cell precursor acute lymphoblastic leukemia (eg, antibody–drug conjugates [ADCs], T-cell bispecifics, and CAR T cells), translation to the myeloid setting is further challenged by an immune-dysregulated tumor microenvironment (TME) consisting of dysfunctional T cells enriched with regulatory T cells, accumulated myeloid-derived suppressor cells (MDSCs), immunosuppressive cytokines (eg, transforming growth factor β [TGF-β], interleukin [IL]-10), soluble inhibitory factors (eg, soluble Fas ligand [sFasL], soluble tumor necrosis factor related apoptosis inducing ligand [sTRAIL], and AML cells expressing indoleamine 2,3-dioxygenase, inhibitory checkpoint molecules, as well as a downregulated antigen-presentation machinery.[9] Considering the biologic, phenotypic, and genetic heterogeneity of AML in adults and AML's impact on the leukemia microenvironment, including T-cell fitness, it is likely that various immunotherapy platforms need to be developed to improve outcomes.[10,11] Here, we review the various emerging immunotherapy platforms and summarize some of the early clinical trial data. Current challenges in development and optimal time points for application will be addressed.

Target antigens

In stark contrast to B-cell malignancies, in which the B-lineage-restricted antigens CD19, CD20, and CD22 have successfully been targeted by various immunotherapy platforms, the ideal target antigen for AML is still to be identified. Such an antigen would be strongly expressed not only on every AML cell in each individual AML case, but also on leukemic stem cells (LSCs) with self-renewal capacities. In addition, healthy tissues would not express the antigen, thus avoiding on-target off-leukemia toxicity. Finally, ideal targets would also be involved in leukemogenesis, and downregulation of the target antigen would lead to a survival disadvantage for the individual cell.

Target antigens usually derive from one of the following categories (Table 1, adapted from Daver et al[12]):

- lineage-restricted antigens: antigens confined to the myeloid lineage
- leukemia-associated antigens: mostly non-lineage-specific antigens frequently overexpressed on AMLs cells relative to healthy tissue
- leukemia-specific antigens: neoantigens resulting from leukemia-specific mutations, usually expressed intracellularly and presented in the context of HLA molecules.

Currently, the majority of immunotherapy approaches in AML target myeloid-lineage antigens (mainly CD33, CD123, and CLL-1), which are hardly ideal targets.[13] As AML derives from myeloid progenitor cells, many potential target antigens for immunotherapy are also expressed on hematopoietic stem cells, imparting the risk of long-lasting or even permanent myelosuppression. This leads to the more common application of CAR T-cell therapy to patients who are also candidates for subsequent allo-SCT. Combinatorial targeting is one way to circumvent toxicity against vital tissues, but can increase the risk of immune escape (when used in an AND-gate strategy). Logic-gating strategies make use of differential expression patterns of several targets by employing, for example, AND, OR, or NOT gates for CAR T-cell activation,[14] and first-in-human clinical trials using such strategies started enrollment in 2023. Other platforms, including T-cell-recruiting bispecifics and adapter CAR T cells, offer the possibility to switch cytotoxicity on and off and hence hold the promise to mitigate ongoing myelotoxicity. However, these platforms still face the challenge that the majority of myeloid lineage antigens are internalized, contributing to an "antigen sink" effect, which might ultimately impair their efficacy. Finally, the role of the targeted myeloid antigens in inducing immune-related adverse events, particularly cytokine release syndrome (CRS), is not fully understood. Their potential contribution to the occurrence and severity of CRS might necessitate the extensive step-up dosing regimens used in the early clinical trials using bispecifics.

Hence, there is still a great medical need to identify novel AML-specific target antigens, or, alternatively, adapt targeting approaches so that flexible multitargeting can overcome target antigen–related toxicities and, at the same time, counteract immune escape. Multiomic approaches[15,16] have identified novel AML-associated surface antigens, and clinical trials targeting such target candidates have recently been initiated. Moreover, a plethora of possible novel target antigens will be derived from intracellular proteins that are presented in the context of defined human leukocyte antigen (HLA)-molecules. These include leukemia-specific neoepitopes of recurrent mutations in genes such as *NPM1* or *TP53*, which could be targeted by T-cell receptor (TCR)-mimic bispecifics or TCR-transgenic T cells.[17]

Antibody–drug conjugates, antibodies, and bispecific antibodies

The only immunotherapeutic agent approved for AML thus far is gemtuzumab ozogamicin (GO), an ADC administered in

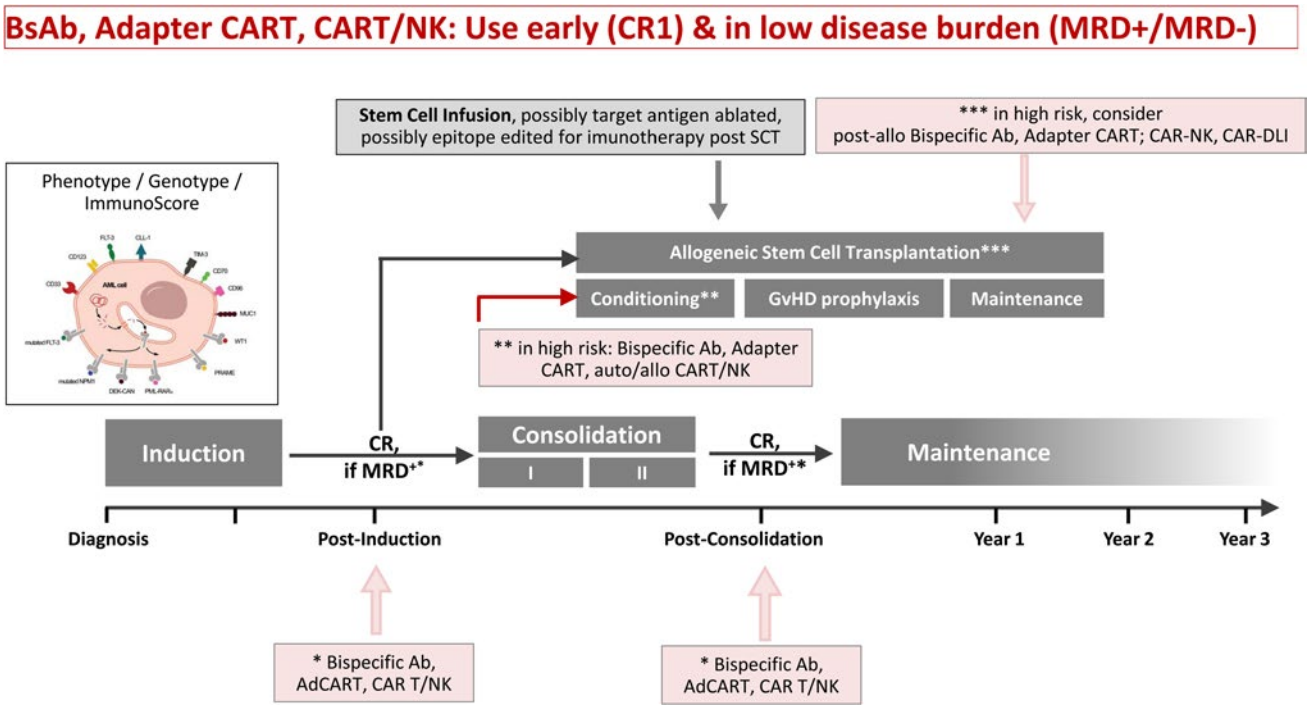

Figure 1. Mode of action of the different immunotherapy platforms in AML. BsAb, bispecific antibody.

combination with daunorubicin and cytarabine for the treatment of newly diagnosed CD33-positive AML, with most significant activity in AML patients in the ELN-favorable risk group. However, its efficacy as a monotherapy in the R/R setting is limited (only the FDA approved its use in R/R). In addition to GO, ADCs directed against CD33 and CD123 and carrying alternative payloads are under investigation (eg, IMGN632 [pivekimab sunirine], a CD123-targeting ADC with a DNA-alkylating payload). In patients with blastic plasmacytoid dendritic cell neoplasia, tagraxofusp—a fusion molecule of IL-3, which targets CD123, with a truncated form of diphteria toxin—was recently approved. Several clinical trials are currently testing the safety, tolerability, and efficacy of tagraxofusp and related ADCs in combination with standard chemotherapy or hypomethylating agents (HMAs) in *de novo* and in relapsed AML patients.[18] Evolving data suggest that combination strategies are especially advantageous for patients eligible for allo-SCT, as they are associated with a greater likelihood of achieving MRD negativity and, consequently, a reduced risk of relapse after allo-SCT. Such approaches could have been advantageous in the context of the case we have presented in this article.

Other monoclonal antibodies include naked antibodies directed against CD33 (eg, lintuzumab), CD123 (eg, talacotuzumab), and CD70 (eg, cusatuzumab). Cusatuzumab was administered in conjunction with azacitidine to patients with newly diagnosed AML ineligible for intensive chemotherapy. Nineteen of 38 patients achieved an objective response and 14/38 a complete response with a median duration of response of 4.5 months and median OS of 11.5 months.[19] In contrast to monoclonal antibodies, bispecific antibodies are recombinant proteins that recruit T cells through CD3 engagement and bind to target antigens on tumor cells, typically with higher affinity. Many different formats of bispecific antibodies have been developed, such as bispecific T-cell engagers (BiTEs), half-life-extended BiTEs, dual-affinity retargeting (DART) proteins, tandem diabodies, DuoBody, affinity-tailored adaptors for T cells, and tetravalent bispecific antibodies.[12] Despite differences in pharmacokinetics due to molecule size, it remains unclear to what extent the different formats impact efficacy and toxicity. Validated by GO, CD33 is one of the antigens most commonly targeted by the bispecifics. Sixty patients with highly advanced AML (48/60 had >4 prior lines of treatment, 27/60 had prior allo-HSCT) were treated with a CD33-directed BiTE (AMG 330, continuous intravenous infusion [CIV]) in a ramp-up approach together with dexamethasone prophylaxis in order to manage cytokine release syndrome (CRS). The complete response (CR)/CRi rate was 17% with doses ≥120 µg/day and the median duration of response was 58.5 days. Responders were more likely to have higher AMG 330 exposure and lower baseline leukemic burden, with no correlation between CD33 expression on AML blasts and response.[20] Instead of a continuous application, AMG 673, a half-life-extended CD33-BiTE construct (fusion of the N terminus of a single-chain IgG Fc region), is given as a short-term infusion (1 h) twice weekly. A reduction in blasts was observed in 16/38 patients, however none of the responses were sustainable. Neither of these trials nor those of related drugs (eg, JNJ-67571244, AMG 427) were able to achieve the projected exposure level within a reasonable timeframe, partly due to integration of multiple dose steps and trial interruptions to mitigate immune-related toxicity as well as hematotoxicity.

Flotetuzumab is the most advanced investigational bispecific antibody directed against CD123. This DART molecule is given as continuous infusion and was tested in a phase 1/2 trial in R/R AML. Notably, within the refractory/early relapse patient cohort, flotetzumab achieved an objective response rate (ORR) of 30%, including a CR rate of 24% and MRD negativity in 21%. This was

Table 1. Selected AML target antigens used in clinical trials

Target antigen	Target antigen category	Expression localization	Physiological function	Expression on bulk AML cells/LSCs	HSCs	Expression on nonhematopoietic cells	Immunotherapy platforms used/evaluated in AML
CD33	Lineage restricted	Surface	Cytoadhesion	+++/+	+	Kupffer cells, microglia	ADC, bispecifics, CAR T
CD123	Lineage restricted	Surface	Interleukin-3 receptor	++/++	(+)	Endothelial cells (upon inflammation), lung, GI	ADC, bispecifics, CAR T
CLL-1/CLEC12A	Lineage restricted	Surface	Inhibitory lectin-like receptor	++/+	–	Not reported	CAR T
CD135/FLT3	Lineage restricted	Surface	Cytokine receptor	+/++	(+)	CNS, GI, testis (intracellular)	Bispecifics, CAR T
IL1RAP	Lineage restricted	Surface	Interleukin-1 receptor accessory protein	++/+	–	GI	CAR T
CD44v6	Leukemia associated	Surface	Cell–cell/cell–matrix interactions	++/+	–	Keratinocytes	CAR T
CD70	Leukemia associated	Surface	T-cell coactivation	(+)–++*/(+)–++*	(+)-++*	Thymic epithelial cells	ADCC-optimized antibody, CAR T
TIM-3	Leukemia associated	Surface	Immunoregulatory protein	++/++	–	Not reported	High-affinity antibody
WT1	Leukemia associated	Intracellular	Transcription factor	++/++	+	Kidney, spleen, heart, lung, prostate	Vaccination, TCR-transgenic T cells
PRAME	Leukemia associated	Intracellular	Cancer testis antigen	+/+	(+)	Testis	Vaccination, TCR-transgenic T cells
NPM1 (mut)	Leukemia specific	Intracellular					Preclinical
FLT3-ITD	Leukemia specific	Intracellular					Preclinical
IDH1^{R132H}	Leukemia specific	Intracellular					Preclinical
TP53^{R175H} (and other TP53 mutations)	Leukemia specific	Intracellular					Preclinical

Expression levels: +++ ubiquitous; ++ frequent; + present; (+) rare; – absent; *reported expression varies significantly between publications.

ADCC, antibody-dependent cellular cytotoxicity; GI, gastrointestinal; HSC, hematopoietic stem cells; LSC, leukemic stem cells.

Adapted from Daver et al.[12]

Table 2. Results of early clinical trials on bispecific antibodies for AML

Clinical trial no.	Target	Construct design	Dosing	Safety	Efficacy (CR/CRi)	No. patients treated to date	Enrollment stage
NCT02520427	CD33	BiTE	0.5–720 µg/day; 0–3 dose steps; 14–28 days CIV	CRS 67% (≥G3 13%)	7/42	96	Terminated
NCT03224819	CD33	HLE-BiTE	0.05–72 µg per dose, 2 IV infusions in 14 days	CRS 50% (≥G3 13%)	1/27	46	Terminated
NCT03144245	CD33	TandAb	0.5–300 µg/day; 14 days CIV; 28-day cycle	CRS NA (≥G3 0%)	2/35	53	Completed
NCT02152956	CD123	DART	RP2D: 500 ng/kg/day; 7 dose steps; 28 days CIV; then 4 days/week	CRS 50% (≥G3 7%)	8/30	246	Terminated
NCT02715011	CD123	DuoBody	0.6–6 µg/kg Q2W IV; 0.15-4.8 µg/kg twice weekly IV; 2.4–4.8 µg/kg twice weekly SC; 0–4 dose steps	CRS 44% (≥G3 15%)	0/62	62	Completed
NCT02730312/ NCT05285813	CD123	XmAb	1.7 µg/kg IV; 4 dose steps on days 1, 3, 5, and 8 followed by weekly administration	CRS 44% (≥G3 15%)	5/51	106	Dose finding completed/ phase 2 initiated
NCT05086315	CD123	Trifunctional NK cell engager	10–3000 µg/kg/dose in cycle 1; 100–3000 µg/kg QW for the rest of induction cycles	CRS 9% (≥G3 n.r.), IRR 43%	3/23	23	Recruiting
NCT03038230	CLL1	Biclonics IgG format	0.675–240 mg weekly after initial ramp-up dosing; 3–4 dose steps	CRS 36% (≥G3 9%)	0/58	62	Active, not recruiting

IV, intravenous; IRR, infusion-related reaction; NA, not applicable; QW, once weekly; Q2W, once every 2 weeks; SC, subcutaneous injection; TandAb, tandem diabody.

correlated to higher density of CD123 receptors on AML blasts, higher inflammatory chemokine signature score, and higher interferon gamma signaling gene expression score compared to patients with late AML relapse.[21] Other bispecific constructs of different design targeting various antigens (eg, FLT3, CLL-1, CD70) are being tested in early clinical trials (Table 2). In addition, bifunctional and trifunctional natural killer (NK)-cell engagers are currently in the early stages of clinical development. Preliminary data from a phase 1/2 trial involving patients with R/R AML showed promising results for SAR'579, a novel CD123 targeting and coengaging NKp46 and CD16a trifunctional NK-cell engager. That trial enrolled 23 patients across 6 dose levels and demonstrated an excellent safety profile.[22] Across the various clinical trials using bispecifics, reasonable safety data have been obtained for integrating prophylactic anti-inflammatory drugs (eg, tocilizumab, corticosteroids) and step-up dosing. Combinatorial approaches might improve the efficacy of bispecifics by modulating TME and boosting T-cell function.[23] The strategies that are currently used in this as well as other disease entities include the following: application of 2 bispecifics, one of which provides a positive costimulatory molecule (thereby mimicking second-generation CAR T); small-molecule modulators of T-cell function (eg, bruton tyrosine kinase [BTK] inhibitors, immunomodulatory drugs [IMiDs], B-cell lymphoma 2 [bcl-2] inhibitors); conditional bispecifics that are only active within the TME[24]; combination with AML-approved drugs (eg, HMAs); integration into the conditioning regimen prior to and in conjunction with DLI after allo-SCT. Importantly, bispecifics appear to work best at an earlier line of therapy in a low disease burden/MRD+ disease setting. In the future, it will be necessary to assess the optimal clinical context for bispecifics. This could involve their use as a transitional strategy to eradicate MRD before allo-SCT, as illustrated in our case, or potentially in conjunction with DLIs to preempt an impending relapse after allo-SCT. Another potential application is as a standalone treatment approach following cytoreductive treatment to eliminate refractory disease.

Adoptive cellular therapy

Similar to bispecifics, the antigens most commonly targeted by CAR T cells in trials are CD33, CD123, and CLL-1 (Table 3).[25] In contrast to the common target antigen denominators, there is great variability in cell source, CAR T-cell constructs, and study design between these clinical trials. Some of the variables include cell source (autologous versus allogeneic), heterogenous lymphodepletion regimens, cell number, manufacturing method (traditional viral vectors versus rapid manufacturing), and the modifications made to products to enhance killing and/or safety (Table 3).

For example, PRGN-3006 CAR T cells are manufactured in a decentralized and rapid process via electroporation of the *Sleeping Beauty* plasmid (from apheresis to patient infusion within 2 days); these cells target CD33 and have membrane-bound IL-15 incorporated into the product to enhance *in vivo* expansion.[26] The completed phase 1 dose escalation data for PRGN-3006 CAR T cells were presented at the American Society of Hematology (ASH) annual meeting 2022. Lymphodepletion was mandatory, as expansion was significantly lower, and 0% efficacy was observed in the nonlymphodepletion cohort. In AML patients treated in the lymphodepletion cohort (n=11), the

Table 3. Results of early clinical trials on CAR T cells for AML

Clinical trial no.	Target	Costimulatory domain	Source	LD regimen	Additional features	Safety	Efficacy	No. patients treated to date	Enrollment stage
NCT03927261	CD33	Second generation (not defined)	Autologous	Flu 30 mg/m^2; Cy 500 mg/m^2×3 days	MB IL-15; electroporation via SB plasmid	CRS 69% (G3 6%); ICANS 6% (0 G3/G4)	3/11 CR/CRi in AML	16 (LD cohort)	Dose expansion
NCT03190278	CD123	41BB	Allogeneic	Flu 30 mg/m^2; Cy 750 mg/m^2; alemtuzumab 12 mg/day×4 days	TCRαβ and CD52 disruption	CRS 100% (≥G3 24%; G5 12%); ICANS 6% (1 G3)	FCA Arm 1/9 CR; 1 90% BM blast reduction	17 (LD cohort)	Dose expansion
NCT04230265	CD123	CD28	Allogeneic	Flu and Cy×3 days (dose not listed)	Universal CAR T cell with CD123-targeting module	CRS 86%; 7% G3; 7% G2 ICANS; 1 DLT ↓ fibrinogen*	2/14 CRi; 1 MRD-negative conversion	14	Dose expansion
NCT02623582	CD123	Not defined	Autologous	Optional Cy 1 g/m^2×1 dose	Manufacturing by mRNA electroporation	100% CRS (40% G3)	0% response (all PD by day 28)	5	Completed
NCT02159495	CD123	CD28	Autologous	Flu 30 mg/m^2; Cy 300 mg/m^2×3 days		83% CRS (0% G3/G4); 0% ICANS	1 CR	6 (AML cohort)	? Dose expansion
NCT03222674	CLL1	CD28 and CD27	Autologous	Flu 30 mg/m^2; Cy 900 mg/m^2×4 days	Inducible caspase 9 motif	CRS 75% (No G3/G4); 25% G1/2 ICANS	3/4 MLFS with MRD negativity	4	? Completed
ChiCTR2000041054	CLL1	41BB	Autologous	Flu 30 mg/m^2; Cy 500 mg/m^2×3 days		100% CRS (60% G3); ICANS 0%	70% CRi (all but 1 transplanted days 18–34)	10	? Completed
NCT03795779	CD33 & CLL1	Not defined	Autologous**	Flu 30 mg/m^2; Cy 300 mg/m^2×3 days		89% CRS (22%); ICANS 44% (33% G3)	7/9 MRD negative CR (? count recovery)	9	? Completed
NCT03018405	NKG2D ligands	Endogenous DAP10	Autologous	None		94% CRS [25% G3, 13% G4 {1 DLT}]; 0% ICANS	3/12 in AML patients	16	Completed

* ICANS and low fibrinogen resolved with targeting-module cessation. ** One patient had a matched sibling donor.

BM, bone marrow; Cy, cyclophosphamide; DLT, dose-limiting toxicity; FCA, fludarabin/cyclophosphamide/alemtuzumab lymphodepletion; Flu, fludarabine; IV, intravenous; LD, lymphodepletion; MB, membrane bound; MLFS, morphological leukemia-free state; PD, progressive disease; SB, Sleeping Beauty.

product was very well tolerated (1 G3 CRS), and 27% of patients showed a response, although no patient with myelodysplastic syndrome (MDS) responded (0/3). However, responses were nondurable outside of a bridge to allo-SCT concept. The trial has since moved to dose expansion at 1×10^6 cells/kg and notably now incorporates a second infusion of CAR T cells in an attempt to augment efficacy.

UCART123 cells are a non-HLA-matched allogeneic product targeting CD123 that have been additionally modified to prevent graft-versus-host disease via TCRαβ elimination as well as CD52 knockout to allow for intensified lymphodepletion by alemtuzumab.[27] Optimal kinetics were observed in the cohort of patients treated with fludarabine, cyclophosphamide, and alemtuzumab; this cohort is the current one in the ongoing in clinical study. Cytokine release syndrome was observed in all patients; notably, events of severity ≥G3 occurred in 24% of patients (4/17), including 2 G5 events. (G stands for grade.) Immune effector cell–associated neurotoxicity syndrome (ICANS) was rare, a finding consistent across AML CAR T studies. Early evaluation of efficacy showed responses in several patients, albeit most notably in a heavily pretreated AML patient (including prior allo-SCT) who achieved MRD-negative CR beyond 12 months without DLI/second allo-SCT at the time of data reporting. That study is also evaluating multiple dosing strategies.

To date, anti-CLL1 CAR T-cell data have been primarily collected from pediatric populations with MRD-negative responses, although the degree of hematopoietic recovery is unclear and challenges with durability exist.[28,29] So far, the only adult anti-CLL1 CAR T data was from a cohort of 10 patients with a reported CR/CRi rate of 70%, although all patients but 1 were transplanted very early in the course of therapy (days 18-34). Cytokine release syndrome occurred in 100% of these patients (60% G3).[30] Perhaps the most exciting results to date concern the CLL1-CD33 compound CAR, which were most recently updated at the European Hematology Association (EHA) annual meeting 2020.[31] Notably, 7/9 patients achieved MRD-negative remission, with 1/2 nonresponding patients being CLL1-negative. These data support that aggressive "OR gating" strategies might be optimal for efficacy in order to cover the majority of AML blasts/LSCs and prevent antigen escape. Notably, allo-SCT appears to be required in the setting of CLL1 CAR T, secondary to a lack of neutrophil recovery.

Saar Gill and colleagues proposed a novel approach in which they initiated the treatment sequence by utilizing a CD33 clustered regularly interspaced short palindromic repeats (CRISPR)-Cas-knockout allogeneic stem cell graft followed by CD33-directed CAR T cells.[32] More recently, the first-in-human trial demonstrated successful engraftment of CD33-deleted stem cells and tolerance to post-allo-SCT GO without incidence of cytopenia.[33]

Given the lack of a single tumor-specific antigen in AML, the development of CAR T-cell therapies that could be agnostic to phenotypic targets are of considerable interest. CYAD-01 is an autologous CAR T-cell product expressing NKG2D, the critical killing receptor of NK cells, and targets 1 of 8 ligands that are overexpressed on nearly 100% of blasts (ie, MICA/MICB and the ULBP family). In the completed phase 1 dose escalation study, 25% (3/12) of AML patients had objective responses, although these were nondurable with the exception of 1 patient bridged to allo-SCT.[34] Additional efforts to enhance efficacy with CYAD-01 have been the incorporation of lymphodepletion chemotherapy as well as a second-generation product (CYAD-02) to abrogate potential fratricide *in vivo* via CAR T-cell upregulation of NKG2D ligands. However, a key factor in the limited efficacy with this approach is that LSCs have been shown to lack NKG2D ligands, which raises the potential for combinatorial therapy with agents that can upregulate NKG2D ligands on LSCs (eg, PARP1 inhibitors).[35]

As critical as T-cell dysfunction is to the impaired efficacy of CAR T cells, universal or adapter CAR T programs might be optimal in overcoming these challenges, as recently shown.[36] These programs might also have therapeutic advantages by helping prevent antigen escape while ameliorating CRS/ICANS and/or on-target toxicity. Notably, we now have supportive clinical data in the recent presentation of a universal CAR T cell with a CD28 costimulatory domain (UniCAR T) and a CD123 targeting module (TM).[37] Although clinical activity was modest (3/14; 2 CRi, 1 MRD-negative conversion), a G2 ICANS was reversed with TM interruption, and a reexpansion of CAR T cells was noted with re-initiation of TM. There will remain clinical obstacles with adapter programs because multiple factors require optimization, undoubtedly a challenge in the clinical investigation of AML.

Optimal timing of adoptive cellular therapy in the context of the clinical case

One paramount question in the field is how the impact of disease burden and past therapies impact the outcomes of CAR T and other cellular therapies. Given that overall efficacy, and particularly durability of response, has been low, with overall enriched responses in patients with lower blast burdens, intervention at the time of MRD positivity and prior to overt relapse is potentially critical. Notably, the T-cell fitness is also likely enhanced in MRD-positive settings, which is most relevant to autologous products. As with our patient, who ultimately relapsed after allo-SCT, the future paradigm is likely intervening at the time of MRD positivity, with the goal of improving the outcome of transplantation. Furthermore, applying T-cell based immunotherapy platforms, such as bispecific antibodies and CAR T cells, earlier in treatment lines with low disease burden should enhance their safety profiles and result in lower rates of immune-related adverse events such as CRS or macrophage activation syndrome.

Checkpoint blockade

Immune checkpoint inhibitors have been investigated in AML with the intent of harnessing components of the immune microenvironment to generate an immune response against AML.[12] Single-agent immune-checkpoint-inhibitor therapies have demonstrated almost no activity in patients with R/R AML who have not undergone allo-SCT. Liu et al conducted a multicenter, randomized phase 2 study to assess the efficacy of nivolumab (every 2 weeks for up to 46 doses) as a maintenance therapy for patients with AML with CR/CRi who were not candidates for allo-SCT.[38] A total of 79 patients were randomized. The primary endpoint of progression free survival (PFS) was not met, with median PFS of 13.2 versus 10.9 months (P=0.38) and 2-year PFS rates of 30.3% versus 30% in the nivolumab and observation arms, respectively. Similarly, median OS and 2 year OS were not improved. The grade 3 adverse event rate was significantly higher in the nivolumab arm.

However, Zeidner et al conducted a phase 2 single-arm study of high-dose cytarabine followed by pembrolizumab in

R/R AML, demonstrating a CR + CRi rate of 38% and median OS of 11.3 months, with patients with primary refractory/first salvage having further improved OS compared to later salvage patients (13.2 vs 11.3 months).[39] The authors considered this an encouraging response rate in this R/R AML population.

Further encouragement can be taken from the results of giving the anti-CTLA4 antibody ipilimumab to patients with hematologic malignancies relapsing following allo-SCT, including 12 with AML and 2 with MDS. Among these 14 patients, CR was achieved in 5 (36%), including 4 of 5 with extramedullary disease, with all responses being observed at the higher ipilimumab dose of 10 mg/kg. Grade 3/4 immune-related adverse events (irAEs) were noted in about 20% of patients and were reported to be generally manageable.[40]

Hypomethylating agents upregulate genes in the interferon-gamma pathway, increase the expression of HLA class I antigens, and activate viral defense pathways, thereby promoting antitumor immune signaling; data suggest they increase the expression of PD-1 and PD-L1 on T cells via methylation.[41] The anti-PD-1 antibody nivolumab was combined with azacitidine in a single-arm phase 2 study ($n = 70$) with an ORR of 33% (CR + CRi 22%) in a heavily pretreated population.[42] A higher response rate was noted in patients with higher CD3 and CD8 T-cell levels in the blood and bone marrow prior to therapy, suggesting that to be a useful and simple biomarker for selecting patients more likely to benefit from anti-PD-1-centered approaches in future trials.[43] In a subsequent phase 2 study, 129 AML patients ≥65 years old and ineligible for intensive chemotherapy were randomized (1:1) to azacitidine with durvalumab or azacitidine alone with no significant difference in CR rate (17.2% vs 21.5%) or OS (13.0 vs 14.4 months).[44] Based on these data, interest in further developing HMAs with PD-1-, PD-L1-, or CTLA-4-targeted interventional trials has waned in recent years.

Novel AML- and MDS-specific checkpoints, such as TIM3 antibodies, in combination with azacitidine have entered clinical development. Although the CR rate in newly diagnosed AML was only 25% (CR + CRi: 30%) in the initial single-arm phase 1B trial, the durability of response in patients with ELN adverse-risk mutations (*TP53/RUNX1/ASXL1*) was encouraging (median duration of response [DOR] 12.6 months).[45] In a subsequent randomized phase 2 study of azacitidine with sabatolimab or with placebo in newly diagnosed higher-risk myelodysplastic syndrome (MDS), the primary endpoint of improved CR rates and/or PFS was not reached.[46] Sabatolimab is currently being investigated in an ongoing frontline randomized phase 3 trial in higher-risk MDS, and the data are eagerly awaited.

The cell-surface molecule CD47 functions as a phagocyte immune checkpoint, impeding phagocytosis and serving as a "don't eat me" signal.[47] CD47 is overexpressed in most cancer cells, especially in AML and MDS, which is associated with adverse prognosis in AML.[48] Azacitidine upregulates prophagocytic signals on AML cells and was combined with magrolimab (a first-in-class humanized monoclonal antibody against CD47) in a recently completed phase 1B study in patients with previously untreated AML or higher-risk MDS. A total of 87 patients were treated in the AML cohort, with an unplanned enrichment of patients with TP53 mutations ($TP53^m$; $n = 72$); the unplanned enrichment was initiated because azacitidine and magrolimab showed encouraging signs of early efficacy in $TP53^m$ patients, emerging data show the limited benefit of adding venetoclax in $TP53^m$ frontline AML, and an extremely high but unmet need exits for effective therapies in this population.[49] The combination of azacitidine and magrolimab was well tolerated. The most significant adverse event was on-target anemia, seen predominantly in the first 7-10 days, postulated to be due to the removal of CD47 antibody-coated sensescent red blood cells by the reticuloendothelial system. The median hemoglobin drop from baseline to first postdose magrolimab assessment was −0.9 g/dL (range: −3.6 to 2.5 g/dL); however, it is important to note that 5%-6% of patients could have a >2.0 g/dL early drop in hemoglobin, and close monitoring and transfusion to try to maintain hemoglobin at >8.5-9.0 g/dL during the first 7-10 days of therapy is strongly recommended. The ORR and CR rates in the 15 $TP53^{wt}$ patients were 47% and 33%, respectively, and, interestingly, these were similar (47% and 32%) in the 72 $TP53^m$ patients. The median DOR in $TP53^m$ patients was about 8 months and median OS was around 11 months. These CR, CR + CRi rates, and median OS intervals appear favorable compared to those recently published with azacitidine and venetoclax[50] and to those in a retrospective observational study of outcomes in frontline $TP53^m$ AML patients. Badar et al. looked at patients treated with 6 different frontline induction regimens in 291 patients across 8 US academic centers between 2012 and 2021.[51] The outcomes were unfortunately dismal, with an overall CR + CRi rate of 29%, ranging from 17% to 41%, and with HMA + venetoclax showing the highest CR + CRi rate, 41%. The median OS ranged from 2.0 to 9.5 months, with allo-SCT being the only factor associated with improved OS in a multivariate analysis. By analyzing survival outcomes for $TP53^m$ AML over time, the authors observed a maintained but modest response to induction therapy and poor OS that has not improved in the era of novel therapies. The combination of azacitidine and magrolimab might be a step forward, but the phase 1B results require confirmation in a randomized study. Interestingly, in that study, the median OS was 18.2 months in the 15 $TP53^{wt}$ patients, suggesting magrolimab is also active in $TP53^{wt}$ patients. Another single-arm study combining azacitidine with venetoclax and magrolimab was initiated based on observed preclinical synergy of this triplet beyond either doublet in both $TP53^m$ and $TP53^{wt}$ patient-derived xenograft (PDX) models.[52] The ongoing phase 1B study has demonstrated encouraging response rates; CR + CRi rates were about 60% in $TP53^m$ and about 80% in $TP53^{wt}$, and median OS was not reached in either population.[53] Based on these results, 2 phase 3 trials are currently enrolling: one with azacitidine with magrolimab in patients with newly diagnosed TP53-mutant AML (ENHANCE-2; NCT04778397) and another with azacitidine and venetoclax with magrolimab in patients with newly diagnosed AML who are ineligible for intensive chemotherapy (ENHANCE-3; NCT05079230). These results are eagerly awaited.

Conclusions

Adoptive cellular therapy: bridge to transplant and beyond

Allogeneic stem cell transplantation has proven effective against AML, harnessing the graft-versus-leukemia effect to eliminate residual disease. Similarly, CAR T-cell therapy has shown promise, as exemplified by the induction of bone marrow aplasia, indicating highly potent antileukemic activity. The most recent developments appear to recapitulate these responses utilizing allogeneic T cell or NK cell sources. Current challenges revolve

around identifying suitable target antigens to elevate these T and NK cell–based platforms beyond a bridge-to-transplant strategy. Besides the obvious on-target off-leukemia toxicity of the healthy hematopoietic compartment and possible other vital tissues, targeting myeloid lineage antigens might contribute to antigen sink, aggravation of CRS and possibly, through continuous antigen exposure, T-cell exhaustion. Given the interindividual and intraindividual heterogeneity of genotypes and phenotypes in AML, antigen-escape variants appear likely. Future strategies to combat AML need to consider multiple targeting, ideally based on the phenotypic profile of the AML of each individual patient.

Bispecifics: moving to earlier treatment lines in low disease burden, preferentially MRD-positive

T-cell-recruiting bispecific antibodies offer the considerable advantages of being off-the-shelf products with increased safety profiles as a result of on–off switchable cytotoxicity. However, despite that and their success against B-cell malignancies, bispecifics have so far failed to deliver sustainable responses in AML. The bispecific antibody platform relies on endogenous T-cell fitness, and hence the interpretation of clinical results must take into account that clinical trials were conducted on patients with advanced AML and high disease burdens after several prior treatment lines. Hence, not surprisingly, and in line with poor outcomes after allo-SCT upon relapse, responses so far have been limited. We propose to promote this therapeutic platform to a first-line therapy in genetically high-risk patients, preferentially in MRD-positive patients, in a clinical scenario involving a less immunosuppressive leukemic microenvironment, better T-cell fitness, and favorable effector:target ratio. In line with this approach are current clinical trials utilizing targeting or adapter molecules in conjunction with universal CAR T cells or DLI to combine the safety and flexibility of bispecific antibodies with a functional adoptive cell therapy.

Tailoring immunotherapy to AML phenotype, AML genotype, and bone marrow inflammatory signature: combination strategies to overcome adversity

Finally, learning the lessons from allo-SCT, we need to particularly adopt combinatorial approaches to improve T-cell function and counteract immune dysregulation. Early clinical trials utilizing additional bispecifics that provide positive costimulation (eg, CD20 x CD3 plus a CD28 x CD3 or 4-IBB x CD3) are currently recruiting participants with B-cell malignancies; other approaches might encompass sequential or in-parallel application of small molecules such as tyrosine kinase inhibitors (TKI), venetoclax–azacitidine, IMiDs, or BTK inhibitors. Recent evidence demonstrates the association between genetics and the inflammatory signature of the bone marrow,[54] supporting the notion that defined immunotherapy platforms might only be successful in certain AML subtypes.[55] We eagerly await the results of randomized clinical trials (currently recruiting) in TP53-mutated AML that will utilize the anti-CD47 antibody magrolimab; this approach might be better suited against AML subtypes less susceptible to T cell–based immunotherapy. Ideally, applied immunotherapy strategies integrate synthetic and natural immunity leading to innate but, importantly, adaptive immunity.[56] In the future, immunotherapy platforms should be applied early in a treatment line; should be based on AML genotype and phenotype, and the inflammatory signature of the bone marrow; and should guide the use of single or combinatorial approaches.

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Off-label drug use

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Novel therapies upon failure of HMA plus venetoclax

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The efficacy and tolerability of the combination of hypomethylating agents with venetoclax (HMA-VEN) in patients with newly diagnosed acute myeloid leukemia has been a practice-changing milestone in the field. However, treatment failure and relapse remain major barriers to prolonged survival. *TP53* mutation is a predictor of primary induction failure and portends especially poor outcomes. Prelinical data suggest that VEN resistance stems from these genetic changes, which lead to increases in antiapoptotic proteins such as MCL-1 and $BCLX_L$. For patients who discontinue HMA-VEN for reasons other than disease progression, such as post allotransplantation, infection, and personal preference, rechallenge with HMA-VEN at the time of relapse may be considered. For those who progress on HMA-VEN, clinical trials with novel agents or rational drug combinations are preferred if available. If no trial option is available, fit patients may benefit from intensive chemotherapy. Emerging therapies aim to overcome venetoclax resistance, target interactions that promote leukemogenesis, and harness the immune system to irradiate leukemic blasts and stem cells.

LEARNING OBJECTIVES

- Review factors predictive of failure following HMA-VEN in patients with previously untreated AML
- Identify mechanisms of venetoclax resistance in AML
- Discuss treatment recommendations for patients with AML who progress after HMA-VEN chemotherapy
- Review novel therapies in development for patients with relapsed or refractory AML following HMA-VEN chemotherapy

CLINICAL CASE 1

A 78-year-old man with a past medical history of hypertension and diabetes was diagnosed with acute myeloid leukemia (AML) 6 months ago. His initial bone marrow biopsy confirmed 70% myeloblasts, cytogenetics were normal, and next-generation sequencing (NGS) detected *DNMT3A* (variant allele frequency [VAF], 11%), *NRAS* (6%), and *NPM1* (23%) mutations. The patient was started on azacitidine at 75 mg/m^2/d on days 1 through 7 every 28 days and VEN at 400 mg/d on days 1 through 28 and achieved a complete remission (CR) with measurable residual disease (MRD) detectable by multiparametric flow cytometry after 1 cycle. He tolerated this regimen well, had a full count recovery, and remained on schedule with treatment until cycle 3, when he developed bacteremia requiring a weeklong hospitalization. Chemotherapy was withheld for 3 weeks due to illness and cytopenia. His absolute neutrophil count, 0.7×10^3/uL, while adequate, never fully recovered. Given the prolonged neutropenia, venetoclax (VEN) administration was shortened to days 1 through 14 every 28 days for the next 2 cycles. Unfortunately, he was found to have circulating peripheral blasts, which prompted a repeat bone marrow biopsy that confirmed morphologic relapse. In addition to the mutations present at diagnosis, there was a new mutation, *FLT3-ITD*.

Hypomethylating agents and VEN failure

Treatment options for older patients with newly diagnosed AML ineligible for intensive chemotherapy (IC) were limited until recent years, when several novel and targeted agents were introduced, perhaps none more paradigm shifting than the combination of hypomethylating agents (HMAs) with VEN. This regimen was found to significantly prolong survival when compared to azacitidine alone in the VIALE-A trial, leading to the US Food and Drug Administration's approval of frontline HMA-VEN in patients aged 75 years or older or unfit for intensive induction chemotherapy.[1] Despite improved outcomes with a median duration

of remission at 22 months in long-term follow-up, most patients will eventually relapse.[2]

Certain molecular patterns appear to predict response and treatment failures to HMA-VEN.[3] An analysis of patients enrolled on the VIALE-A (HMA-VEN) and VIALE-C (low-dose cytarabine [LDAC]-VEN) clinical trials (n = 81) reported high rates of CR/CR with incomplete hematologic recovery (CRi) (>80%) in those with *NPM1*, *IDH1/2*, or *DNMT3A* mutations.[3] The *NPM1* and *IDH2* subsets also demonstrated a long duration of response (DoR) from over 20 to almost 50 months.[3] In contrast, patients with *TP53* have a CR/CRi of 55.3% and a shorter DoR for those who do respond.[1] Interestingly, clonal selection for *TP53* while receiving treatment with HMA-VEN and clonal expansion that increases kinase activation were shown to contribute to the adaptive resistance observed.[3] The median time to relapse for patients with adaptive resistance was 6.4 months.[3]

Another important prognostic biomarker is MRD.[4] Of the 164 CR/CRi patients evaluable for MRD in the VIALE-A trial, 67 (41%) had undetectable MRD, defined as residual blasts of less than 0.1% by multiparametric flow cytometry.[5] In patients with undetectable MRD, 25% achieved MRD negativity at the end of cycle 1, 27% at the end of cycle 4 (cumulatively 52%), 27% at the end of cycle 7 (cumulatively 79%), and 21% after cycle 7 (cumulatively 100%). This suggests that early and late responses are possible. Among patients with *IDH1/2*, 49% (21/43) achieved undetectable MRD and had a more favorable DoR, event-free survival, and overall survival (OS) compared to those with MRD. Notably, patients who achieved undetectable MRD at any time point had a higher incidence of *NPM1* mutations at diagnosis (36% vs 3% in detectable MRD).[5] Additionally, those without MRD had better outcomes according to DoR, event-free survival, and OS, with the 12-month estimates being 81.2%, 83.2%, and 94%, respectively. Upon long-term follow-up, the median OS reached 34.2 months in patients without MRD vs 18.7 months in those with detectable MRD.[2]

Mechanism of VEN resistance

VEN is an inhibitor of B-cell lymphoma 2 (BCL2), which is an important cellular protein that modulates intrinsic apoptosis.[6] Normally, antiapoptotic proteins ($BCLX_L$, BCL_2A_2, MCL-1, $BCL\text{-}_W$, and BFL_{-1}/A_1) sequester proapoptotic proteins (eg, sensitizers and activators) via BH3 domain interactions. Sensitizers (BAD, BIK, HRK, and NOXA) and BH3 mimickers such as VEN promote apoptosis by binding to antiapoptotic proteins, thereby releasing activators (BIM, BID, and PUMA) to interact with effectors (BAK and BAX), increasing mitochondrial outer membrane permeabilization and cytochrome c–mediated cell death.[7] VEN is selective for BCL2, with much lower affinity toward other family members such as $BCLX_L$.[8] VEN resistance occurs when displaced BH3-only activator proteins, instead of interacting with BAK/BAX to cause apoptosis, are sequestered by the upregulation of other BCL2 family members including $BCLX_L$, MCL-1, and BCL2A1 (Figure 1).[6] In preclinical studies, this shift of less BCL2 dependency and more $BCLX_L$ and MCL-1 expression was observed in AML cells with prolonged exposure to VEN.[9]

Specific somatic mutations such as those associated with cell signaling, *FLT3-ITD*, *PTPN11*, *KRAS*, and tumor suppression, *TP53*, have been shown to confer VEN resistance.[10-12] *FLT3-ITD* mutation increases the level of $BCLX_L$ and MCL-1 via activation of the STAT5, PI3K/AKT, and RAS/MAPK signaling pathways.[11] Specifically, AKT and ERK, downstream of PI3K and RAS, respectively, inhibit glycogen synthase kinase 3, resulting in decreased MCL-1 degradation.[6] STAT5 regulates *$BCLX_L$* gene expression and also increases MCL-1 indirectly via phosphorylation of AKT. Similarly, *PTPN11* mutation upregulates $BCLX_L$ and MCL-1.[10] In *KRAS*-mutated AML, there is upregulation of MCL-1 and BCL2A1 and downregulation of BCL2 and BAX. Based on clustered regularly interspaced short palindromic repeats/Cas9 screening and preclinical studies, *TP53* knockout cells were found to have a lower level of activator and effector proteins, contributing to VEN resistance in *TP53*-mutated AML.[12]

Several studies have reported that the monocytic differentiation of blasts is a predictor of resistance to VEN-based therapy.[13,14] Ex vivo drug testing of blast populations has observed high sensitivity to VEN in AML M0 and M1 but resistance to BCL2 inhibition in M4 and M5 subtypes.[13] A separate study corroborated this finding and additionally suggested that azacitidine (AZA)-VEN resistance arose from intrinsic biological properties of monocytic AML cells.[14] However, investigators have recently shown that clinical response to AZA-VEN is independent of myeloid differentiation.[15] Rather, it is the disease-driving leukemic stem cells, whose characteristics are mainly based on mutations rather than blast differentiation state, that determine the response to AZA-VEN. Indeed, the expression of BCL2 family proteins including BCL2, $BCLx_L$, and MCL-1 within leukemic stem cell–like cells calculated as a combinatorial score was shown to accurately predict AZA-VEN response.[15]

Treatment options after HMA-VEN discontinuation/failure

In patients with newly diagnosed AML who achieve a clinical response with HMA-VEN but relapse after therapy interruption or discontinuation, rechallenge is an option. In a cohort of 15 patients where reasons for stopping HMA-VEN included allogeneic hematopoietic cell transplantation (alloHCT) and invasive fungal infections where over 50% had adverse-risk disease at diagnosis, retreatment with HMA-VEN resulted in CR/CRi in 5 patients (33%), with 2 of the responders achieving MRD negativity.[16] While the responses were short-lived, 1 patient was able to bridge to a second alloHCT. The median latency between initial treatment and retreatment was 224 days (range, 73-407 days).[16] In a study utilizing data from the VIALE-A/C trials, 13 patients who electively discontinued treatment were identified.[17] These patients were in first CR/CRi and had received at least 12 months of therapy. A total of 6 patients relapsed (67% showed molecular evolution), and 3 were retreated with the same regimen, with 2 out of 3 (66%) successfully recapturing CR/CRi.[17]

Despite these data, once patients progress through HMA-VEN, the overall outcome is poor. Median OS was 2 to 3 months in a retrospective study evaluating outcomes of 2 clinical trials.[18] Of 95 patients, 41 (43%) had relapsed or refractory (R/R) disease to frontline HMA-VEN. Twenty-four patients received salvage therapy and only 5 responded (2 received HMA-*FLT3* inhibitor, 1 received cladribine-idarubicin-cytarabine, 1 received cladribine-idarubicin-cytarabine-gemtuzumab ozogamicin, and 1 received HMA-nivolumab-ipilimumab).[18] Consistent with prior reports, all patients had aggressive disease biology at diagnosis with complex cytogenetics, *TP53*, *FLT3*, and/or *K/NRAS* mutations.[3] In a similar study but using real-world data (n = 103), 71 patients (28 relapsed, 43 refractory) were identified as R/R to frontline HMA-VEN.[19] Further treatment was pursued in

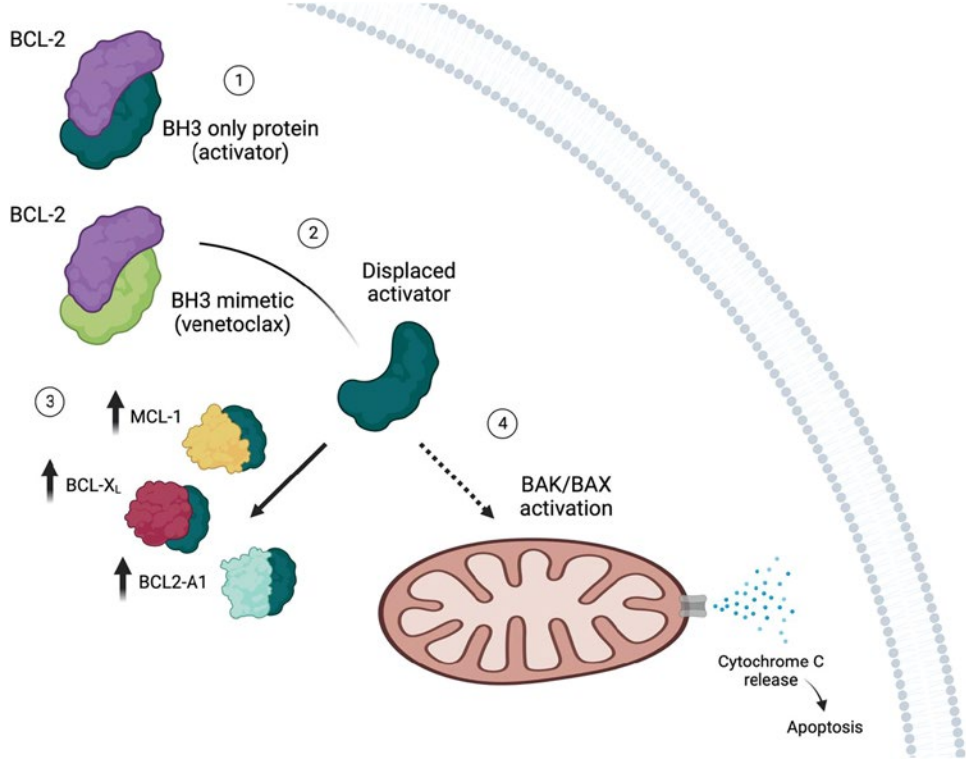

Figure 1. Mechanism of VEN resistance. (1) The proapoptotic BH3-only protein binds to antiapoptotic protein BCL2. (2) VEN is a BH3 mimetic that binds to BCL2, which leads to displacement of BH3-only protein. (3) In the setting of VEN resistance, increased levels of antiapoptotic proteins such as MCL-1, $BCLX_L$, and BCL2A1 sequester BH3-only proteins. (4) Consequently, there are fewer BH3-only proteins available to induce apoptosis. Created with BioRender.com.

11 patients, mostly in the form of targeted therapy, and CR was achieved in 3 patients. Multivariable analysis revealed that the presence of *K/NRAS*, *TP53*, and refractoriness to HMA-VEN were predictors of inferior survival.[19]

CLINICAL CASE 1 (continued)

This patient had a disruption of HMA-VEN due to infection and resumed therapy at a modified dose due to continued neutropenia. The patient relapsed with evidence of clonal evolution including an actionable mutation. While rechallenge with HMA-VEN at the full dose is a possibility, the likelihood of response is low. Retreatment, if chosen, should be limited to no more than 1 additional cycle with repeat bone marrow biopsy afterward to assess disease status. If no significant improvement is seen, it is unlikely that further HMA-VEN will be beneficial. Targeted therapy, specifically gilteritinib, is an option. However, if available, clinical trials using novel agents or a combination of agents including an *FLT3* inhibitor should be prioritized.

CLINICAL CASE 2

A 56-year-old previously healthy woman presented to the emergency room with several weeks of worsening fatigue and easy bruising. Laboratory studies were notable for pancytopenia with circulating blasts. Bone marrow biopsy and ancillary testing revealed a 25% myeloblast marrow infiltration, complex cytogenetics, and a monoallelic *TP53* (VAF, 30%) mutation (the only mutation) consistent with AML. The patient received azacitidine on days 1 through 7 every 28 days per cycle with VEN at 400 mg/d and achieved CR with undetectable MRD by flow cytometry but detectable *TP53* on NGS. Unfortunately, while waiting for alloHCT, she developed a morphologic relapse and a rising *TP53* VAF on NGS. Her performance status remains robust.

HMA and VEN in fit patients with adverse-risk AML

Although HMA-VEN is approved for patients who are not IC candidates, there are more patients receiving HMA-VEN who would otherwise be considered fit for IC. In a retrospective study comparing newly diagnosed AML patients treated with HMA-VEN (n = 143) or IC (n = 149), the response rates were not significantly different at 76.9% and 70.5%, respectively ($P = .2109$).[20] Median OS was superior in the IC arm (29 vs 16 months in HMA-VEN; $P = .002$). However, in a propensity-matched cohort accounting for age, European LeukemiaNet risk, and alloHCT status, median OS for IC became 24 months and was not reached for HMA-VEN, with a trend toward significance ($P = .0667$).[20] Further analysis showed that adverse risk, *RUNX1* mutation, and older age were predictors of better outcomes with HMA-VEN. In a multivariate

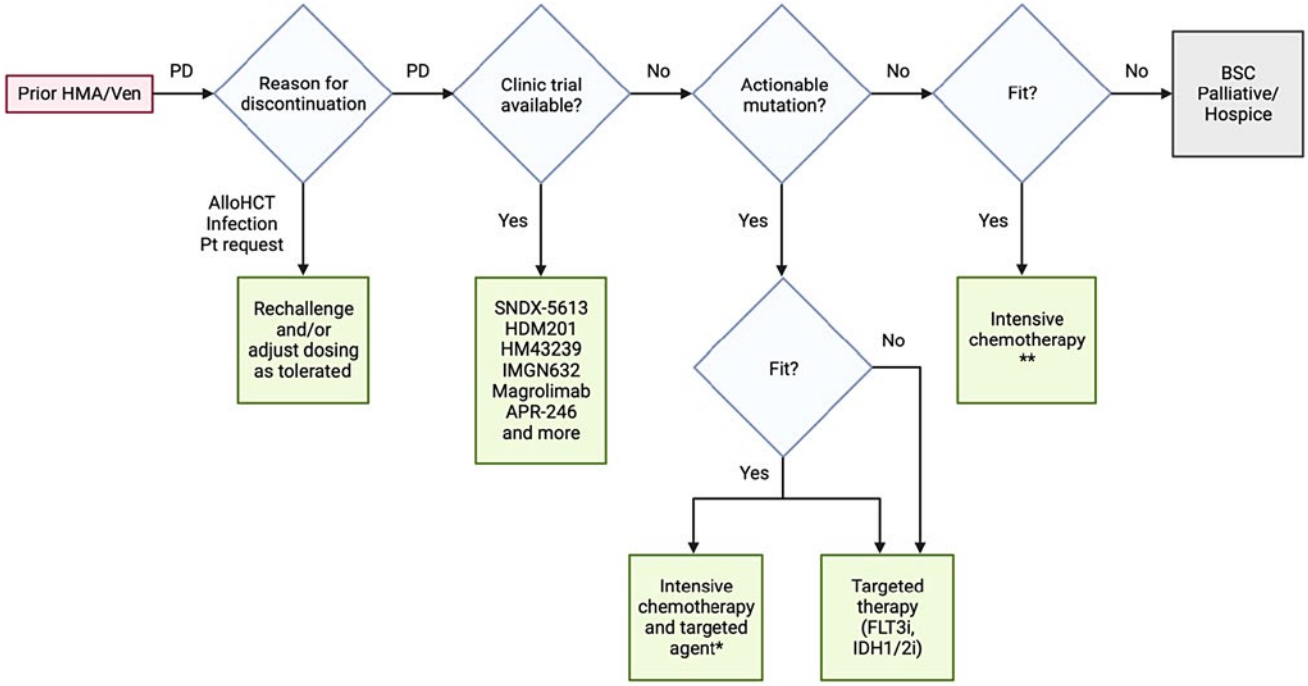

Figure 2. Treatment algorithm after HMA-VEN failure. *Combining intensive chemotherapy with a targeted agent represents an area of active research, and this treatment approach is being used off-label. **More studies are needed to assess whether adding VEN to IC can improve outcomes in the setting of post-HMA-VEN failure. BSC, best supportive care; PD, progressive disease. Created with BioRender.com.

analysis, alloHCT was found to be the sole positive predictor of OS, underscoring the importance of considering this treatment modality for patients treated with either regimen. These findings provide the rationale for the ongoing clinical trial (NCT03573024) evaluating frontline HMA-VEN therapy for younger patients (aged 18-59) eligible for IC but with adverse-risk disease by European LeukemiaNet guidelines.[21]

Another study retrospectively evaluated the outcomes of using HMA-VEN (n = 488) or 7 plus 3 (cytarabine and anthracycline; n = 312) in newly diagnosed AML patients who were older (60-75 years) and fit.[22] The median OS was 10 and 22 months for HMA-VEN and 7 plus 3, respectively (P<.0005). No subset with improved OS was identified in either regimen. Notably, in a sensitivity analysis in which only patients with a 30% to 70% chance of receiving either treatment were included (HMA-VEN: n = 120 and 7 plus 3: n = 135), the median OS was not significantly different, close to 17.5 months for both cohorts.[22] This suggests that select older patients can substantially benefit from IC, while many do not. In a related study, the same authors assessed the real-world effectiveness of HMA-VEN (n = 439) and CPX-351 (n = 217) in older patients with newly diagnosed AML.[23] Interestingly, the median OS for HMA-VEN (11 months) and CPX-351 (13 months) did not significantly differ (P = .22). This is despite multiple sensitivity analyses and controlling for various variables.[23] A randomized clinical trial to address this question of HMA-VEN vs IC for frontline AML is ongoing (NCT04801797).

In patients with R/R disease after frontline HMA-VEN, IC as a second-line therapy is often an approach chosen for select patients. In a study of 208 newly diagnosed AML patients who received frontline HMA-VEN, 19 were relapsed (n = 6) or refractory (n = 13) and subsequently received 7 plus 3 or another cytarabine-based IC regimen.[24] The response rate in this subset was 57.9% (11/19), and 81.8% (9/11) of responders proceeded to alloHCT.[24] The majority had adverse-risk disease (15/19, 78.9%), as expected. Of note, these were patients treated in the second-line setting. If a patient has progressed through multiple lines of therapy, including salvage with a VEN-based regimen, the outcome is dismal. One study examined this population (n = 28), and among those who received further therapy (n = 22), only 18% had a CR.[25] Treatment using *IDH/FLT3* inhibitors (enasidenib, sorafenib, and gilteritinib) in 6 patients, decitabine-VEN in 5 patients, and LDAC-glasdegib in 10 patients did not yield an objective response. However, 3 out of 6 patients achieved CR with VEN-actinomycin D plus or minus LDAC despite previous VEN exposure, suggesting some patients may benefit from VEN with intensification of chemotherapy. However, the number of patients treated with this regimen is small, and results should be interpreted cautiously.[25] A treatment algorithm for patients who progress after HMA-VEN is shown in Figure 2.

Novel therapies in development for R/R AML

Treatment options for R/R AML, particularly after the failure of HMA-VEN, are a well-recognized area of unmet need. Clinical trials using the combination of BCL2 and MCL-1 inhibitors to overcome VEN resistance based on sound preclinical data were limited by cardiotoxicities (NCT03218683, NCT03465540, and NCT03672695).[26] Navitoclax, a BCL2 and BCLXL dual inhibitor, in combination with VEN and decitabine (NCT05455294), is another potential regimen that might overcome VEN resistance; however, the severe thrombocytopenia associated with

BCL-x_L inhibition could be limiting. Other novel agents are being explored in early-phase trials in the R/R setting, including but not limited to small-molecule targeted inhibitors and immune-based therapies (Table 1). SNDX-5613 is a highly selective inhibitor of the menin-*KMT2A* interaction, which plays an essential role in regulating *HOXA9*, a potent transcription factor that promotes leukemogenesis.[27] Menin inhibitors have been shown to promote differentiation of blast cells in *MLL1(KMT2A)*-rearranged or *NPM1*-mutated AML.[28] In the first-in-human phase 1 study, 68 patients with R/R acute leukemia (n = 56 AML) were enrolled, with 89% having either an *NPM1* mutation or a *KMT2A* rearrangement.[29] The overall response rate (ORR) in the efficacy cohort (n = 60) was 53%, with *NPM1* at 36% (5/14) and *KMT2A* at 59% (27/46). CR/CRi was 30%, with 78% of these patients achieving MRD negativity. The drug was well tolerated, and a phase 2 clinical trial is ongoing (NCT04065399). Ziftomenib is another menin inhibitor recently shown to have high response rates in patients with *NPM1* mutations (a CR rate of 35% and an ORR of 45% at the recommended phase 2 dose).[30] HDM201 is a potent inhibitor of the MDM2-p53 interaction and represents another promising antileukemic approach.[31] The upregulation of MDM2, a ligase that degrades the tumor suppressor p53, leads to a reduction of p53 activity. In cancers with wild-type or functional p53, HDM201 promotes p53 reactivation and thereby suppresses cancer formation.[31] Among 28 of the 91 R/R AML patients in a phase 1 study of HDM201, 22.2% achieved CR/CRi.[32] Clinical trials using HDM201 in combination with either MBG453, an anti-TIM-3, or VEN for AML/MDS are in process (NCT03940352). *FLT3* inhibitors are an important class of targeted agents with proven efficacy in select subsets of AML patients.[33,34] HM43239 is a novel FLT3/SYK inhibitor that has demonstrated activity in both *FLT3*-mutated and wild-type AML.[35] Preclinical studies suggest it can overcome resistance to prior *FLT3*-targeted therapy.[36] In the first-in-human phase 1/2 study, 28 patients with R/R AML (*FLT3*-positive, 10; -negative, 16; and unknown, 2) were treated with HM43239.[35] The CR/CRi rate at the expansion dose was 26.3% (5/19) with *FLT3*-mutated patients at 37.5% (3/8) and wild type at 18% (2/11). One of the responding patients had received gilteritinib in the past.[35] This trial is currently active with a phase 2 component that combines HM43239 with VEN (NCT03850574).

Aside from targeted agents, immunotherapies that are antibody based are actively being explored. IMGN632 is a CD123-directed antibody-drug conjugate with the payload indolinobenzodiazepine dimer, which causes DNA alkylation.[37] Synergistic effects were observed when used in combination with azacitidine and/or VEN in preclinical models.[38] A phase 1b/2 study of IMGN632-AZA-VEN in R/R AML patients (n = 35) has been reported.[39] Notably, over 50% had received prior VEN, and efficacy was seen across all doses and schedules. Among those evaluable for efficacy (n = 29), the ORR and CR/CRi were 55% and 31%, respectively. The CR/CRi rate was over 70% in VEN-naive, higher-intensity cohorts.[39]

TP53-mutated AML is a distinct disease entity with poor outcomes regardless of treatment. Compared to its wild-type counterpart, response rates are lower to any of the approved agents, and median OS is estimated to be 5 to 10 months.[40] Magrolimab, a novel antibody that inhibits CD47, thereby blocking the "do not eat me" signal expressed on leukemic cells, has shown promising results even in the *TP53*-mutated subset.[41] A total of 74 AML patients (45 newly diagnosed and 29 R/R) received magrolimab-AZA-VEN.[42] Among the newly diagnosed (ORR, 80%), *TP53*-mutated AML patients (n = 27) had an ORR

Table 1. Emerging novel therapies in R/R AML

Phase	Regimen	Mechanism of action	Number of patients	Median age (range)	Response	Reference
1	SNDX-5613	Menin inhibitor	68 (56 R/R AML)	51 (19–79) among adults	ORR: 53% (32/60) CR/CRi: 38% (23/60) ORR in *KMT2A*: 59% (27/46) ORR in *NPM1*: 36% (5/14)	Wang et al[26]
1	HDM201	MDM2 inhibitor	208 (91 R/R AML)	~70 (23–85)	CR/CRi: 13.2% (12/91) CR/CRi in 45mg: 22.2% (6/28)	Klossowski et al[28]
1/2	HM43239	FLT3/SYK inhibitor	28	60 (35–83)	CR/CRi in 80mg: 26.3% (5/19) CR/CRi in *FLT3* 80mg: 37.5% (3/8)	Konopleva et al[31]
1b/2	IMGN632 + AZA + Ven	CD123 antibody-drug conjugate	35 (29 efficacy evaluable R/R AML)	69 (range not available)	ORR: 55% (16/29) CR/CRi: 31% (9/29)	Daver et al[35]
1/2	Magrolimab + AZA + Ven	CD47 inhibitor	74 (29 R/R, 45 newly diagnosed)	Not available	ORR in R/R Ven-naïve: 75% ORR in R/R Ven prior: 12%	Kuruvilla et al[38]
1b/2	APR-246	p53 reactivator	55 (11 R/R oligoblastic AML)	66 (34–85)	ORR: 64% (n=7) CR: 36% (n=4) Median OS 10.8 months	Daver et al[39]

of 74%; CR/CRi, 63%; and CR, 41%. For R/R patients the ORR was 75% and 12% for VEN naive and VEN exposed, respectively. Another agent with preferential activity in *TP53*-mutated disease is APR-246, which works by restoring mutant p53 back to its functional conformation.[41] In patients with AML (n = 11), the ORR was 64% and CR was 36%.

Other novel agents and rational drug combination studies are in the pipeline. However, it is important to note that the data for these trials, including those discussed thus far, are based upon preliminary results and small sample sizes. Larger cohorts across multiple centers in later-phase trials will help clarify and confirm the effectiveness of these regimens.

CLINICAL CASE 2 (continued)

There is no standard of care for this patient's R/R *TP53*-mutated AML. The preference would be enrollment in a clinical trial that utilizes a novel agent or a targeted approach. Otherwise, IC remains an option for this fit patient.

Conclusion

While the advent of HMA-VEN has transformed the treatment of patients with newly diagnosed AML, primary and acquired resistance prevent the achievement of long-term disease-free and overall survival in most patients. Signaling pathway and tumor suppressor mutations represent a major source of VEN resistance, leading to HMA-VEN treatment failure and relapse. While a subset of these patients may benefit from IC or even rechallenge with HMA-VEN, the overall prognosis is poor and options are limited. Enrollment in clinical trials with novel agents and rational combinations is needed to improve outcomes for this group of patients.

Conflict-of-interest disclosure

Onyee Chan: no competing financial interests to declare.
Alison R. Walker: no competing financial interests to declare.

Off-label drug use

Onyee Chan: Figure 2 mentions label use of intensive chemotherapy + targeted agent but doesn't specify which agents.
Alison R. Walker: Figure 2 mentions label use of intensive chemotherapy + targeted agent but doesn't specify which agents.

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How old is too old? Frailty and geriatric assessments of older patients undergoing allogeneic HCT

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Allogeneic hematopoietic cell transplantation (HCT) is a curative-intent treatment for many hematologic malignancies but carries a significant risk of morbidity and mortality. An increasing number of older adults are receiving HCT, but current pretransplant evaluations overlook the unique vulnerabilities that older adults face. Oncology-specific geriatric and frailty assessments provide a comprehensive evaluation of older adults, help better weigh the risks of HCT with patients, and guide personalized optimization strategies to minimize vulnerabilities. Geriatric assessments evaluate seven domains: comorbidities, physical function, mental health, cognition, nutrition, medications, and social support. Frailty indices provide unique evaluations into a patient's overall status. Various standardized measures have been used to evaluate these areas in older adults prior to HCT. Different care models exist for the integration of geriatrics and geriatric principles into HCT evaluation: a multidisciplinary consultative clinic, a geriatrician alongside the HCT clinic, or a primary geriatric hematologist/transplant physician. Future studies are needed to investigate the use of geriatric assessments in selecting the conditioning regimen and intensity and measuring the impact of geriatric assessment–driven interventions on quality of life and toxicities post transplant.

LEARNING OBJECTIVES
- Describe the domains of a geriatric assessment
- Assess for vulnerabilities in HCT recipients utilizing standardized measures

CLINICAL CASE

Jane is a 71-year-old woman with acute myeloid leukemia with a complex karyotype. Treatment was initiated with venetoclax and azacitidine. She achieved a complete response, and reduced-intensity matched unrelated peripheral blood allogeneic hematopoietic cell transplantation (HCT) was pursued. She underwent HCT evaluation per institutional standards, including a geriatric assessment.

Geriatric and frailty assessments in HCT

HCT is a curative treatment for many hematologic malignancies but carries a significant risk of morbidity and mortality. With advances in disease treatment, transplant techniques, and supportive measures, an increasing number of older adults are receiving HCT. Current evaluation for HCT eligibility is standardized regardless of age, overlooking the unique vulnerabilities older adults face. The lack of standardized evaluation of older adults has led to physicians arbitrarily determining eligibility for HCT.

Oncology-specific geriatric and frailty assessments provide in-depth evaluations of older adults, with a growing evidence base in HCT.[1] These assessments help better weigh the risks of HCT and guide personalized optimization strategies. Geriatric assessment-driven interventions decrease toxicities and falls in the nontransplant geriatric oncology population.[2,3] An area not evaluated by these assessments is the transplant ecosystem, which has recently been published.[4] Here, I focus on geriatric and frailty assessments and touch on models to incorporate into clinical practice. An in-depth discussion of care models and interventions are outside the scope of this article.

Geriatric assessment

Oncology-specific geriatric assessments typically comprise 7 domains (Table 1). The Practical Geriatric Assessment is

Table 1. Geriatric assessment domains, measures, and possible interventions

Domains	Tools	Interventions[5,10,40]
Comorbidity	HCT-CI Cumulative Illness Rating Scale-Geriatric OARS comorbidity	Referral to specialist familiar with transplant
Physical function	ADLs IADLs TUG 4-meter gait speed 6MWT	Physical therapy Occupational therapy Address uncontrolled pain
Psychological	PHQ-9 Hospital Anxiety and Depression Scale Mental Health Inventory-17 Short Form-36 (mental component summary) PROMIS Anxiety Geriatric Depression Screen	Referral to psychiatry Cognitive behavioral therapy Initiate antidepressants
Cognition	MiniCog Mini-Mental Status Exam MoCA Blessed-Orientation-Memory-Concentration Test	Review medications for possible contributing cause Address depression if present Delirium precautions when admitted
Nutritional status	MNA Preoperative Nutrition Screen Albumin Weight loss[a]	Refer to dietician Encourage nutrition supplement use Address possible contributing factors
Polypharmacy and potentially inappropriate medications	≥7 medications Beers Criteria	Stop potentially inappropriate and unnecessary medications Review medications for possible drug-drug interactions Consult pharmacist
Social support	Medical Outcomes Study Social Support Survey	Complete health care power of attorney Identify short-term and long-term needs

[a]Over the preceding 3 months.

6MWT, 6 minute walk test; ADLs, activities of daily living; IADLs, instrumental activities of daily living; MNA, mininutrition assessment; MoCA, Montreal cognitive assessment; OARS, Older Americans Resources and Services; PHQ-9, patient health questionnaire-9; TUG, timed up and go.

Adapted from Jayani et al.[1]

a concise tool built to overcome barriers to implementation.[5] The Practical Geriatric Assessment provides a foundation for more in-depth assessments of vulnerabilities in older adults undergoing intensive therapies, including HCT. Summarized below are the standardized measures utilized in HCT.

Comorbidities

Standard HCT evaluation includes an assessment of comorbidities, most commonly using the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI).[6] This tool calculates a weighted score of comorbidities and organ function that is predictive of nonrelapse mortality (NRM) and overall survival (OS).[6] It was originally validated in a cohort of young patients and was updated to incorporate chronologic age with the Comorbidity-Age Index.[7] The advantage of this tool in patients beyond their sixth decade of life remains uncertain.[8] In addition, this tool has yet to be validated with the recent advancements in HCT such as posttransplant cyclophosphamide-based graft-versus-host disease prophylaxis.

Physical function

Two categories of physical function measures have been studied: patient-reported and objective measures.

Patient-reported measures

Activities of daily living (ADLs) and instrumental activities of daily living (IADLs). ADLs are activities needed to independently care for one's self; IADLs are activities needed to independently live in the community. Impairments in these measures are frequently seen in older adults prior to HCT, with reports of impairments in ADLs and IADLs as high as 42% and 50%, respectively.[9,10] These findings indicate that prior to HCT, the majority of older adults require assistance to care for themselves or live in the community. Multiple single-institution studies have shown that older adult HCT recipients with impairments in ADLs and/or IADLs have increased NRM, decreased OS, and decreased progression-free survival.[11-13] However, these findings were not replicated in a multicenter retrospective study.[14]

Health-related quality of life (QOL). Up to 42% to 64% of older adults report low physical function prior to HCT, which has been correlated with inferior survival outcomes.[11-13,15] These findings are also seen in adults of all ages, suggesting that patient-reported physical function provides an additional perspective into a patient's function not otherwise captured.[15,16] However, these studies utilize different measures. Some measures focus on physical function in terms of health-related quality of life (QOL)—for example, the Short Form-36 (SF-36)—while others, such as the International Physical Activity Questionnaire, focus on the level of activity.

The National Institutes of Health–sanctioned Patient-Reported Outcomes Measurement Information Systems (PROMIS) aims to overcome this variability by standardizing the collection and reporting of patient-reported measures.

Objective measures

Objective measures of physical function are not subject to recall bias and may measure a patient's physical function with more accuracy and reproducibility, but these measures have not consistently correlated with survival post transplant. Studied measures range from evaluation of fall risk to cardiopulmonary fitness. The 6-minute walk test (6MWT) is not predictive of post-transplant survival despite evaluating cardiopulmonary fitness.[17,18] However, studies show that a decline in 6MWT early post transplant identifies patients at risk of inferior survival. Additionally, these studies, which included adults of all ages, found that the majority of patients scored lower than age-matched norms. This finding raises the need for a threshold specific for adults (or older adults) with hematologic malignancies that may be more predictive of outcomes.

Geriatric-specific objective physical function measures show impairments in 3% to 45% of older adults prior to HCT.[11-14,19,20] These measures include the 4-meter gait speed, timed up-and-go (TUG), and short physical performance battery (SPPB). The 4-meter gait speed is a timed measure of gait over 4 meters. TUG is a timed measure assessing gait and balance. The SPPB evaluates 3 components of physical function: balance, gait speed, and lower extremity strength. A slow 4-meter gait speed has been associated with decreased OS.[11] There are mixed reports on the correlation between an abnormal TUG and survival outcomes post HCT.[12-14,19] There are limited data on the prognostication of SPPB in HCT; however, older adults with AML and low SPPB prior to intensive chemotherapy have inferior survival.[21]

Mental health

Increased rates of depression and anxiety are seen in those with cancer, and HCT recipients are no exception. Uncontrolled depression and anxiety in older adults with cancer are associated with treatment nonadherence, decreased health-related QOL and survival, and increased hospital length of stay.[22,23] These mental health disorders are often overlooked, with up to 56% of older adults reporting uncontrolled depression or anxiety prior to HCT.[11]

The tools currently utilized focus on patient-reported measures, with discordant findings on the impact of uncontrolled depression or anxiety on transplant outcomes. These differences may in part be due to the varying measures used. The Patient Health Questionnaire (PHQ_) evaluates depression symptoms but has some limitations. It focuses on symptoms that older adults may not present with and does not evaluate anxiety.[24] Measures like the Hospital Anxiety and Depression Scale evaluate for anxiety and depression. Yet other measures evaluate global mental health, including the Mental Health Inventory and SF-36. Of these, only the SF-36 Mental Component Summary has been associated with decreased OS post transplant. Here again, the PROMIS measures help decrease the variability in measures used.

Cognition

The risk of cognitive impairment increases with age and is seen in up to 20% of older adults prior to HCT.[19] A number of tools exist to screen for cognitive impairment. These range from the MiniCog, which consists of a clock drawing and 3-word recall, to the Montreal Cognitive Assessment (MoCA), covering 8 domains of cognitive function. The MoCA detects subtle changes in cognition, such as mild cognitive impairment. Thus, the MoCA may identify patients at increased risk of complications such as delirium who would benefit from additional support. In a single-institution study of adults of all ages undergoing autologous or allogeneic HCT, the MoCA was not predictive of posttransplant outcomes, but extrapolation to older adults is limited.[25] However, impaired cognition identified by the 6-item Blessed Orientation-Memory-Concentration Test correlated with decreased OS in a multicenter retrospective study.[14]

Nutrition

Malnutrition places older adults at increased risk of inferior survival with many medical procedures, including HCT.[13,26] Prior to HCT, up to 36% of older adults are malnourished, and 76% are at risk.[13,27] In addition, malnutrition increases during the first 6 months post transplant.[27] Various measures of malnutrition have been investigated in the older adult HCT population. These range from simple measures such as serum albumin to more extensive tests such as the Perioperative Nutrition Screen and the Mini-Nutrition Assessment (MNA). The Perioperative Nutrition Screen focuses on weight loss, body-mass index, and oral intake, whereas the 6-item MNA incorporates neuropsychiatric and physical components. Low scores on these measures have been associated with inferior survival post-transplant.[11,13,26]

Polypharmacy and potentially inappropriate medications

The definition of polypharmacy varies, from 5 or more to 9 or more medications. Regardless, the concerns with polypharmacy remain the same: increased risk of adverse events and drug-drug interactions. Post-transplant medication regimens are complex and include medications with a narrow therapeutic index and the risk of severe and long-term consequences of noncompliance, such as acute and severe chronic graft-versus-host disease (GHVD). Polypharmacy in older adults prior to HCT is associated with lower OS, regardless of age and comorbidities.[28] With older adults on a median of 7 medications prior to HCT, ongoing efforts in deprescribing and the impact on HCT-related outcomes are needed.[28]

Older adults are also at increased risk of adverse effects from medications that may overshadow the potential benefit. Resources are available to help identify these so-called potentially inappropriate medications—for example, the American Geriatric Society Beer's Criteria.[29] Some of these medications are commonly used during HCT (Table 2). Nearly 50% of older adults are taking potentially inappropriate medications prior to or during HCT, which has been associated with inferior survival and increased severe toxicities post-transplant.[28,30,31]

Social support

HCT recipients are required to relocate close to the transplant center with an informal caregiver for the early posttransplant period. During this time and even after returning home, many remain on immunosuppression with increased risk of infection. As a result, many isolate themselves to decrease this risk. However, social isolation places older adults at increased risk of additional issues, including dementia. Additionally, informal

Table 2. Examples of potentially inappropriate medications commonly used post transplant[a]

Promethazine
Scopolamine
Lorazepam
Oxybutynin
Dicyclomine
Cyclobenzaprine
Proton pump inhibitors[b]
Sliding scale insulin
Diphenhydramine[b]

[a]Potentially inappropriate medications based on American Geriatric Society Beers Criteria.[29]

[b]May be appropriate in certain circumstances.

caregivers have other responsibilities or burdens. Children often have other commitments such as work or their own families. Spouses or siblings may have medical conditions or be frail as well. A limited number of studies have evaluated the impact of social support on HCT outcomes in older adults. After transplant, the majority of older adults report inadequate social support and decreased social well-being.[27,32]

Frailty index

Two models of frailty index exist, with different theoretical constructs.

Fried Frailty Index

The Fried Frailty Index evaluates patients as frail, prefrail, or fit.[33] This index consist of 3 patient-reported (weight loss, energy, physical activity) and 2 objective hand grip, gait speed) measures with adaptations (Table 3).[27,33-35] Up to a quarter of older adults undergoing HCT are frail, and over half are prefrail.[11,19,27] Although the Fried Frailty Index has not consistently been shown to predict OS in older adults, a study of adult HCT recipients showed that frailty predicted for an increased risk of severe or life-threatening nonhematologic toxicities and mortality.[11,27,35]

Cumulative Frailty Index

The Cumulative Frailty Index focuses on the accumulative impact of health deficits.[36] This index includes symptoms, comorbidities, laboratory values, and disabilities. It has been associated with hospitalization and survival in the general older adult population. Further studies are needed to understand the advantage of this frailty model in evaluating HCT recipients.

CLINICAL CASE (continued)

We return to Jane, our 71-year-old woman with high-risk AML. Her standard HCT evaluation revealed a history of coronary artery disease, supraventricular tachycardia, a normal echocardiogram, and a low forced expiratory volume and diffusing capacity of lung for carbon monoxide. Her HCT-CI score was 4. Her geriatric assessment revealed additional areas of vulnerability. She reported low physical function and was found to have an abnormal TUG and low 6MWT. The PHQ-9 identified severe depression symptoms. The PROMIS Anxiety measure showed no increased anxiety symptoms. The MoCA revealed mild cognitive impairment. On review of her medications, she was found to be on 12 medications, with the use of a potentially inappropriate medication, zolpidem.

Table 3. Fried Frailty Index

Component	Original Tool[33]	Adapted measures[27-35]
Exhaustion	Effort for and difficulty starting activities over preceding week (from Center for Epidemiologic Studies Depression Scale)	Need for assistance or unable to carry out normal activity or work or Self-reported weakness
Low physical activity	Kilocalorie expenditure over a week	Rapid Assessment of Physical Activity MOS-SF-10 physical function or Minnesota Leisure Time Activity Questionnaire- short version or Frequency of intensive exercise over preceding week
Weight loss	Self-report	Self-report
Low grip strength/ weakness	Grip strength	Ability to lift and carry groceries. or Self-reported upper extremity weakness
Slow gait speed	15-feet walk time	3-meter walk or TUG or Self-report of health limiting walking

MOS-SF-10, Medical Outcomes Survey Short Form 10-Item.

Table 4. Care models for implementation of geriatrics into HCT

Model type	Description
Multidisciplinary consultative clinic	Patient is evaluated by multiple disciplines in 1 day or separate days Allows for more patients to be seen Limited follow-up Treatment plan finalized by primary hematologist/transplant physician Resource intensive
Geriatrician alongside HCT clinic	Allows patients to be seen longitudinally by geriatrics or with new geriatric syndromes
Shared care	Division of medical management of medical care between the geriatrician and transplant physician Providers located in different clinic spaces with different support staff
Embedded	Incorporation of the geriatrician within the same clinical space as the transplant team May take on a consultative role
Geriatric hematologist/transplant physician primary provider	Evaluated prior to HCT and geriatric syndromes addressed early Monitor and address new geriatric syndromes Treatment modifications as appropriate

Models for integration of geriatrics into HCT

Different care models exist for integrating geriatrics and geriatric principles into the care of older adults with cancer and have shown improvement in treatment outcomes in transplant and nontransplant patients (Table 4).[2,3,10,37-39] This integration may also benefit younger HCT recipients who experience accelerated aging.[34] Each health care system has unique needs best addressed by different care models. In these models, evaluations may be completed through a combination of patient-reported tools, objective measures, and clinical evaluation.

Multidisciplinary consultative clinic

A clinic involving providers from other disciplines such as geriatrics, physical therapy, occupational therapy, social worker, or psychiatry, among others, may be ideal in assessing older adults prior to HCT. This model might require multiple trips or a lengthy clinic day for patients but is aligned with traditional comprehensive geriatric assessments. This model is more resource intensive but has been shown to improve OS and NRM in single-institution retrospective studies.[10,39] These clinics are typically consultative clinics where patients are evaluated, recommendations are made, and final treatment plans rest with the treating hematologist/transplant physician. A limitation to this approach is that these clinics may focus on evaluation and optimization prior to transplant due to limited resources, but there is a need to support older adults post transplant as well. However, one benefit to this approach is the expanded access for patients. This model can be incorporated into the standard institutional HCT evaluation of older adults or as a referral through the use of geriatric screening tools to identify patients at highest risk of vulnerabilities.

Geriatrician alongside HCT clinic

Another care model is to incorporate geriatricians into HCT clinics, which may be done in 2 ways: 1) shared care, 2) embedded. Both methods allow longitudinal geriatrics support for older adult HCT recipients, including for new geriatric syndromes that arise post transplant. The shared care model comprises a division of medical management between the geriatrician and transplant physician, who may be located in different offices with different support staff. The embedded model incorporates a geriatrician within the same offices as the transplant team but who may take on a consultative role.

Primary geriatric hematologist/transplant physician

Another model is to have a geriatric-hematologist/transplant physician as the primary provider for the patient. In this model, the primary provider evaluates patients prior to transplant, addresses geratric syndromes prior to transplant and any that arise after, and treatment modifications are made where appropriate. This model is limited by the number of geriatric-trained hematologists/transplant physicians.

CLINICAL CASE (continued)

Based on the vulnerabilities identified on the geriatric assessment, prior to HCT Jane was referred to physical therapy to build muscle strength and improve her physical function and to psychiatry for management of depression. The zolpidem was stopped and melatonin trialed. By HCT, her physical function had improved both on the TUG and in self-report, and her depression symptoms had significantly improved. Other than one planned 3-day admission, she completed the majority of her transplant care as an outpatient. She had an initial decline in 6MWT post transplant but recovered by Day+ 100. She and her caregiver were provided written instructions with any medication changes. Unfortunately, she relapsed at Day+ 180. Her salvage treatment course was complicated by recurrent infections. She ultimately developed progressive disease, at which point she transitioned to hospice and passed at 1 year post-transplant.

Conclusion

In conclusion, geriatric and frailty assessments identify vulnerabilities in older adult HCT candidates not captured on routine HCT assessment. These vulnerabilities place them at increased risk of poor outcomes, but oncology-specific geriatric assessments can guide interventions to minimize vulnerabilities. Different care models exist for implementing geriatrics into HCT evaluations. Future studies are needed to investigate the use of geriatric assessments in selecting the conditioning regimen and intensity and the impact of geriatric assessment-driven interventions on QOL and toxicities post-transplant.

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Reena V. Jayani: no competing financial interests to declare.

Off-label drug use

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The sum of the parts: what we can and cannot learn from comorbidity scores in allogeneic transplantation

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Allogeneic hematopoietic cell transplantation (alloHCT) requires the comprehensive evaluation of patients across multiple dimensions. Among the factors considered, comorbidities hold great significance in the pretransplant assessment. As many as 40% of alloHCT recipients will have a high burden of comorbidities in contemporary cohorts. To ensure a standardized evaluation, several comorbidity scores have been developed; however, they exhibit variations in properties and performance. This review examines the strengths and weaknesses associated with these comorbidity scores, critically appraising these models and proposing a framework for their application in considering the alloHCT candidate. Furthermore, we introduce the concept that comorbidities may have specific effects depending on the chosen transplantation approach and outline the findings of key studies that consider the impact of individual comorbidities on alloHCT outcomes. We suggest that a personalized transplantation approach should not rely solely on the overall burden of comorbidities but should also take into account the individual comorbidities themselves, along with other patient, disease, and transplantation-related factors.

LEARNING OBJECTIVES

- Incorporate comorbidity data into a comprehensive framework for risk-informed decision-making
- Identify the main comorbidity-based indices used to assess patients undergoing allogeneic HCT
- Critically appraise comorbidity indices, identifying their strengths and weaknesses
- Introduce the concept that the impact of comorbidities on alloHCT outcomes can vary according to transplantation platform

CLINICAL CASE

A 59-year-old man was diagnosed with de novo normal-karyotype acute myeloid leukemia (AML) 3 months ago. Molecular testing at diagnosis detected an fms-like receptor tyrosine kinase 3 internal tandem duplication (*FLT3*-ITD) mutation. He achieved complete remission without evidence of measurable residual disease after initial induction therapy with "7 plus 3" and midostaurin and went on to receive a consolidation cycle with high-dose cytarabine and midostaurin. He is now presenting for an evaluation to determine suitability for an allogeneic hematopoietic cell transplantation (alloHCT). His past medical history is notable for diabetes mellitus type 2 controlled with metformin, stage IIa colon adenocarcinoma resected 4 years ago without adjuvant treatment, and nonalcoholic fatty liver disease. He has a good performance status (Karnofsky Performance Status 90), and his physical exam is remarkable for a body mass index of 37. Blood laboratory measurements show slightly elevated liver enzymes (aspartate aminotransferase [AST] 74, alanine aminotransferase [ALT] 66), a creatinine level of 1.3 (estimated glomerular filtration rate [eGFR] of 63 mL/min/1.73 m^2), a lactate dehydrogenase (LDH) level of 240 µL, and a platelet count of 112 K/µL. His predicted forced expiratory volume in 1 second (FEV1) and hemoglobin-adjusted diffusing capacity of the lungs for carbon monoxide (DLCo) are 92% and 89%, respectively. The left ventricular ejection fraction (LVEF) is 55%. He is cytomegalovirus immunoglobulin G–positive. He has an available 10 out of 10 human leukocyte antigen–matched unrelated donor who is also cytomegalovirus-seropositive.

Introduction

AlloHCT provides a potentially curative treatment for hematologic malignancies and disorders. However, despite improvements in transplantation outcomes over time, significant risks of morbidity and mortality persist.[1] Careful consideration of benefits and risks is essential in candidate evaluation and transplantation planning. Pretransplantation comorbidities (ie, coexisting medical conditions) significantly impact alloHCT recipient prognosis.[2-4] Individual comorbidities, as well as their cumulative burden, are integral factors that need to be considered when deciding if and how to perform the transplantation. The introduction of reduced-intensity conditioning and improvement in supportive care has made it possible and commonplace to transplant patients with high comorbidity burdens. Indeed, a survey of the US and European transplantation registries shows that among patients receiving myeloablative and reduced-intensity conditioning, 20% to 40% and 31% to 42% had a Hematopoietic Cell Transplantation-Specific Comorbidity Index (HCT-CI) score of 3 or higher, respectively (Figure 1A, B). Here we review current knowledge about the role of pretransplantation comorbidity-based indices and individual comorbidity in alloHCT.

Comorbidity-based prognostic models

Comorbidity scoring systems standardize the approach to quantifying patients' burden of comorbid conditions and evaluating the risks associated with transplantation. These scores also facilitate adjustments for confounding factors in statistical analyses and serve as quality assurance benchmarks. We expect that higher scores, indicative of a greater pre-HCT comorbidity burden, correspond to recipients' lower capacity to withstand the physiological demands and complications associated with transplantation. Moreover, these scores may offer insights into the recipients' later ability to endure cancer maintenance therapy or receive "salvage" treatment in the event of disease

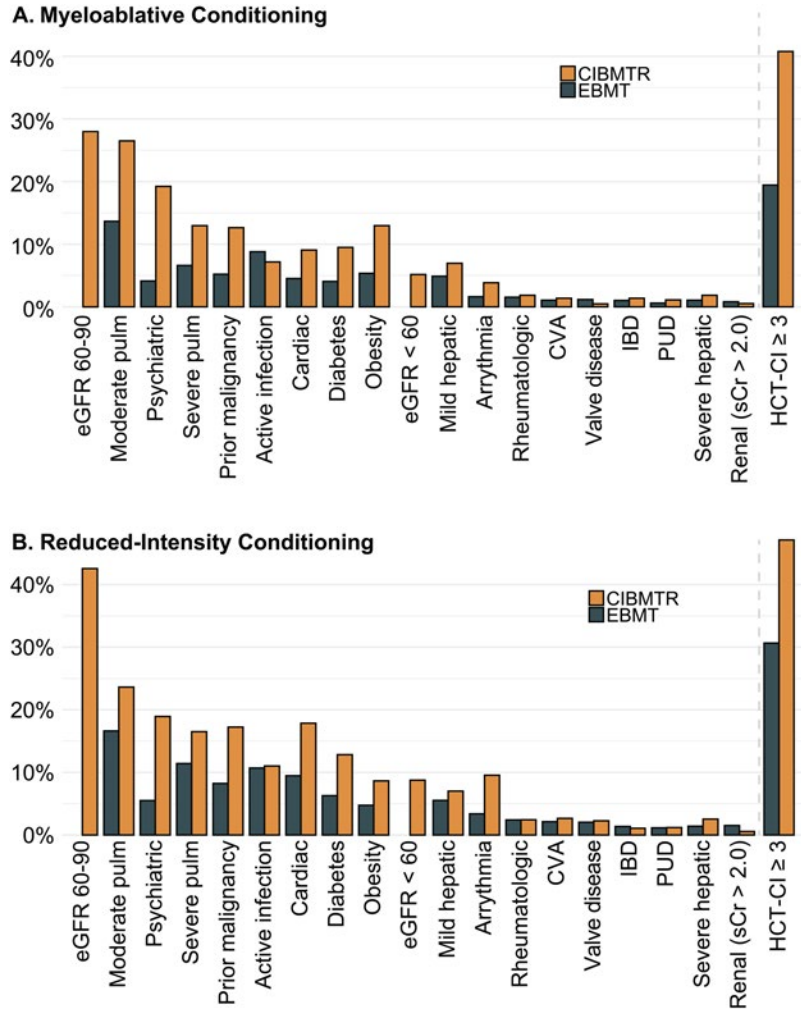

Figure 1. Prevalence of comorbidities in myeloablative and reduced-intensity conditioning cohorts from the CIBMTR and EBMT. The CIBMTR cohort consists of 3685 patients, median age 57.5 (IQR, 50.5, 63.9); the EBMT cohort consists of 9323 patients with a median age of 51.5 years (42.8, 62.1). Both cohorts were selected to include only patients with AML in complete remission. CVA, cerebrovascular accident; IBD, inflammatory bowel disease; IQR, interquartile range; PUD, peptic ulcer disease; pulm, pulmonary disease; sCr, serum creatinine. Data adapted from Fein et al.[3] and Farhadfar et al.[46]

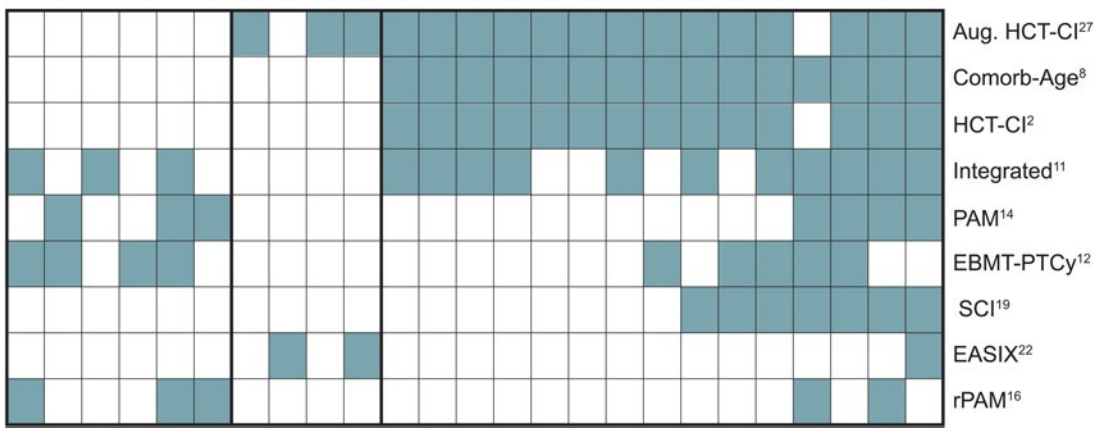

Figure 2. Comorbidity and noncomorbidity components of individual scores. CMV, cytomegalovirus; CVA, cerebrovascular accident; DM, diabetes mellitus; IBD, inflammatory bowel disease.

recurrence after transplantation. An ideal comorbidity score serves as a decision-support tool for personalizing interventions. However, evidence-based application of these scores to optimize HCT is challenging since the bulk of our knowledge stems from retrospective studies with inherent biases. Prospective studies considering comorbidities as a pivot for therapeutic decisions are lacking. Nonetheless, insights from numerous retrospective analyses have guided management in the absence of large-scale clinical trials. Over the past 2 decades, various comorbidity-based prognostic models, or *risk scores*, have emerged, incorporating various comorbidities and other factors to quantify mortality risk following HCT (Figure 2). These scores diverge in the selection and weight of component comorbidities, consideration of determinants beyond comorbidities, and characteristics of the cohorts used for development (Table 1). Here we present a nonexhaustive overview of the predominant comorbidity-based scores.

Table 1. Characteristics of the development cohort for individual scores

		HCT-CI[2]	Comorb-age[8]	Integrated score[11]	Aug-HCI[26]	PAM[14]	rPAM[16]	EASIX[22]	SCI[19]	EBMT-PTCy[12]
No. of pts		708	3033	812	3917	1401	1549	311	573	1,861
Median age		45 (1–73)	n/a	58 (20–76)	n/a	n/a	51 (15–79)	53 (17–70)	56 (46–64)[a]	51 (18–79)
Years		1997–2003	2000–2006	2000–2011	n/a	1990–2002	2003–2009	2001–2013	2008–2018	2010–2018
Donor type	Related	612 (58%)	1677 (55%)	432 (53%)	1962 (50%)	663 (47%)	584 (38%)	109 (35%)	195 (34%)	254 (14%)
	Unrel.	443 (42%)	1356 (45%)	380 (47%)	1942 (50%)	738 (53%)	886 (57%)	189 (61%)	378 (66%)	1607 (86%) 56% haplo
Stem cell source	Marrow	306 (29%)	660 (22%)	n/a	n/a	1059 (76%)	n/a	202 (6%)	0 (0%)	507 (27%)
	PB	749 (71%)	2373 (78%)	n/a	n/a	329 (23%)	n/a	291 (94%)	573 (100%)	1354 (73%)
	Cord	0 (0%)	0 (0%)	n/a	n/a	0 (0%)	n/a	20[a] (6%)	0 (0%)	0 (0%)
Regimen intensity	MAC	760 (72%)	1,889 (62%)	0 (0%)	2083 (53%)	1314 (94%)	940 (61%)	72 (23%)	573 (100%)	561 (30%)
	RIC	295 (28%)	295 (28%)	812 (100%)	1810 (46%)	87 (6%)	609 (39%)	239 (77%)	0 (0%)	1300 (7%)
T-cell depletion	None specified	708 (100%)	2790 (92%)	472 (58%)	n/a	n/a	n/a	122 (39%)	0 (0%)	0 (0%)
	ATG	n/a	243 (8%)	340 (42%)	n/a	n/a	n/a	189 (61%)	0 (0%)	0 (0%)
	PTCy	n/a	n/a	n/a	n/a	n/a	n/a	n/a	0 (0%)	1861 (100%)
	CD34 selection	n/a	n/a	n/a	n/a	n/a	n/a	n/a	573 (100%)	0 (0%)

[a]Provided in the original article as median and interquartile range.

[b]described in the original article as "bone marrow or unrelated cord blood."

ATG, antithymocyte globulin; Aug-HCI, augmented HCT-CI; Comorb-Age, comorbidity-age score; unrel, unrelated; PB, peripheral blood; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning.

Hematopoietic Cell Transplantation-specific Comorbidity Index (HCT-CI)

The Charlson Comorbidity Index pioneered comorbidity-based prognostic modeling in medicine, assigning weights to 19 medical conditions based on their impact on mortality. The Index proposed a standardized comorbidity classification scheme validated in various medical conditions. However, the score was not well fit to the role of comorbidity in the unique physiologic stressor of alloHCT, providing an incentive for the development of the HCT-CI by Mohamad Sorror and colleagues from the Fred Hutchinson Cancer Research Center (FHCRC).[2] The HCT-CI was developed on a cohort of 708 patients transplanted in the late 1990s at FHCRC and came to include 15 comorbidities weighted according to their association with nonrelapse mortality (NRM). The HCT-CI is typically categorized into three intervals (0, 1-2, ≥3), corresponding with increasing NRM risk. The HCT-CI has been validated repeatedly across various transplantation indications, conditioning regimens, and donor types, demonstrating its utility in stratifying NRM and overall mortality risk.[5-7] It has also been expanded to nontransplant settings and modified to include age, performance status, and disease features.[8-10] While the HCT-CI remains the most widely used comorbidity score, it has important limitations in the current era. It was developed on a heterogeneous cohort of patients transplanted over 2 decades ago. The threshold for including comorbidities in the HCT-CI was not based on statistical significance, leading to the incorporation of less common comorbidities for which the association with mortality may be unstable. Finally, the HCT-CI prognostic capacity has been variably reproduced, with C statistic values ranging from approximately 0.55 to 0.65.[5-7,11-13]

Pretransplant assessment of mortality score

Similar to the HCT-CI, the Pretransplant Assessment of Mortality (PAM) score was developed in alloHCT patients (n=1401) transplanted at the FHCRC between 1990 and 2002, largely with myeloablative conditioning.[14] It includes measures of physiological reserve (age, serum creatinine level, serum alanine aminotransferase level, and pulmonary function metrics) as well as disease- and transplantation-related features selected based on their association with overall mortality in a multivariable Cox regression model. The score ranged from 9 to 44 and was broken into 4 categories (9-16, 17-23, 24-30, and 31-44), corresponding with increasing mortality risk across the development and validation cohorts. C statistic values ranged from 0.69 to 0.76 across validation cohorts.[13-15] Due to evolving alloHCT strategies with more frequent application of nonmyeloablative conditioning regimens, the PAM score was reevaluated and streamlined on a cohort transplanted between 2003 and 2009.[16] The revised PAM score includes only 5 elements, of which only 1 (FEV1) represents a comorbidity. It has been validated in multiple cohorts, with C indices ranging from 0.64 to 0.68.[13,17,18]

Simplified comorbidity index

The Simplified Comorbidity Index (SCI) was developed in a cohort of 573 patients who underwent CD34-selected alloHCT after myeloablative conditioning at the Memorial Sloan Kettering Cancer Center.[19] It incorporates 4 comorbidities and age. Comorbidities were included if their prevalence exceeded 5% and showed a statistically significant association with NRM in multivariable analysis. The SCI score components differ from their HCT-CI counterpart definitions in several ways. The HCT-CI definition of renal disease, a creatinine of greater than 2 mg/dL, was replaced with the estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[20] None of the patients in the development cohort met the renal dysfunction criteria according to HCT-CI, while 24% and 5% met the SCI criteria for mild (eGFR, 60-89.9 mL/min per 1.73 m^2) and moderate to severe (eGFR <60 mL/min per 1.73 m^2) renal comorbidities, respectively. SCI utilizes a composite definition of cardiac comorbidity, encompassing cardiac disease, arrhythmia, and valve disease, each as defined by HCT-CI. Moderate to severe hepatic comorbidity was included, while mild hepatic disease was not. A higher SCI score was strongly associated with increased NRM risk, as validated in an independent data set from another center utilizing lower-intensity conditioning regimens in unmodified transplants. The score was also validated in an independent cohort of patients undergoing alloHCT following reduced-intensity conditioning.[21] Notably, patients with an SCI score of 0 who had elevated HCT-CI scores did not have greater NRM risk, suggesting that the SCI effectively captures the most prognostically meaningful information.

Endothelial activation and stress index

Endothelial dysfunction significantly contributes to complications encountered in alloHCT, including graft-versus-host disease (GVHD), sinusoidal obstruction syndrome, and transplant-associated microangiopathy. Luft et al. developed the endothelial activation and stress index (EASIX) as a score for mortality prediction in patients with GVHD.[22] This index includes laboratory tests ([creatinine×LDH]/platelets) intended to serve as indicators of endothelial dysfunction and complement activation.[23] EASIX also demonstrated utility as a pre-alloHCT predictor of survival following transplantation.[13,23] However, discrimination was only reported by one group (C statistic of approximately 0.65 for NRM).[13] A notable advantage of EASIX is that it is composed of objective, readily available measurable markers. However, EASIX is a continuous metric with no consistent threshold for defining high-risk vs low-risk scores. Instead, studies have divided EASIX scores into quartiles, which are cohort dependent, and have demonstrated that patients in the highest-scoring quartiles experience excess mortality.

How to evaluate the performance of comorbidity scores

Extracting the most useful and actionable information from these prediction models in the clinical setting depends on a critical appraisal of their respective literature. Transparent reporting of the development and validation process allows us to evaluate each score's generalizability, risk of bias, and replication potential.[24] The most common 2 metrics for describing the performance of a prediction model are calibration and discrimination. Calibration describes how well the predicted probabilities or risk estimates align with the actual outcomes observed in a given population. In other words, it assesses whether the risk score accurately reflects the true probabilities of an event occurring. Calibration is rarely reported in HCT scores.[13] Discrimination describes the ability of a risk score to accurately distinguish between individuals who experience an event and those who do not. The most general and widely reported measure of discrimination is the concordance index (C index or C statistic),

which ranges from 0.5 to 1. Values below 0.6 and above 0.9 represent poor and excellent discrimination, respectively.[24,25] In HCT, most comorbidity-based indices have discrimination in the range of 0.6 to 0.7, underlining their limitations as clinical decision-making tools. Discrimination can be evaluated at different time landmarks after transplantation (ie, 100-day or 2-year overall survival), potentially revealing sets of predictors particularly relevant to shorter- or longer-term outcomes.[13] Critically, c-statistic can only be directly compared within the same cohort of patients, though HCT scores tend to fall in this range across numerous studies.[7]

We ascribe the generally suboptimal discrimination with comorbidity scores to several important factors. First, comorbidities are a single aspect among many influencing transplant outcomes, including other patient characteristics, disease profile, and transplantation factors (eg, donor type and conditioning). However, it is noteworthy that even incorporating these additional dimensions only marginally improves predictive accuracy.[11-13,26] Second, the subjective nature of defining a patient's comorbidities and the potential for interobserver variation may lead to less accurate risk prediction even when these indices are used correctly. For this reason, we believe that future scores based on objective biomarkers of organ function and physiological reserve may have improved performance and reproducibility. Third, the effects of individual comorbidities within an index might interact with the selected transplantation approach, such as conditioning or GvHD prophylaxis regimens.[3,27] This potential interaction points to the need for indices validated within, or perhaps even designed for, specific transplant contexts rather than relying on universal scores. Lastly, transplantation may be inherently unpredictable over a long timescale, with random and unanticipatable events limiting precise prediction. Dynamic models that adjust predictions over time based on incorporating posttransplantation events such as GVHD and infections may mitigate this predictive uncertainty.

From composite scores to individual comorbidities

We have described the need for a single and comprehensive quantity to represent a patient's fitness for transplantation. The cumulative burden of comorbidities allows for risk-stratification across cohorts. However, the consideration of comorbidities in aggregate risks losing vital prognostic information which should bear upon clinical decision-making. In a recent study, we identified that in a low-conditioning-intensity comorbidity setting (ie, one enriched for patients preselected due to comorbidities and other adverse features) several but not all of the individual comorbidities included in the HCT-CI still contributed to an elevated hazard of NRM.[3] Cardiac and psychiatric disease contributed meaningfully to further increased risk. In contrast, moderate pulmonary disease had a hazard ratio of approximately 1.0 despite a large sample size (n=275/1663, 17%). This suggests that moderately diminished FEV1 or DLCo does not further increase risk beyond the baseline NRM anticipated in this low-conditioning-intensity setting. In another study from the European Society for Blood and Marrow Transplantation (EBMT) encompassing over 38 000 transplantations and including reduced-intensity and myeloablative approaches, the only comorbidities significantly associated with NRM were pulmonary, obesity, cardiac, infection, diabetes, and renal.[4] With the exception of renal disease (defined in the HCT-CI primarily by a serum creatinine >2.0g/dL, which applies to few HCT recipients), hazard ratios for these comorbidities ranged from 1.13 to 1.24, suggesting that the specific risk of any individual comorbidity remains modest. Results of selected publications describing the risk associated with common individual comorbidities are described in Table 2.

Table 2. Selected studies of outcomes associated with individual comorbidities

Article	Brief summary
Pulmonary disease	
Parimon 2005[29]	2852 patients 1990–2001; decreasing FEV1 or DLCo, greater A-a gradient associated with early respiratory failure and overall mortality
Parimon 2006[14]	**PAM score**; FEV1<80% and DLCo <70% associated with increased risk of overall mortality
Tran 2011[30]	845 adult patients 2005–2009; former or current smoking not associated with higher risk of NRM
Shibasaki 2016[31]	101 adult patients 2005–2014; Dinakara equation preferred for adjusting DLCo for hemoglobin when compared to Cotes equation
Fein 2018[27]	875 adult patients 2006–2015; "severe" but not "moderate" pulm disease associated with NRM; significantly worse outcomes in severe pulm disease if conditioned with Flu/Mel
Yang 2022[32]	923 myeloablative recipients 2015–2018; decreased FEV1/FVC ratio associated with chronic pulmonary GVHD
Schierbeck 2023[33]	663 adult patients 2012–2019; severe pulmonary disease associated with NRM
Penack 2022[4]	38 760 patients treated 2010–2016; pulm disease associated with increased NRM risk
Fein 2023[3]	1663 adults receiving low-conditioning intensity 2008–2018; no significant association between moderate disease and NRM
Hermans 2023[12]	1861 adult patients receiving PTCy 2010–2018; no association between pulm comorbidity and NRM
Alhomoud 2023[34]	511 adult patients treated 2013–2020; abnormal chest computed tomograpy findings before transplantation associated with increased NRM and pulm morbidity
Hepatic disease	
Parimon 2006[14]	**PAM score;** ALT >49 but not total bilirubin >1.3 associated with increased risk of overall mortality
Barba 2011[35]	455 adult patients 1998–2008; elevated bilirubin and GGT associated with 100-day NRM; elevated ALT associated with increased chronic GVHD

Table 2. Selected studies of outcomes associated with individual comorbidities (*Continued*)

Article	Brief summary
Fein 2018[27]	875 adult patients 2006–2015; "severe" but not "mild" disease associated with worse NRM, especially in patients conditioned with Flu/Treo
Fein 2023[3]	1663 adults receiving low-conditioning intensity 2008–2018; neither mild nor moderate/severe hepatic disease significantly associated with increased NRM
Hermans 2023[12]	1861 adult patients receiving PTCy 2010–2018; moderate/severe hepatic disease associated with increased NRM
Psychiatric disease	
El-Jawahri 2017[36]	7433 CIBMTR patients 2008–2012; psychiatric comorbidity associated with overall mortality and severe acute GVHD
Solh 2020[37]	556 adult patients 2003–2017; increased Transplant Evaluation Rating Scale associated with increased NRM and overall mortality
Fein 2023[3]	1663 adults receiving low-conditioning intensity 2008–2018; psychiatric disease associated with increased NRM
Prior solid malignancy	
Fein 2018[27]	875 adult patients 2006–2015; prior solid malignancy not associated with increased NRM
Portuguese 2023[38]	1193 adult patients 2010–2018; prior solid malignancy not associated with NRM or overall survival
Fein 2023[3]	1663 adults receiving low-conditioning intensity 2008–2018; prior solid malignancy not associated with increased NRM
Active infection	
Campbell 2015[39]	458 patients with respiratory nasal wash collected pretransplant 2005–2010; ≥ 1 respiratory virus in 116 (23%) of adult patients; among asymptomatic patients, no difference in mortality but increased duration of hospitalization
Versluys 2018[40]	179 pediatric patients who underwent routine BAL 2004–2013; asymptomatic URTI did not progress to lower respiratory tract infections; positive BAL associated with allo-mediated lung syndromes (see also Zinter 2021)[47]
Ottaviano 2020[41]	586 pediatric patients 2007–2016; 12% with positive nasal swab; 53 transplanted with active URTI. Increased NRM among patients with URTI who did not delay transplant, though these were potentially higher-risk patients for other reasons
Kim 2022[42]	946 adult mostly myeloablative transplants 2010–2016; significantly lower day-100 survival with pretransplant lower respiratory disease including rhinovirus; URTI not significantly associated with NRM
Cardiac disease	
Qazilbash 2009[43]	217 adult patients (25% with LVEF <45%) 2000–2006; no association between decreased LVEF and OS
Stillwell 2011[44]	1178 adult patients 1999–2009; no association between pretransplantation coronary artery disease and mortality, ICU admission, length of stay, or posttransplant cardiac events
Fein 2018[27]	875 adult patients 2006–2015; cardiac disease, arrhythmia, and valve disease associated with increased NRM; elevated risk seen in cardiac disease with Flu/Bu4 but not Flu/Bu2 or Flu/Treo conditioning
Penack 2022[4]	38 760 patients treated 2010–2016; cardiac disease and arrhythmia significantly associated with increased NRM
Fein 2023[3]	1663 adults receiving low-conditioning intensity 2008–2018; combined cardiac disease (including arrhythmia and valve disease) associated with elevated NRM risk
Hermans 2023[12]	1861 adult patients receiving PTCy 2010–2018; cardiac disease associated with significantly increased NRM
Renal disease	
Shouval 2018[45]	1217 adult patients treated 2003–2015; eGFR <60 mL/min/1.73 m^2 associated with increased NRM
Fein 2018[27]	875 adult patients 2006–2015; eGFR <60 mL/min/1.73 m^2 associated with increased NRM
Farhadfar 2021[46]	13 305 adult patients ≥40 years 2008–2016 (in two groups); decreased eGFR associated with increased NRM
Shouval 2022[19]	**SCI Score;** 573 adult patients undergoing CD34+ selected transplant 2008–2018; eGFR <60 and 60–89.9 associated with increased NRM

BAL, bronchoalveolar lavage; Bu2, 2 days of Busulfan; Bu4, 4 days of Busulfan; Flu/Mel, Fludarabine/Melphalan; FVC, forced vital capacity; GGT, gamma-glutamyl transferase; Treo, Treosulfan; URTI, upper respiratory tract infection.

One compelling approach is to consider whether the impact of individual comorbidities is mediated by conditioning regimen–specific toxicities. Such findings might guide the personalization of conditioning chemotherapy for each patient. This possibility was considered by Fein et al. in a single-center study of 875 patients, where the NRM risk of prevalent comorbidities was studied within conditioning-regimen cohorts.[27] The authors noted a striking increase in NRM for patients with cardiac comorbidity conditioned with fludarabine and 4 days of busulfan; in contrast, no statistically significant difference was observed among patients who received fludarabine and treosulfan. Overall, weighing individual comorbidities when considering a transplantation approach may contribute to treatment personalization, whereas aggregating comorbidities in a score potentially leads to prognostic information loss.

CLINICAL CASE (continued)

We calculated several comorbidity indices for our patient. With the HCT-CI, he has a total score of 6 (3 points for prior malignancy and 1 each for obesity, diabetes mellitus, and mild hepatic

disease). In comparison, by the SCI score he has a total of 2 points for reduced eGFR only. His revised PAM (rPAM) score is 13.75, in the second quartile of scores in 1 large external validation,[13] and his EASIX score is 2.12, in the third quartile of scores.[13] These indices stratify his risk range from the lower moderate (SCI, rPAM) to higher (EASIX) to very high (HCT-CI) given the stark differences in what factors they include and how they are weighted. Multiple discussions with the patient and his family, informed by NRM-predictive comorbidity scores and accounting for his individual comorbidities alongside the consideration of the relapse potential of MRD-negative intermediate-risk AML, lead to a shared decision to proceed with transplantation. Based on the results of the BMT CTN 0901 trial along with what is felt to be an intermediate comorbidity burden, conditioning with fludarabine and melphalan (140 mg/m^2) is selected.[28] GvHD prophylaxis with post-transplant cyclophosphamide is chosen, drawing on the results of the BMT CTN 1703, with consideration to the possible benefit of an earlier withdrawal of calcineurin inhibitors given his chronic renal disease. He experienced stage I gut and skin GVHD, which improved with steroids and topical therapies, and is alive and relapse-free at 14 months posttransplantation.

Conclusions

NRM remains a tenacious challenge in alloHCT, and the development of instruments that can help anticipate its occurrence is crucial. Physician intuition plays a vital role in evaluating patient suitability and eligibility for transplantation. Objective, reproducible measures such as comorbidity scores help to guard against anecdotal bias when estimating the likelihood of transplantation success. Nevertheless, caution is needed in using these scores for clinical decision-making—as we have shown, they do not fully account for variability in transplantation outcomes. Comorbidity scores account for one among many axes, together with other patient-, disease-, and transplant-related features, along which the pretransplantation evaluation is conducted and a transplantation approach selected. The extreme ends of these scores may often predict good or poor outcomes, and thus the scores are most valid when used for risk-stratification rather than individualized prediction. Decision-making should also be informed by considering the patient's individual comorbidities and how they interact with the chosen transplantation platform. In conclusion, a comprehensive approach that integrates multiple factors, including comorbidity scores and individual comorbidities, is necessary to enhance risk assessment and improve patient care in alloHCT.

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Conflict-of-interest disclosure

Roni Shouval: no competing financial interests to declare.
Joshua A. Fein: no competing financial interests to declare.

Off-label drug use

Roni Shouval: nothing to disclose.
Joshua A. Fein: nothing to disclose.

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Approaches to optimize outcomes in transplant recipients

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Curative therapy with an allogeneic hematopoietic cell transplant (HCT) can now be offered to a wider patient population due to improvements in donor selection, transplant conditioning regimens, and supportive care measures. However, risk of transplant-related morbidity and mortality remains, and thus appropriate transplant candidate workup pre-HCT for risk stratification and a management plan after HCT is crucial for success of the procedure. These include understanding and identifying risk of underlying malignant disease relapse, graft-versus-host disease, and infectious complications a patient may be predisposed toward, irrespective of allogeneic donor type. Progress in these domains with new therapeutic paradigms allows for development of a treatment plan prior to HCT to mitigate these potential risks tailored to the patient's case. Herein, we present case studies to focus on factors that influence decision-making in HCT and the approaches and strategies used to optimize post-HCT outcomes based on the individual HCT recipient's clinical scenario to improve on these high-risk scenarios.

LEARNING OBJECTIVES

- Identify the most common causes of allogeneic hematopoietic cell transplant treatment failure
- Illustrate treatment options available to mitigate risk based on individual hematopoietic cell transplant recipient's risk
- Define novel strategies used to improve outcomes of high morbidity and mortality in hematopoietic cell transplant

Introduction

Transplantation of healthy hematopoietic stem cells from an allogeneic donor (HCT) is a treatment option to improve outcomes for otherwise incurable hematologic diseases. With the development of safer transplant conditioning regimens, supportive care measures, and enhanced donor selection methodologies, HCT can now be offered to a wider population. This is reflected by the rising number of annual HCT procedures (over 9000 performed domestically).[1,2] As HCT poses risks (relapse, organ failure, treatment toxicity, graft-versus-host disease [GVHD]) (Figure 1),[2] assessment entails a detailed patient evaluation to determine the most appropriate HCT treatment algorithm.[3]

CLINICAL CASE 1

A 32-year-old woman with FMS-like tyrosine kinase 3–internal tandem duplication (FLT3-ITD) mutant acute myeloid leukemia (AML) is being considered for HCT. She underwent induction chemotherapy along with FLT3-ITD inhibitor followed by consolidative chemotherapy. She continues to have measurable residual disease via FLT3-ITD polymerase chain reaction–next-generation sequencing with a sensitivity of ~1×10^{-6}. Her sister was identified as a fully matched sibling donor, and she proceeds to HCT using myeloablative conditioning (MAC). Day +30 bone marrow biopsy specimen confirms she is in morphologic remission and FLT3 polymerase chain reaction–next-generation sequencing is negative. She begins maintenance with gilteritinib, continues this for 2 years, and remains in remission post-HCT.

Drug-based maintenance therapies

Disease relapse remains the leading cause of HCT failure,[4,5] irrespective of donor type (Figure 1). Maintenance therapy may decrease the risk of relapse post-HCT, but broad application to all patients risks overtreatment.[6] Thus, questions remain regarding identification of ideal patient subsets, minimal residual disease testing, duration of treatment, and drug intervention. Other considerations include (1) the

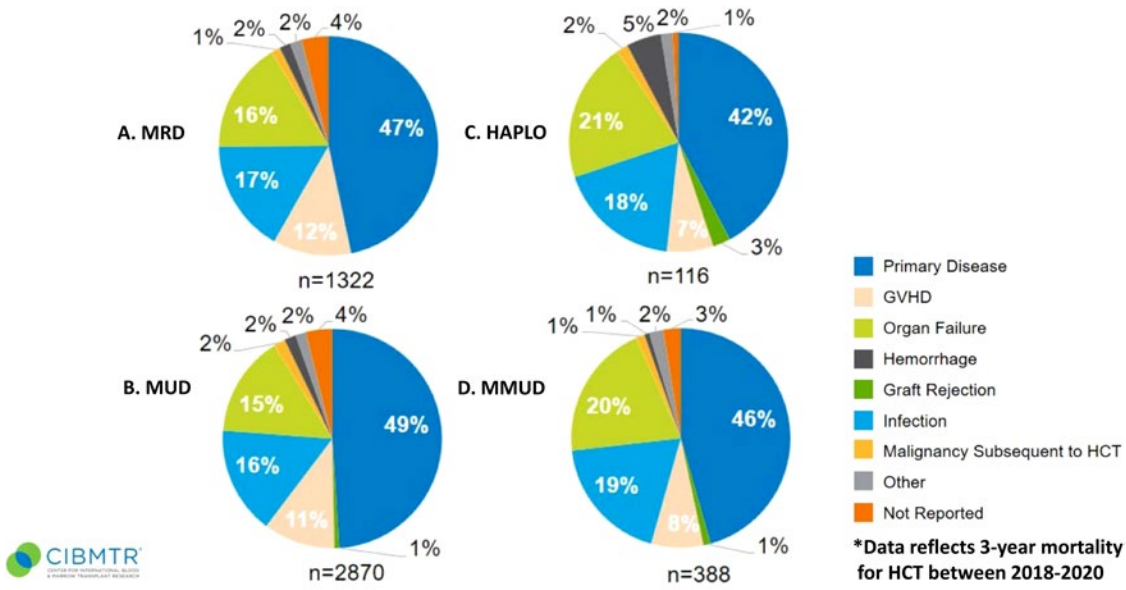

Figure 1. Center for International Blood and Marrow Transplant Research–reported causes of death after HCT for adults in the United States by donor type at or beyond day 100. HAPLO, haploidentical; MMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor.

underlying disease risk itself, (2) conditioning intensity, (3) remission status, (4) patient post-HCT recovery, and (5) toxicity risk of the maintenance therapy.[6] Promising advances have been achieved with commercially available pharmacologic agents, with the majority in targeted maintenance therapy for AML and myelodysplastic syndrome (MDS) (Figure 2).

Maintenance post-HCT for AML/MDS

FLT3-ITD is common (~25% of AML), and incorporation of FLT3 inhibitors to upfront induction chemotherapy and routine use of HCT as consolidative therapy have improved outcomes.[7] Despite these advances, risk of relapse post-HCT ranges from ~30% to 40%. Several prospective randomized trials evaluated efficacy of tyrosine kinase inhibitor maintenance with varying degrees of benefit in different patient populations. The SORMAIN (SORafenib Maintenance) trial enrolled 83 patients and closed early due to poor accrual after 5 years but showed a 2-year relapse-free survival (RFS) benefit with maintenance therapy given up to 2 years after HCT vs placebo (85.0% vs 53.3%, $P = .002$).[8] In a larger phase 3 randomized placebo controlled trial (n = 202), Xuan et al[9] demonstrated a similar benefit favoring a 6-month sorafenib maintenance regimen initiated 30 to 60 days after HCT with a 2-year leukemia-free survival to be 78.9% vs 56.6% over placebo ($P<.0001$). The RADIUS study, evaluating midostaurin, did not show an RFS benefit in the maintenance setting, although the study was underpowered to determine this.[10] More recently, the highly anticipated results of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 1506/MORPHO trial (NCT02997202) were presented. This study was a phase 3, randomized, double-blind, placebo-controlled multicenter, multinational, trial, randomizing 356 patients with FLT3-ITD AML to receive either gilteritinib 120 mg or placebo for a total of 2 years. While the study did not achieve its primary end point of RFS (hazard ratio [HR], 0.679; 95% CI, 0.46-1.005; 2-sided $P = .052$), the study did demonstrate the benefit of gilteritinib maintenance in patients with FLT3-ITD minimal residual disease pre- and post-HCT (HR, 0.2; 95% CI, 0.32-0.8; $P = .0065$) compared to placebo. Thus, maintenance therapy may be most beneficial for those with minimal residual disease.[11]

Data for other targetable mutations for myeloid neoplasms are in earlier stages of development. This includes mutations of IDH1 and IDH2. Several early phase studies have evaluated orally available small-molecule inhibitors, ivosidenib and enasidenib, IDH1 and IDH2 inhibitors, respectively, post-HCT. Fathi and colleagues[12] reported a promising tolerance profile with maintenance enasidenib in a phase 1 setting with 63% of patients (n = 12) completing all 12 planned cycles of treatment with a 2-year relapse rate of 16% (95% CI, 3.7%-36%) for the cohort at a median follow-up for surviving patients of 25 months. Similarly, post-HCT maintenance ivosidenib was well tolerated when evaluated in the phase 1 setting (n = 18), with only 1 patient discontinuing maintenance due to an adverse event. Additionally, the study had a promising 2-year incidence of relapse, nonrelapse mortality (NRM), and overall survival (OS) of 19%, 0%, and 88%, respectively.[13] Ongoing clinical trials will further delineate efficacy of maintenance with these agents.

As many high-risk mutations may not be targetable by current pharmacologic agents,[14] other more broadly acting agents are of interest. Hypomethylating agents may be an attractive option given their tolerance and potential for increasing the graft-leukemia effect through increased tumor antigen expression and expanding immunomodulatory T regulatory cells.[15] Existing data are conflicting and represent a mixture of patient populations and dosing schedules. Early-phase trials demonstrated promise with both 5-azacitidine (AZA) and decitabine.[16-18] Oran and colleagues[19] conducted a phase 3 randomized study of AZA

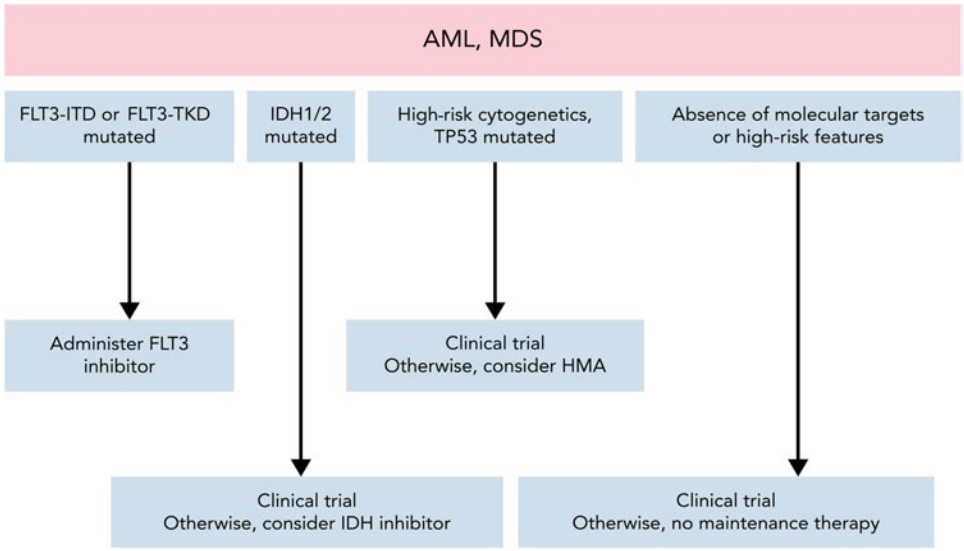

Figure 2. Approach to maintenance therapy for AML and MDS after HCT. Adapted from DeFilipp and Chen.[6]

maintenance (32 mg/m^2/d intravenously×5 days every 28 days for 12 cycles; median number delivered, 4) vs observation in 187 patients after HCT in high-risk AML and MDS but found that RFS and OS were not different in the 2 groups. Challenges in delivering this intravenous therapy post-HCT may be addressed by the oral formulation AZA, CC-486, which is currently being studied in a phase 3, randomized, placebo-controlled trial (Table 1; NCT04173533). Last, to potentiate the efficacy of hypomethylating agents, combination therapy with the BCL-2 inhibitor and BH3 mimetic, venetoclax (VEN), is being explored in high-risk myeloid diseases. Wei and colleagues evaluated the combination of decitabine/VEN prospectively in patients with high-risk AML (n = 17) and MDS (n = 3) based on prespecified prognostic criteria in phase 1 followed by a phase 2 study showing 2-year OS and event-free survival of 85% for both in this high-risk population.[20] Additionally, phase 4 studies with AZA/VEN are also under way (NCT04161885).

Conclusion 1: Maintenance strategies are a promising approach for relapse prevention. Further randomized clinical trials are needed to characterize benefit and delineate the patient population best suited for such treatments with several ongoing clinical trials (Table 1).

CLINICAL CASE 2

A 68-year-old African American man was diagnosed with high-risk MDS. An unrelated registry donor search revealed no human leukocyte antigen (HLA)–matched donors. His son was identified as a haploidentical (haplo) donor, and he subsequently proceeded to HCT using reduced intensity conditioning (RIC) and received peripheral blood mobilized stem cells (PBSCs). GVHD prophylaxis includes posttransplant cyclophosphamide (PTCY) (days +3 and +4) along with tacrolimus (TAC) and mycophenolate mofetil (MMF). MMF was stopped on day +35. He had mild grade 1 skin acute GHVD that was controlled with topical steroids. TAC was ultimately able to be discontinued by day +180. At 12 months post-HCT, he had no evidence of MDS, with 100% donor chimerism, and no evidence of GVHD.

Approaches to GVHD prevention in HCT

One of the challenges of HCT is the interplay of disease relapse prevention and controlling GVHD. The usage of calcineurin inhibitors, such as TAC in combination with other GVHD prophylaxis therapies, including methotrexate (MTX), has been an established standard. Advances in GVHD prophylaxis with PTCY have led to its subsequent widespread adoption based on initial studies in the haplo setting.[21-24] The monumental success of the PTCY platform to prevent GVHD in HLA disparate haplo transplants with promising survival outcomes changed the landscape for the transplant community at large. This approach paved the path for utilization of PTCY for mismatched unrelated donors and now more recently to well-matched donors, which will be discussed in this section (Table 2).

PTCY as a preventative measure for GVHD in matched related, matched unrelated, and mismatched unrelated donor HCT

The BMT CTN previously compared the efficacy of 3 GVHD prophylaxis regimens comparing TAC/MMF/PTCY (n = 92) or TAC/MTX/bortezomib (n = 89) or TAC/MTX/maraviroc to contemporaneous controls of TAC/MTX (n = 224 controls) in a phase 2 randomized study to determine the most promising approaching in the RIC setting using matched related and matched unrelated donors (BMT CTN 1203). The study demonstrated a benefit of using PTCY/TAC/MMF compared to TAC/MTX.[25] Thus, the BMT CTN subsequently launched the 1703 study, a phase 3 study of standard TAC/MTX (n = 217) vs PTCY/TAC/MMF (n = 214) for RIC HCT using matched related or 7-8/8 matched unrelated PBSC donors. GVHD/relapse or progression-free survival (GRFS) at 1 year was improved in the PTCY group (HR, 0.641; 95% CI,

Table 1. Active trials for drug maintenance therapy after HCT for AML or MDS

ClinicalTrials.gov identifier	Phase	Status	Drug/schedule	N	Age	Primary outcome
Post-HCT FLT3-ITD maintenance						
NCT02997202, CTN 1506	3	Active, not recruiting	Gilteritinib 120 mg daily vs placebo for 2 years	356	>18	96-month RFS
NCT03690115, PONALLO trial	2	Active, not recruiting	Ponatinib 30 mg daily for 1 year	77	18–70	24-month RFS
Post-HCT IDH 1/2 maintenance						
NCT03728335	1	Active, not recruiting	Enasidenib (dose unspecified) daily Q28 days for 24 cycles	15	>18	Incidence of AEs
NCT03564821	1	Active, not recruiting	Ivosidenib 500 mg daily dose escalation study	18	18–75	MTD
NCT04522895	2	Active, not recruiting	Enasidenib 100 mg daily Q28 days for 12 cycles	50	>18	Incidence of AEs
Post-HCT HMA-based maintenance						
NCT01995578	2	Active, not recruiting	Azacitadine 32 mg/m^2 Q5 days every 28 days starting days +60–120 post-HCT up to 1 year post-HCT for ~8–10 cycles	32	1–75	24-month relapse rate
NCT04173533	3	Recruiting	Oral azacitidine (CC-486) 14 days for each 28-day cycle for 12 cycles	324	≥16	12-month RFS
NCT03613532	1	Recruiting	Azacitidine 5 doses with venetoclax 14 doses for 8–12 cycles or oral decitabine/cedazuridine 3 doses with venetoclax 14 doses for 8 cycles	68	≥18	MTD
NCT04128501	2	Recruiting	Azacitidine days 1–5 + venetoclax days 1–7 Q4–8 weeks for up to 12 cycles. Dosing unspecified	125	18–75	RFS
NCT04161885, VIALE-T	3	Recruiting	Azacitidine days 1–5 + venetoclax days 1–28 Q28 for 6 cycles. Dosing unspecified	424	>18	MTD and RFS
NCT03843528	1	Recruiting	Azacitidine days 1–5 Q28 days. Vorinostat concurrently on days 1–7 and 15–21 of 28-day cycles up to 1 year	15	1–21	MTD
NCT04980404	1	Recruiting	Decitabine/cedazuridine on days 1–3 Q42 days	22	≥18	DLT
Additional disease-based maintenance approaches						
NCT03267186	2	Active, not recruiting	Ibrutinib PO daily up to 18 months post-HCT	8	18–70	18-month relapse rate
NCT03932643	1	Recruiting	Onc201 at various dose levels weekly for up to 52 weeks	20	≥19	MTD
NCT04168502	3	Recruiting	Glasdegib 100 mg daily for 1 year vs clinical observation	414	18–60	DFS
NCT03286530	2	Recruiting	Ruxolitinib orally twice daily Q28 days for up to 24 cycles	64	60–80	12-month GRFS rate

Accessed on April 23, 2023, using search terms "maintenance post allogeneic transplant," "maintenance post allo transplant," "maintenance post allo aml or mds" and "relapse prevention after allo."
AE, adverse events; DLT, dose limiting toxicity; DFS, disease free survival; MTD, maximum tolerated dose; Q_, every.

Table 2. Pivotal studies using PTCY for GVHD prophylaxis

Study	Population	Design	Comparison	Summary findings
Haploidentical				
Luznik et al. (2008)[20]	0.5–70 years old receiving NMA BM HCT with haplo donors for hematologic disorders (n = 68)	Single-arm prospective multicenter	Single Arm PTCY/MMF/TAC	The CI of grades II-IV and III-IV acute GVHD by day +200 was 34% and 6%, respectively. Graft failure rate was 13%. CI of NRM and relapse at 1 year post-HCT was 15% and 51%, respectively.
Ciurea et al. (2015)[21]	Adults with de novo or secondary AML who received their first HCT	Retrospective registry study	Haplo BM graft with PTCY prophylaxis (n = 192) vs PBSC mobilized or BMT graft 8/8 HLA-matched MUD receiving calcineurin inhibitor GVHD backbone (n = 1982)	CI of acute grades II-IV (16% vs 33%, *P*<.0001) and 3-year chronic GVHD (30% vs 53%, *P*<.0001) was lower after haplo vs MUD in patients who received MAC. Similarly, CI of acute grades II-IV GVHD was 19% vs 28% (*P* = .05) and 34% vs 52% (*P* = .002) for chronic GVHD for RIC. OS was similar for haplo and MUD.
Fuchs et al. (2021)[22]	Adults aged 18–70 (n = 368) with lymphoma or acute leukemia receiving RIC with Flu/Cy/TBI	Two parallel phase 2 trials	Umbilical cord blood (n = 186) with GVHD prophylaxis with cyclosporine and MMF vs haplo (n = 182) with GVHD prophylaxis with PTCY/TAC/MMF	Two-year PFS was 35% (95% CI, 28%-42%) vs 41% (95% CI, 34%-48%) after UCB compared to haplo, respectively (*P* = .41). Two-year NRM was significantly higher for UCB at 18% (95% CI, 13%-24%) vs 11% (95% CI, 6%-16%) or haplo, *P* = .04. Two-year OS after UCB was lower at 46% (95% CI, 38%-53%) vs 57% (95% CI, 49%- 64%) for haplo, respectively (*P* = .04).
Related/unrelated				
Bolaños-Meade et al. (2019)[2]	18–75 years old RIC HCT with related and MUD donors	Randomized phase 2	TAC/MMF/PTCY (n = 92) or TAC/MTX/bortezomib (n = 89) or TAC/MTX/ maraviroc (n = 92) vs TAC/MTX (n = 224 controls)	PTCY/TAC/MMF had the best GRFS benefit with an HR of 0.72 (90% CI, 0.54–0.94, *P* = .044) compared to TAC/MTX. Results from this study prompted launch of phase 3 BMT CTN 1703 comparison of PTCY with MTX and calcineurin inhibitor for GVHD prophylaxis.
Luznik et al. (2022)[26]	≤65 receiving 8/8 HLA-matched MAC HCT for AML in CR or MDS with <5% blasts	Randomized phase 3	(1) Ex vivo CD34 selected T-cell–depleted PBSC graft (n = 114), (2) unmanipulated BM graft followed by single-agent Cy (n = 114), and (3) TAC/MTX (n=118)	Intent-to-treat 2-year CRFS was 50.6% for CD34 selection (HR in comparison to TAC/MTX, 0.80; 95% CI, 0.56–1.15; *P* = .24), 48.1% for PTCY (HR, 0.86; 0.61–1.23; *P* = .41), and 41.0% for control. Calcineurin-free regimens did not translate to improved survival.
Bolaños-Meade et al. (2022)[25]	≥18 with hematologic malignancies receiving RIC using a 6/6 MRD, (n = 128), 8/8 MUD (n = 288), or 7/8 single mismatched (n = 15) PBSC donor	Randomized phase 3	TAC/MMF/PTCY (n = 214) vs TAC/MTX (n = 217)	The PTCY group had significantly lower hazard GRFS than TAC/MTX arm (HR, 0.641; 95% CI, 0.492–0.835; *P*=.001). Day 100 grade III-IV acute GVHD was 6.3% vs 14.7% (*P* = .001), and chronic GVHD rate at 1 year was 21.9% vs 35.1% (*P* = .005) for PTCY vs TAC/MTX, respectively. No difference in risk of relapse 1-year post-HCT (20.8% vs 20.2%, *P*=.9) was noted.
MMUD				
Malki et al. (2021)[29]	Adults ≤75 years old receiving PBSC MMUD HCT using RIC or MAC with MMUD HLA matched ≥6/8 (N = 38)	Single-arm prospective	None. Single-arm PTCY/MMF/TAC	One-year OS and GRFS were 87% (95% CI, 71%-94%) and 68% (95% CI, 51%-81%), respectively. CI of NRM at 100 days and 1 year was 0% and 11% (95% CI, 4%-27%), respectively. CI 100-day acute GVHD grades II-IV and III-IV and 1-year chronic GVHD were 50% (95% CI, 36%-69%), 18% (95% CI, 9%-36%), and 48% (95% CI, 34%-68%), respectively.
Shaw et al. (2021)[30]	15–71 with hematologic malignancies using a BM graft with either MAC or RIC, mismatched in at least at least 1 allele (4–6/8) (N = 80)	Prospective phase 2	PTCY/MMF/sirolimus vs CIBMTR contemporary controls with PTCY	Nearly 50% enrollment of ethnic minorities. The 1-year OS of 76% (90% CI, 67.3%-83.3%) was for all patients in the cohort and 72% and 79% for MAC and RIC, respectively. In regard to GVHD, patients receiving MAC had grade III-IV acute and chronic GVHD of 18% and 36% at 1 year, respectively. For RIC, no grade III-IV acute GVHD was noted with 18% chronic GVHD at 1 year.

BM, bone marrow; CI, cumulative incidence; CIBMTR, Center for International Blood and Marrow Transplant Research; CR, complete remission; CRFS, chronic relapse-free survival; Cy, cyclophosphamide; Flu, fludarabine; MMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; NMA, nonmyeloablative; PFS, progression-free survival; TBI, total body irradiation; UCB, umbilical cord blood.

0.492-0.835; P = .001). The adjusted 1-year GRFS rate was 52.7% (95% CI, 45.8%-59.2%) for PTCY vs 34.9% (95% CI, 28.6%-41.3%) for TAC/MTX. The day 100 grade III to IV acute GVHD was 6.3% vs 14.7% (P = .001), and chronic GVHD rate at 1 year was 21.9% vs 35.1% (P = .005) for PTCY vs TAC/MTX, respectively. This landmark study supports a new standard of care for GVHD prophylaxis for matched donors receiving RIC.[26]

In the MAC setting, the BMT 1301 evaluated (1) ex vivo CD34$^+$ selected T-cell depleted PBSC graft (n = 114), (2) unmanipulated bone marrow graft with single-agent cyclophosphamide 50 mg/kg on days +3 and +4 post-HCT (n = 114), or (3) unmanipulated bone marrow graft with TAC/MTX (n = 118). The intent-to-treat 2-year chronic GVHD (moderate or severe) and chronic relapse-free survival was 50.6% for CD34 selection (HR in comparison to TAC/MTX, 0.80; 95% CI, 0.56-1.15; P = .24), 48.1% for PTCY (HR, 0.86; 95% CI, 0.61-1.23; P = .41), and 41.0% for control. One of the most important findings from the study was that the 2-year cumulative incidence of NRM was surprisingly low at 7.9%, and 2-year OS for the TAX/MTX cohort was 76.1%. Despite the highest rate of chronic GVHD in this arm (33.7% at 2 years), the low NRM and relapse rates made it comparable to the other approaches. For example, the CD34$^+$ selected arm had the lowest 2-year cumulative incidence of chronic GVHD at 8.9%, with the highest NRM at 2 years at 21.5%. Thus, study was not able to demonstrate that calcineurin-free prophylaxis as administered to that study population resulted in improved chronic relapse-free survival when compared with TAC/MTX. However, additional GVHD preventive therapy is likely helpful in conjunction with PTCY in preventing GVHD.[27] Further studies to elucidate the efficacy of PTCY in patients receiving MAC with PBSC grafts are needed to address this knowledge gap. Combination of PTCY and novel agents such as abatacept and JAK-inhibition is additionally being explored to build on the efficacy of PTCY to improve GRFS.[28,29]

These successes have led investigators to evaluate PTCY in more HLA-disparate donors, including mismatched unrelated donors.[30,31] In a phase 2 study done by the National Donor Marrow Program, using at least a single HLA allele mismatched donor (4-6/8) with PTCY/MMF and sirolimus for GVHD prophylaxis was evaluated in patients receiving either MAC or RIC for conditioning chemotherapy. All patients (n = 80) received fresh bone marrow grafts on day 0, PTCY on days +3 and +4 after HCT, and sirolimus with MMF starting on day +5. The 1-year OS was 76% (90% CI, 67.3%-83.3%) for all patients in the cohort and 72% and 79% for MAC and RIC, respectively. In regard to GVHD, patients receiving MAC had grade III to IV acute and chronic GVHD of 18% and 36% at 1 year, respectively. For RIC, no grade III to IV acute GVHD was noted with 18% chronic GVHD at 1 year. Notably, this study had 48% enrollment of racial or ethnic minorities.[31] The ACCESS study (NCT04904588) is further expanding on this experience, evaluating the efficacy of mismatched unrelated donor PBSCs using MAC and RIC in adult patients and evaluating a pediatric stratum. This highly anticipated study addresses an unmet patient need to further mitigate risk of NRM and GVHD with expected accrual to be complete in 2023.[32]

Toxicity considerations with PTCY

Given that cyclophosphamide is a nitrogen mustard alkylating agent, it is historically associated with hemorrhagic cystitis, renal insufficiency, and notably cardiac toxicity, including cardiomyopathy, arrythmias, and pericarditis. With the expansion of PTCY usage, there have been some reports of early cardiac events after HCT, with the incidence of cardiac toxicity ranging from 7% to 19% in patients having received PTCY.[33-35] Pre-HCT workup includes detailed cardiac history and function evaluation. Close monitoring of cardiac function should be considered in patients chosen to receive these medications with noted pre-HCT cardiac abnormalities. Studies to determine the lowest effective dose of PTCY that facilitates GVHD reduction but also minimizes toxicity are currently under way (NCT05436418). Immune dysregulations considerations resulting in infectious complications will be discussed later in this article.

Conclusion 2: Advances in GVHD prophylaxis with PTCY have improved GVHD outcomes, with several prospective studies showing promising outcomes. Given improvements in GVHD outcomes and survival, this broadens the curative potential of HCT to a wider patient population. Ongoing prospective studies are under way and give hope to the continued possibility to improve GVHD outcomes with novel combination therapies.

CLINICAL CASE 3

A 42-year-old woman presents for myeloablative conditioning followed by HCT using her haploidentical sibling donor (sister) for high-risk monosomal karyotype AML. Planned GVHD prophylaxis includes PTCY to be administered on days +3 and +4. Both recipient and donor are cytomegalovirus (CMV) seropositive. Along with standard prophylactic antimicrobials, she will begin letemovir daily beginning between day 0 and day +28 post-HCT and continue through day +100 for CMV prophylaxis.

Novels approaches to infection prevention

Detailed pretransplant evaluation includes planning for specific tailored antimicrobial prophylaxis post-HCT. Nonetheless, infections with CMV have remained clinically significant.[36] Reactivation is influenced by both recipient and donor CMV serostatus prior to HCT, with recipients who are CMV seropositive being at the greatest risk for reactivation and occurrence of CMV disease after HCT. Notably, CMV infection risk is highest in PTCY recipients irrespective of donor type. In a Center for International Blood and Marrow Transplant Research analysis, CMV infection was highest for CMV-seropositive HCT recipients irrespective of donor type. When further evaluating risk of CMV infection in seropositive recipients by GVHD prophylaxis type, matched related siblings (n = 1065) who received calcineurin inhibitors had the lower risk (HR, 24.4), followed by matched related siblings (n = 279) who received PTCY having a higher risk (HR, 47.7) and haploidentical recipients (n = 545) who received PTCY having the highest risk (HR, 50.3) (P < .001).[37]

Given toxicities of agents like ganciclovir, CMV monitoring and preemptive therapy has been a standard of care. Investigational agents have been evaluated in the HCT setting, but both orally available brincidofovir and mirabavir did not confirm benefit in the phase 3 setting.[38,39] More recently, a phase 3, double-blind, placebo-controlled trial in CMV-seropositive transplant recipients evaluated the efficacy of letemovir, a viral terminase complex

inhibitor, for CMV prophylaxis. Patients on the letemovir treatment arm were found to have a significantly lower rate of infection (n = 122 of 325 treated [37.5%]) than recipients of placebo (n = 103 of 170 [60.6%]) at 24 weeks after HCT,[40] had no excess toxicity, and had lower all-cause mortality compared to placebo.

PTCY can also increase the risk of non-CMV herpes viral (NCHV) infections. In the largest such study to date (over 1100 haploidentical and matched sibling recipients), NCHV-related infection was 11% with PTCY in comparison to 4% in matched siblings who received calcineurin-based GVHD prophylaxis (P<.001), with HHV-6 being the most common viral infection noted (P = .004).[41] Development of NCHV infection was also associated with NRM. Current prospective studies are evaluating the efficacy of additional platforms beyond pharmaceuticals, including the usage of directed concentrated antibodies and adoptive cell immunotherapies for antiviral control for both CMV (NCT05370976; NCT04056533) and NCHV infections (NCT05305040) to address this ongoing need.

Conclusion 3: Patients who have received a T-cell–depleting GVHD preventative strategy are at risk for CMV reactivation and infection. Thus, usage of an efficacious aggressive CMV preventative strategy, letemovir, should be considered in such patients, as demonstrated by its efficacy in the PTCY-based setting. Additional prospective studies are needed to evaluate mechanisms to improve immune reconstitution and identify infectious risk in new transplant treatment platforms.

Summary

By identifying the most common causes of HCT treatment failure, risk mitigation strategies can be considered in individual HCT recipients to optimize HCT outcomes. In conjunction with pre-HCT workup, a treatment plan can be tailored to delineate a comprehensive treatment plan. Novel agents and combination therapies for patients in these high-risk scenarios are currently being developed to further improve HCT outcomes that will broaden the availability of this treatment option to a wider patient population based on underlying disease, age, racial disparities, comorbid conditions, and underlying risk factors such as infection.

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Conflict-of-interest disclosure

Asmita Mishra: no competing financial interests to declare.

Off-label drug use

Asmita Mishra: cyclophosphamide, 5-azacytidine, decitabine, enasidenib, and ivosidenib are discussed.

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EVIDENCE-BASED MINIREVIEW

What makes a pediatric or young adult patient an appropriate transplant candidate?

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A 3-year-old child with chronic granulomatous disease was brought to the transplant clinic by his parents. The patient has a history of *Aspergillus fumigatus* pneumonia, which required mechanical ventilation, and sepsis, resulting in several intensive care stays. He has failure to thrive and developmental delay. His parents are seeking guidance whether allogeneic hematopoietic cell transplantation (HCT) is a reasonable treatment option given concerns about his upfront major health limitations. Based on the original HCT-Comorbidity Index (CI), this child's risk for nonrelapse mortality (NRM) would be negligible with a score of 0. With use of the validated youth-nonmalignant HCT-CI, the score increases to 5, due to prior mechanical ventilation (+3), history of fungal infection (+1), and being underweight (+1), with at least 2-fold increase in risk of NRM. The role of developmental delay is unclear and not currently validated to prognosticate survival. While HCT was ultimately recommended in this case, the family was counseled to have a more realistic sense of NRM risk.

LEARNING OBJECTIVES

- Comorbidity assessment via the youth-HCT-CI is key in understanding risks of mortality before HCT
- Other factors, such as neuropsychiatric, socioeconomic, and metabolic risks, should be taken into account when counseling youth HCT candidates

CLINICAL CASE

A 3-year-old child with chronic granulomatous disease was brought to the transplant clinic by his parents. The patient has a history of *Aspergillus fumigatus* pneumonia, which required mechanical ventilation, and sepsis, resulting in several intensive care stays. He has failure to thrive and developmental delay. His parents are seeking guidance whether allogeneic hematopoietic cell transplantation (HCT) is a reasonable treatment option given concerns about his upfront major health limitations.

Introduction

Despite improvements in HCT procedures and supportive care, nonrelapse mortality (NRM) remains a major contributor to death after allogeneic HCT, accounting for >60% of early deaths in those ≤18 years of age.[1] To assist in determining an individual patient's risk for NRM, the Hematopoietic Cell Transplantation-Comorbidity Index (HCT-CI) was created to capture incremental organ impairments and was validated to predict overall survival (OS) in both malignant[2] and nonmalignant[3] diseases. While its discriminative appeal has been proclaimed for its value in helping transplant physicians counsel recipients of all ages on transplant risks, the HCT-CI score has been rarely used by pediatric transplant physicians due to concern of its applicability to this age group.[4] Children have been found to have fewer comorbidities per the HCT-CI compared to adults, resulting in relatively lower scores (0-1),[5] despite the fact that they continue to experience substantial NRM.[1] This suggests that either the definitions used to assess HCT-CI do not comprehensively apply to children or that there are additional risk factors that are missing for this age group.[6] This perspective also applies to adolescents and young adults (AYA)[7]; although they are older and therefore more likely to have testable organ dysfunction compared to infants and children, AYA also tend to have other nondisease related concerns, such as environmental and social dysfunction, that can affect OS after HCT. "Sentiment" tends to be more emotional. Finally, nonmalignant diseases represent a large percentage of

indications for allogeneic HCT in children and AYA, but many of these diseases are individually unique and are predisposed to developing distinct organ toxicities that may not be fully reflected in the HCT-CI. It is also possible that these disease-defining comorbidities may be treated or cured by HCT, therefore becoming less problematic for predicting OS. Here, we discuss 1) updated applications of the HCT-CI in children and AYA populations (collectively named *youth*) and 2) other ways to improve pretransplant risk assessment in youth.

Updating the HCT-CI to be more inclusive of youth risk factors

Strategy 1: Expanding the HCT-CI definitions

One method for improving applicability of HCT-CI for youth is to expand definitions and include new items that are prevalent in this age group.[8,9] For example, lack of pulmonary function testing in young children limited proper evaluation of their pulmonary comorbidities. To overcome this limitation, the definition was expanded to include history of mechanical ventilation. History of previously treated fungal infection was counted toward expanding infection comorbidity. Definitions for renal comorbidities were expanded to include the estimated glomerular filtration rate (eGFR) using either the Bedside Schwartz equation for children or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation for young adults. Finally, failure to thrive or being underweight was added to the index to capture nutritional deficiencies more comprehensively. These additions were first tested in training sets and then validated in independent validation sets with discriminatory capacity confirmed by C-statistic (Table 1).

Strategy 2: Simplifying the HCT-CI to remove comorbidities that are not prevalent in pediatrics

Despite being developed and validated in both adult and pediatric populations, adult patients and their risk factors were overly represented in the development of the HCT-CI, therefore creating the impression that it is a more adult-focused scale.[2] To address this issue, comorbidities with hazard ratios <1.2 were eliminated, as these would indicate comorbidities that had minimal contribution to the predictive capacity of the model in the youth population.[8,9] Comorbidities that were eliminated in both the malignant and nonmalignant groups were arrhythmia, psychiatric disease, mild hepatic disease, moderate pulmonary disease, and peptic ulcer disease. Additional

Table 1. Comorbidity list with corresponding weight distributions compared between HCT-CI and youth HCT-CI

Comorbidity	Definition	HCT-CI	Expanded youth HCT-CI		Simplified youth HCT-CI	
			Malignant	Nonmalignant	Malignant	Nonmalignant
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	1	1	1	0	0
Cardiac disease	Coronary artery disease, congestive heart failure, myocardial infarction, or EF ≤50% on most recent test	1	1	1	1	1
Heart valve disease	Except asymptomatic mitral valve prolapse	3	3	3	3	3
Inflammatory bowel disease	Crohn disease or ulcerative colitis	1	1	1	0	1
Diabetes	Requiring treatment with insulin or oral hypoglycemics, but not diet alone	1	1	1	1	1
Psychiatric disease	Requiring psychiatric consult or treatment in the last 4 weeks	1	1	1	0	0
Cerebrovascular	Any history of transient ischemic attack, subarachnoid hemorrhage, or cerebrovascular accident	1	1	1	0	1
Infection*	Requiring antimicrobial treatment for serious infection that continues through conditioning	1	1	1	1	1
	Or history of invasive fungal disease (proven, suspected, and/or documented)	N/A				
Obesity*	>35 kg/m^2	1	1	1	1	0
	BMI >95th percentile by CDC guidelines for (≤18 years old)	N/A				
Underweight*	Underweight: BMI <5th percentile by CDC guidelines for (≤18 years old) or <18 kg/m^2 (>18 years old)	N/A	0	1	0	1
Mild hepatic disease	Chronic hepatitis, bilirubin >upper limit of normal to 1.5 × upper limit of normal, or AST/ALT upper limit of normal to 2.5 × upper limit of normal	1	1	1	0	0
Moderate/severe hepatic disease	Liver cirrhosis, bilirubin >1.5 × upper limit of normal, or AST/ALT >2.5 × upper limit of normal	3	3	3	3	3

Table 1. Comorbidity list with corresponding weight distributions compared between HCT-CI and youth HCT-CI (*Continued*)

Comorbidity	Definition		HCT-CI	Expanded youth HCT-CI		Simplified youth HCT-CI	
				Malignant	Nonmalignant	Malignant	Nonmalignant
Mild renal disease*	Creatinine >2 mg/dL or prior renal transplant	or on dialysis	2[a]	N/A	N/A	N/A	N/A
		or eGFR 60–89 mL/min/1.73 m^2 (by Bedside Schwartz calculation for <18 years old, CKD-EPI calculation for ≥18 years old)	N/A	2	N/A	2	N/A
	eGFR 60–89 mL/min/1.73 m^2 (by Bedside Schwartz calculation for <18 years old, CKD-EPI calculation for ≥18 years old)		N/A	N/A	1	N/A	1
Moderate/severe renal disease*	Creatinine >2 mg/dL, or prior renal transplant, or on dialysis		2[a]	N/A	N/A	N/A	N/A
	Creatinine >2 mg/dL, on dialysis, or prior renal transplant	or eGFR <60 mL/min/1.73 m^2 (by Bedside Schwartz calculation for <18 years old, CKD-EPI calculation for ≥18 years old)	N/A	N/A	2	N/A	2
	On dialysis		N/A	3	N/A	3	N/A
Moderate pulmonary disease	Corrected diffusion capacity of carbon monoxide and/or FEV_1 66%-80%, or dyspnea on slight activity		2	2	2	0	0
Severe pulmonary disease*	Corrected diffusion capacity of carbon monoxide and/or FEV_1 ≤65%, dyspnea at rest, or requiring oxygen		3	3	3	3	3
	Or prior history of mechanical ventilation		N/A				
Peptic ulcer disease	Confirmed by endoscopy and requiring treatment		2	2	2	0	2
Rheumatologic disease	Systemic lupus, rheumatoid arthritis, polymyositis, mixed connective tissue disease, or polymyalgia rheumatica requiring treatment		2	2	2	0	2
Prior solid tumor	At any point in patient's history, excluding nonmelanoma skin cancer, leukemia, lymphoma, or multiple myeloma; does not count if patient is being transplanted for indication of solid tumor		3	3	3	3	3

This is a summary table combining the three different HCT-CI scores that have been validated in assessing pre-HCT comorbidities. Definitions of comorbidities and weighted scores are listed. Refer to original citations for accompanying hazard ratios and confidence intervals that justify weights assigned. New definitions that apply to the pediatric/AYA population (or youth scores) are indicated with an *. Please note that youth classification is further divided into 2 groups based on malignant or nonmalignant disease status. In validation models, having an underlying malignant or nonmalignant disease made a difference in weighted scores when using the simplified youth HCT-CI to assess inflammatory bowel disease, cerebrovascular disease, obesity, being underweight, renal disease, peptic ulcer disease, and rheumatologic disease. N/A is listed for those new definitions that are part of the youth HCT-CI measures that are not part of the original HCT-CI. For renal disease, the definitions are different for malignant compared to non-malignant conditions. Adapted from Sorror et al., Blood 2005; Friend et al., TCT 2023; and Broglie et al., TCT 2023.[2,8,9]

[a] In the original HCT-CI, there was only one category listed for renal disease. With the pediatric definitions, the renal category is now split into mild or moderate/severe. The renal disease HCT-CI score is currently listed in both the mild and moderate/severe categories to reflect this.

* Items modified for youth population (pediatric/AYA) include revised definitions or new definitions.

ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; CDC, Centers for Disease Control and Prevention; N/A, not applicable.

items removed for malignant diseases included inflammatory bowel disease, cardiovascular disease, being underweight, peptic ulcer disease, and rheumatologic disease, while obesity was the only additional factor removed for nonmalignant diseases (Table 1).

The new HCT-CI for youth

Applying these two strategies, two studies were performed. Youth with nonmalignant diseases (n=2815) who received their first allogeneic HCTs between 2008 and 2017 were included in the first study to develop the youth-nonmalignant-HCT-CI (ynHCT-CI).[9] These modifications resulted in 39% of patients having an increase in their ynHCT-CI scores with an increased hazard of mortality compared to those whose score remained the same (hazard ratio, 1.41; 95% CI, 1.01-1.98). Performance of the new model was slightly better than that of the original HCT-CI (C-statistic estimates of 65.8 versus 64.3, respectively), but the main advantage was that it represented a more youth-focused scale. Likewise, 5790 youths with malignant diseases contributed to development of the youth-malignant-HCT-CI.[8]

The youth-maliginant-HCT-CI led to an increase in comorbidity scores for 23% of youth patients and was associated with a significant risk of NRM (HR, 1.34; 95% CI, 1.02-1.74). The 3-year survival rates were 62.9%, 53.3%, and 50.1%, respectively for scores 0, 1-2 and ≥3.

Future directions and other considerations

Further fine-tuning of the youth HCT-CI will require thoughtful attention to data collected in future prospective studies. Psychiatric comorbidities could be expanded to include certain behaviors (eg, aggression) that have that been connected to untreated depression or anxiety in children.[10] Another example is better defining comorbidities linked to specific primary nonmalignant diseases. For instance, redefining iron overload by using T2* magnetic resonance imaging or including the number of intensive care hospitalizations could better capture the burden of hemoglobinopathy-associated risk factors. It is also unknown whether elevated pre-HCT baseline biomarkers, such as urine protein to creatinine ratio or soluble C5b-9 levels, could capture individuals at higher risk for life-threatening post-HCT complications such as transplant-associated microangiopathy (Table 2).[11]

Aside from comorbidities, other risk factors could further enhance our understanding of HCT risks in youths. These include categories such as neuropsychiatric conditions and socioeconomic factors.[12] Finally, genetic variants found in recipients and their donors could adversely impact outcomes and are an emerging area of research.[13,14] A list of these potential factors is detailed in Table 2. Future prospective studies are needed to enhance risk-assessment potential for youth recipients of allogeneic HCT.

Table 2. Additional variables that could be validated as pre-HCT risk factors in pediatric patients

Cardiac
Prolonged QTc
Endocrinopathies
Metabolic syndrome
Thyroid dysfunction
Low cortisol production
Growth delays
Hypertriglyceridemia
Low activity levels/sedentary lifestyle
Gastrointestinal
Pneumatosis
Pancreatitis
Poorly-diversified microbiome
Hypoalbuminemia/hypoproteinemia
Genetic variants (HLA and non-HLA genes)
Intensive care
Number of hospitalizations
Extracorporeal membrane oxygenation
Iron overload
Number of red blood cell transfusions received
T2*MRI
Neuropsychiatric concerns
Developmental delay
Isolation
Poor resiliency
Behavior concerns
Attention deficit hyperactivity disorders
Poor sleep patterns
Low scores on validated, age-appropriate patient-reported outcomes
Pain requiring scheduled opioid medications

Table 2. Additional variables that could be validated as pre-HCT risk factors in pediatric patients (*Continued*)

Predisposition to transplant-associated microangiopathy
High inflammatory markers (e.g., C reactive protein) preceding conditioning
High baseline terminal complement system pathway factors (eg, soluble C5b-9)
High urine protein to creatinine ratio
Socioeconomic factors/access to care
Noncompliance with treatments
Homelessness
Poor family support system
Social isolation
Food insecurity
Financial toxicity/insurance concerns
Amount of school/work missed
Marginalized populations/disparities in care
Vitamin and mineral deficiencies
Vitamin D
Iron
Zinc

HLA, human leukocyte antigen; MRI, magnetic resonance imaging. While not exhaustive, many of these entities have been evaluated as possible contributors to poor health and/or medical complications in transplant and/or nontransplant patients and may contribute to acute and long-term complications in areas of human health that could impact NRM and OS after HCT in youths.

CLINICAL CASE (revisited)

Based on the original HCT-CI, this child's risk for NRM would be negligible with a score of 0. With use of the validated ynHCT-CI, the score increases to 5, due to prior mechanical ventilation (+3), history of fungal infection (+1), and being underweight (+1), with at least a 2-fold increase in risk of NRM. The role of developmental delay is unclear and not currently validated to prognosticate survival. While HCT was ultimately recommended in this case, the family was counseled to have a more realistic sense of NRM risk.

Conclusions

The HCT-CI was introduced almost two decades ago and has been reliable in providing data-driven survival predictions for transplant recipients. The newly validated youth scores can further assist transplant physicians in counseling families with children. When counseling patients about transplants, additional risk factors (Table 2) need to be considered, especially those that may be discovered in future studies and added to the current models.

Recommendations[15]

Based on our review of the literature, we offer the following recommendations:

1. The HCT-CI assessment should be performed in all pediatric patients undergoing HCT to assess risk of NRM. (Grade 1A).
2. Either the expanded or simplified youth HCT-CI, which provide improved definitions for certain pediatric comorbidities, should be assessed in all youth to determine risk of NRM, although real-life experience using this new scale is limited. (Grade 1B).
3. At this time, we suggest consideration of other pretransplant risk factors reported in the literature that could contribute to mortality after HCT. While they have not been validated to date in the transplant setting, future studies could broaden their applicability. (Grade 1C).

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Conflict-of-interest disclosure

Monica S. Thakar: on the scientific advisory boards for Proteios Technology and ImmunoVec.
Mohamed L. Sorror: reports consultancy for and receiving an honorarium from Jazz Pharmaceuticals and receiving a research grant from Massachusetts General Hospital and BlueNote.

Off-label drug use

Monica S. Thakar: Nothing to disclose.
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Labor and delivery: DIC, HELLP, preeclampsia

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Hematologists are often needed to assist with the management of microangiopathic emergencies in pregnancy. A firm understanding of the diagnosis and management of preeclampsia with severe features, hemolysis elevated liver enzyme and low platelet syndrome, and disseminated intravascular coagulation, which are the most common causes of microangiopathic emergencies, is critical. However, being able to consider when other microangiopathic emergencies (acute fatty liver of pregnancy, congenital and acquired thrombotic thrombocytopenic purpura, complement mediated microangiopathy, antiphospholipid syndrome) should be considered is imperative. The hematologist and obstetric team should work together to optimize the care of common as well as rare hematologic emergencies.

LEARNING OBJECTIVES

- Identify the epidemiology and common presentations of labor and delivery microangiopathic emergencies including DIC, HELLP, and preeclampsia
- Outline the diagnosis of preeclampsia, distinguish it from HELLP syndrome, and learn the management similarities and differences between the two
- Review the etiology and typical management of DIC in pregnancy
- Identify when other microangiopathic emergencies should be considered

CLINICAL CASE

A 30-year-old patient, gravida 2 para 1, presents at 29 weeks with shortness of breath, epigastric pain, and headache. She has a history of immune thrombocytopenia in the setting of systemic lupus erythematosus for which she has received multiple immunosuppressive medications. She also has a history of multiple venous thromboembolisms (VTEs), for which she is currently on long-term anticoagulation with enoxaparin 1 mg/kg twice daily. She received a course of high dose prednisone early in pregnancy (developed significant psychiatric side effects) and has received intravenous immunoglobin every 3 weeks for the past 8 to 10 weeks, maintaining a platelet count of around 80 000/μL.

Introduction

Common reasons for hematology consultation in hospitals are anemia and thrombocytopenia beyond what is expected for gestational age[1] and especially when identified in an acutely ill pregnant patient. The differential diagnoses include the usual causes of cytopenias that hematologists are familiar working through as well as pregnancy-specific etiologies with which consultants are less comfortable. The acuity of presentation and speed at which a working diagnosis and treatment plan need to be formulated pose a unique challenge to the clinical team. Prioritization of interventions, which include emergent delivery, requires prompt identification features that support one diagnosis over another, acknowledging that microangiopathic emergencies overlap in their pathophysiology and share many common features.

Endothelial activation and consumptive coagulopathy are the main mechanisms that drive microangiopathies resulting from pregnancy-specific conditions as well as those that occur in pregnancy but are not unique to pregnancy.[2] Hypertensive disorders of pregnancy with hematologic features include preeclampsia and preeclampsia with severe features, which, by definition, occur after week 20

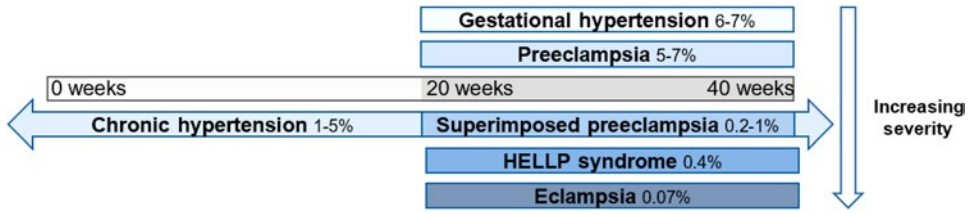

Figure 1. Spectrum of hypertensive disorders during pregnancy and their prevalence. Gestational hypertension is defined by new-onset elevations in blood pressure (<140/90 mmHg) after 20 weeks of gestation, whereas preeclampsia is also accompanied by proteinuria and/or end-organ dysfunction. Chronic hypertension is present prior to 20 weeks of gestation or continues >12 weeks into the postnatal period and can occur in concert with preeclampsia. Hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome is classified as a subset of preeclampsia, and eclampsia is a complication of preeclampsia characterized by the addition of seizures.

of gestation and can present post partum. Hemolysis elevated liver enzyme and low platelet (HELLP) syndrome involves liver endothelial dysfunction that results in consumptive coagulopathy and has some shared features in clinical presentation with acute fatty liver of pregnancy (AFLP). Disseminated intravascular coagulation (DIC) can result from systemic medical illness or trauma in the context of pregnancy but is most frequently observed secondary to placental abruption and postpartum hemorrhage. Congenital thrombotic thrombocytopenia purpura (cTTP), though rare, is most likely to be seen in pregnant patients, especially those that develop severe thrombocytopenia before 20 weeks; in contrast, acquired/immune TTP (iTTP) is the subtype of thrombotic thrombocytopenia purpura (TTP) that most hematologists are more familiar diagnosing and managing.[3] Complement-mediated disorders, in particular those that predominantly affect the kidneys, are seen with increased frequency during pregnancy and especially in the postpartum period but are not exclusive to pregnancy.

Preeclampsia

Preeclampsia impacts 2%-8% of all pregnancies[4] and is part of the spectrum that includes hypertensive disorders in pregnancy, ranging from gestational hypertension to HELLP syndrome (Figure 1), and is the most common microangiopathic emergency in pregnancy.[5] It is important to note that not all cases of preeclampsia include microangiopathy. Most preeclampsia cases present during the last few weeks of pregnancy, with only 10% of patients developing the condition prior to 34 weeks and 5% developing it after delivery.[6,7] The diagnostic criteria of these hypertensive disorders of pregnancy, yet the nuances are important. All of these disorders occur after the first 20 weeks of pregnancy.[8,9] Prior to 20 weeks of pregnancy, other causes should be pursued more seriously, as it is extremely rare that these disorders present earlier.[10,11] Gestational hypertension is elevated blood pressure (>140 mm Hg systolic or >90 mm Hg diastolic) without any other symptoms, evidence of end organ damage, or laboratory criteria.[8,9] It is crucial to be able to distinguish preeclampsia

Table 1. Diagnostic criteria for preeclampsia*

Mild BP	Systolic BP >140 mm Hg or diastolic BP >90 mm Hg on 2 occasions at least 4 hours apart**
Proteinuria#	>300 mg in 24 hr urine OR Protein-to-creatinine ratio of 0.3 or greater
Severe BP elevation	Systolic BP >160 mm Hg or diastolic BP >110 mm Hg^
Thrombocytopenia	Platelet count <100 000/L in the absence of other causes of thrombocytopenia
Renal insufficiency	Serum creatinine >1.1 mg/dL or doubling of baseline creatinine in the absence of other causes for renal disease
Impaired liver function	Liver transaminases greater than twice the normal values
Pulmonary edema	
Headache	New onset, unresponsive to medication, and not accounted for by alternative diagnoses
Visual disturbances	Most commonly scotoma or blurry vision

Characteristics that elevate the diagnosis to preeclampsia with severe features are shaded in pink.

*Gestational age >20 weeks.

#Preeclampsia with severe features can occur in the absence of proteinuria with any of the severe features shaded pink.

^Unlike the 4-hour requirement between BP for mild readings, repeat BP for severe readings should be taking at least every 15 minutes in order to facilitate prompt treatment.

**Should not have previously been diagnosed as having chronic hypertension.

BP, blood pressure.

from preeclampsia with severe features. Preeclampsia is diagnosed with elevated blood pressure (>140 mm Hg systolic or >90 mm Hg diastolic) plus symptoms, end organ dysfunction, or laboratory derangement (Table 1).[8,9] Proteinuria with elevated blood pressure represents most of the cases of preeclampsia without severe features; most of the other diagnostic criteria represent a severe feature, thus elevating the severity of the disease.[8]

CLINICAL CASE (continued)

Vital signs in OB triage were blood pressure, 172/88; pulse, 61 beats per minute; saturation of peripheral oxygen (SpO_2), 83%; and temperature, 98.8 degrees Fahrenheit. Labs were significant for platelets of 32 000/μL, aspartate transaminase (AST) of 1560 U/L, alanine transaminase (ALT) of 1222 U/L, and creatinine of 0.83 mg/dL. Lactate dehydrogenase was markedly elevated at 3452 U/L, and haptoglobin was normal at 39 mg/dL. There were 0–2 schistocytes per high power field on peripheral smear. Fetal heart tracing remained reassuring. Given the blood pressure on presentation and laboratory abnormalities, preeclampsia with severe features was the top diagnosis, with a question of whether she met criteria for HELLP syndrome. With her underlying hematologic disorders, the hematology team was consulted, magnesium sulfate was started, betamethasone was administered (for fetal lung maturation), and antihypertensive medications were administered.

Preeclampsia with severe features and preeclampsia without severe features are managed differently and in a gestational-age-dependent fashion. For all preeclampsia, the only resolution of the disease is birth, as the disease is thought to arise from the placenta.[8,9] Therefore, at premature gestational ages, care is taken to provide supportive care and symptomatic management for the mother in order to gain greater gestational age for the fetus. This tradeoff of risks and benefits is done differently based on gestational age and disease severity.[8] At term (37 weeks or greater), all patients diagnosed with preeclampsia should be promptly delivered.[8,9,12] A diagnosis of preeclampsia, however, is not an indication for a cesarean delivery, which should be reserved for obstetric indications. In pregnancies with preeclampsia with severe features, patients should also be given magnesium sulfate for seizure prevention as well as antihypertensive treatment (labetalol, hydralazine, or nifedipine) to maintain blood pressure less than 160/110 mm Hg.[8,9]

At a preterm delivery (<37 weeks), severity of disease strongly impacts management decisions. For example, in preeclampsia

Table 2. Comparison of clinical features and specific management of preeclampsia with severe features (PES)/HELLP syndrome, DIC, TTP, and C-TMA

Clinical feature	PES/HELLP	TTP	DIC	C-TMA
Incidence (per 10^5 pregnancies)	1000	1	130[$]	Unknown. May be similar to TTP
Time of occurrence during pregnancy/post partum	By definition, occurs after 20 weeks; more common near term and within 3 days post partum	May occur throughout pregnancy, but most common near term and several weeks post partum	Typically at the time of delivery (independent of gestational age) but can occur in the setting of acute illness	May occur throughout pregnancy, but most common post partum
Blood pressure	Typically, >160/110 mm Hg, but could be >140/90 mm Hg	Normal	Normal or hypotensive	High, related to acute kidney injury
Neurologic abnormalities	Minor (headache, vision changes). Less common: eclamptic seizures, PRES, stroke	Severe in 30% (transient focal defects, seizure, stroke); minor in 30%	None	Inconsistent, but up to 50% of patients
MAHA, thrombocytopenia	Moderate	Severe	Variable	Moderate
Kidney injury	Mild	Mild	Mild	Severe
Liver function tests: ALT, AST	Markedly increased ALT, AST	Normal or slightly increased	Normal (as long as liver dysfunction is not the driver)	Normal
Typical course following delivery	Improvement within 24–36 hours	No improvement within 36 hours	Improvement if driven by obstetric complication	Increasing serum creatinine
Specific management	Delivery of infant is curative	Plasma infusion or plasma exchange, immunosuppression if acquired autoimmune TTP suspected	Transfusion support, correction of the underlying cause	Anticomplement agent

The incidence of preeclampsia with severe features is 1 case/100 pregnancies.[3] The incidence of TTP associated with pregnancy is estimated from Oklahoma TTP Registry data. Five patients have had TTP associated with pregnancy during 19 years, 1996–2014. Centers for Disease Control in the US state that in 2013, there were 12 births/1000 population[36]; the Oklahoma TTP Registry region has a population of approximately 2×10^6. Therefore, the Oklahoma TTP Registry region would have approximately 24 000 births/year, 456 000 births/19 years. Five patients with pregnancy-associated TTP/456,000 births is approximately 1 patient/10^5 pregnancies.

[$]Overall prevalence is low, but risk is highest in patients with placental abruption and amniotic fluid embolism.

MAHA, microangiopathic hemolytic anemia; PES, preeclampsia with severe feature; PRES, posterior reversible encephalopathy syndrome.

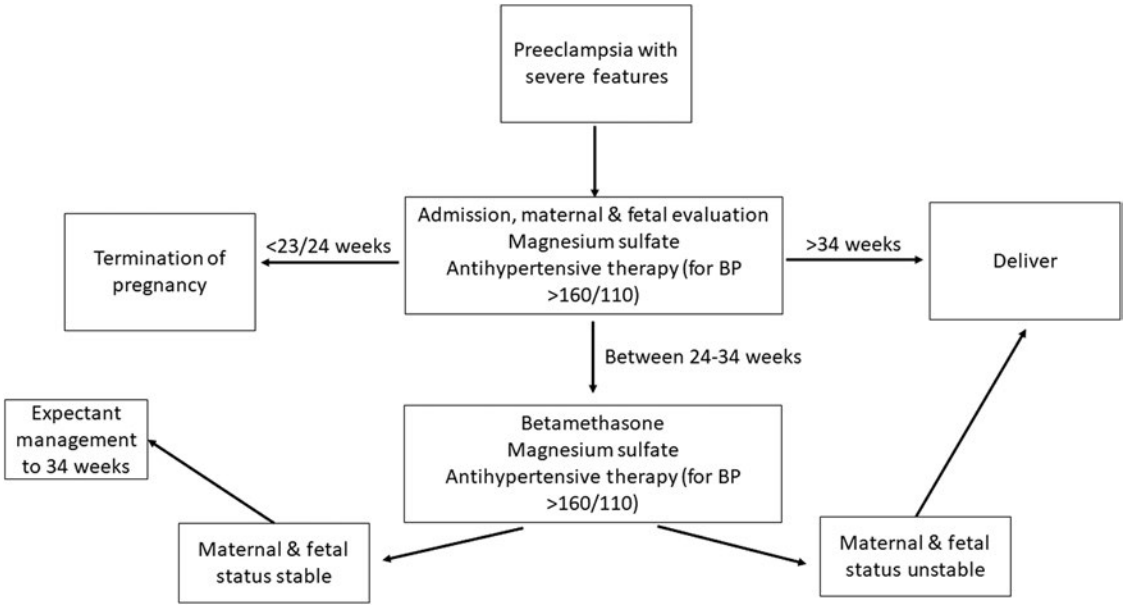

Figure 2. Management of preeclampsia with severe features.

with severe features and a gestational age >34 weeks, delivery is standard of care, while those with preeclampsia without severe features are monitored closely with planned delivery at 37 weeks' gestation.[8,9] In those with preeclampsia with severe features, the stability of the pregnant person dictates whether or not the patient should be managed expectantly or delivered more promptly.[8,9] Additionally, confirming that the diagnosis is preeclampsia with severe features and not another condition is crucial.[3] As there is overlap in the clinical criteria of these syndromes and distinguishing one from another can be challenging (Table 2), maternal fetal medicine and hematology should work together to diagnose the patient.[3,13] Patients with preeclampsia with severe features are particularly at risk for poor outcomes, including seizures, strokes, placental abruption, stillbirth, and death. In patients who are preterm with preeclampsia with severe features, delivery versus expectant management depends on patient stability.[8,9,14] The unstable patient will be delivered immediately, the somewhat stable patient (when the patient's life or that of their fetus is not imminently at risk) will be given a 48-hour course of betamethasone (for fetal lung maturity), and the stable patient will be managed as an inpatient until 34 weeks (Figure 2).[8,9] While delivery is the ultimate cure for preeclampsia, immediate resolution does not always occur. In fact, platelet nadir typically occurs at ~24 hours post partum, and returns toward normal approximately 3 days later.[15] Resolution of elevated creatinine and liver function follow the same trends.[15] Therefore, in patients who continue to have severe, persistent, or worsening thrombocytopenia and/or rising creatinine and have liver transaminases beyond the first 24-48 hours postpartum, alternative diagnoses should be strongly considered.[3,15]

CLINICAL CASE (continued)

After being diagnosed with HELLP syndrome and considering her systemic compromise and overall clinical decline, the patient urgently underwent a cesarean delivery. On the first postpartum day, her lab values were as follows: platelet count, 19 000/μL; AST, 2536 U/L; ALT, 1446 U/L; and creatinine, 1.79 mg/dL. By the second day post partum, platelets and creatinine continued to worsen and were 20 000/μL, and 4.77 mg/dL, respectively, while her liver enzymes began improving with AST of 451 U/L and ALT of 531 U/L. Maternal fetal medicine and hematology consulted the nephrology service to evaluate her for complement mediated thrombotic microangiopathy (C-TMA). A heparin drip was used instead of enoxaparin because of her compromised renal function, while dialysis was considered when her creatinine peaked at 7.17 mg/dL on postpartum day 4. Ultimately, her hemolysis parameters normalized, and her renal function began recovering. By postpartum day 9, her creatinine had dropped to <2.0 mg/dL and was thought to be part of preeclampsia/HELLP and acute kidney injury.

HELLP syndrome

HELLP syndrome occurs in 0.5%-0.9% of pregnancies but in 10%-20% of those with preeclampsia with severe features.[13,16] Most often, HELLP presents prior to delivery, but in up to 30% of cases, it can present post partum, typically within the first 48 hours of delivery.[16,17] The hallmark of HELLP syndrome is microangiopathic hemolytic anemia, so laboratory values are most useful in confirming this diagnosis.[3,15] HELLP is considered to occur on the spectrum of hypertensive disorders of pregnancy, but can also be considered a variant of preeclampsia, as up to 15% present without hypertension or proteinuria.[18] It is critical to determine whether the patient has an obstetric disease, HELLP, or an alternative diagnosis, such as TTP or C-TMA (Table 2). Because C-TMA is rare, collaboration between the hematology and maternal fetal medicine departments is paramount.[13] Determining the etiology is especially important in pregnant patients, as HELLP syndrome requires delivery,

Table 3. Coagulation parameters in the nonpregnant and pregnant states stratified by first, second, and third trimesters

Coagulation parameters	Nonpregnant adult	1st Trimester	2nd Trimester	3rd Trimester
D-dimer (micrograms/mL) factor (%)	0.22–0.74	0.05–0.95	0.32–1.29	0.13–1.7
Factor V	50–150	75–85	72–96	60–88
Factor VII	50–150	100–146	95–153	149–211
Factor VIII	50–150	90–210	97–312	143–353
Factor IX	50–150	103–172	154–217	164–235
Factor XI	50–150	80–127	82–144	65–123
Factor XII	50–150	78–124	90–151	129–194
Fibrinogen (mg/dL)	233–496	244–510	291–538	373–619
INR	0.9–1.04	0.89–1.05	0.85–0.97	0.80–0.94
PTT, activated (s)	26.3–39.4	24.3–38.9	24.2–38.1	24.7–35.0
Protein C, functional (%)	70–130	78–121	83–133	67–135
Protein S, functional activity (%)	65–140	57–95	42–68	16–42
tPA (ng/mL)	1.6–13	1.8–6.0	2.4–6.6	3.3–9.2
tPA inhibitor-1 (ng/mL)	4–43	16–33	36–55	67–92

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INR, international normalized ratio; PTT, partial thromboplastin time; tPA, tissue plasminogen activator.

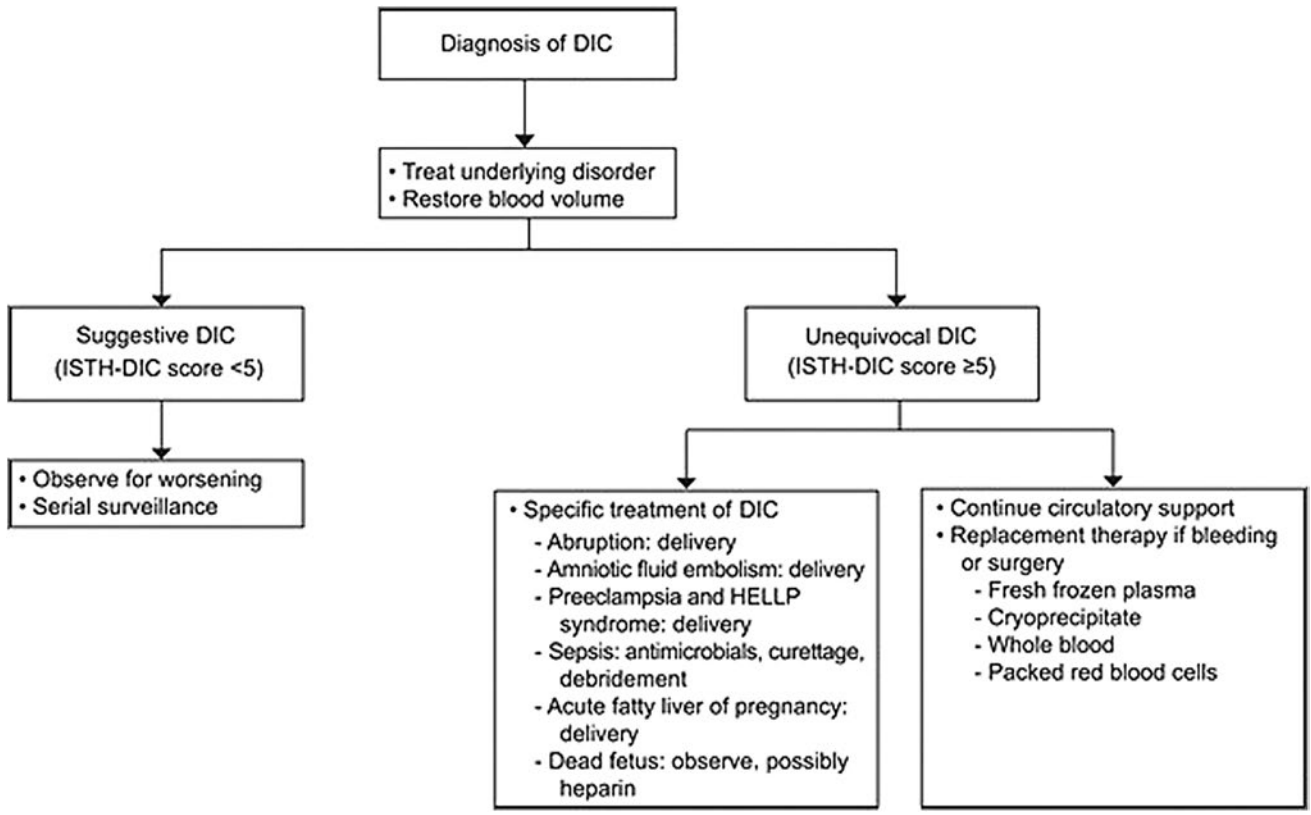

Figure 3. Treatment algorithm for clinical management of DIC in obstetric syndromes. ISTH, International Society of Thrombosis and Hemostasis. Reproduced with permission from Cunningham and Nelson, Disseminated intravascular coagulation syndromes in obstetrics, *Obstet Gynecol*. 2015;126(5):999–1011.[20] Copyright © 2015.

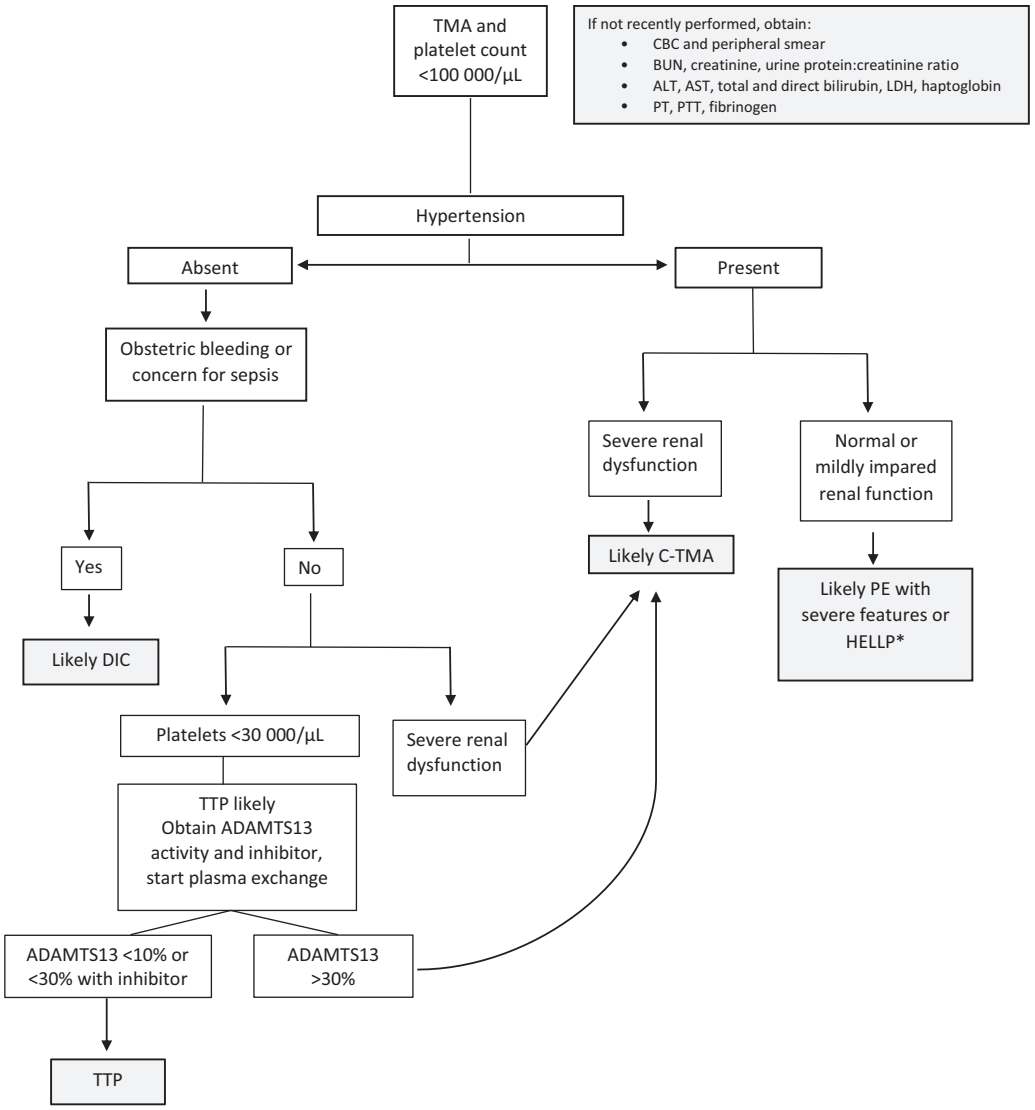

Figure 4. Algorithm for clinical evaluation of microangiopathy in pregnancy. ADAMTS13; BUN, blood urea nitrogen; CBC, complete blood count; C-TMA, complement-mediated thrombotic microangiopathy; LDH, lactate dehydrogenase; PE, preeclampsia; PT, prothrombin time; PTT, activated partial thromboplastin time; TMA, thrombotic microangiopathy. *Occasionally severe renal failure occurs with HELLP, but the recovery is typically more rapid than in C-TMA.

whereas nonobstetric etiologies may benefit from treatment of the underlying problem rather than delivering. In general, HELLP syndrome is managed similarly to preeclampsia with severe features (Figure 2) but often requires further supportive care, transfusion of blood products, and intensive-care-unit admission, as these patients can be critically ill.[8,18] In the postpartum period, HELLP should typically improve within 24-48 hours and rapidly resolve.[3,15] In cases where the patient fails to improve, alternative diagnoses must be considered. Work-up should include TTP or C-TMAs.

DIC

Disseminated intravascular coagulation in pregnancy is uncommon, occurring in less than 1% of pregnancies, yet represents a leading cause of maternal morbidity and mortality worldwide.[19,20] The cause of DIC can be obstetric or nonobstetric; however, obstetric causes of DIC are the primary focus of this discussion. Obstetric causes of DIC include massive obstetric hemorrhage (typically from atony or laceration), HELLP, preeclampsia, spontaneous or missed abortion, intrauterine fetal demise, placental abruption, sepsis, AFLP, and amniotic fluid embolism.[19,20] It is important to note that coagulation parameters change throughout pregnancy, and normal laboratory values during pregnancy are not the same as those outside of pregnancy, or even comparable across all trimesters. Therefore, when evaluating coagulation parameters in a pregnant patient, it is important to reference trimester-specific values (Table 3).[20] Disseminated intravascular coagulation occurs because of a disrupted balance in normal hemostasis and regulatory mechanisms. These pathways are tightly regulated, and a tip toward or away from normal can result uncontrolled thrombin and fibrinogen-fibrin split product generation, and, consequently, endothelial activation and coagulopathy resulting in DIC.[19,20] Scoring systems for DIC that take into account

the expected changes in laboratory parameters, such as the pregnancy-modified International Society of Thrombosis and Hemostasis DIC score, have been developed.[19] Although these scoring systems have not been validated in obstetrics, they can be used to guide management.[19] The diagnosis is typically made based on a combination of clinical and laboratory parameters, with platelet count, prothrombin time and/or partial thromboplastin time, fibrinogen, and fibrin-split products being most commonly used.[20]

Successful management of DIC is grounded on identification and treatment of the underlying cause concurrent with product replacement and circulatory support (Figure 3).[20] Importantly, delivery and pregnancy termination/abortion must be carefully considered in these situations, as evacuating the uterus often saves the pregnant patient's life. Early initiation of the massive transfusion protocol and use of tranexamic acid in cases of obstetric hemorrhage have significantly improved maternal morbidity and mortality and are considered standard of care in the U.S.[19,20] The on-call hematologist may be called to help identify or confirm the underlying coagulopathy and may be asked for assistance in balancing ongoing risks of bleeding and thrombosis.

Other microangiopathic emergencies

Though less common, the following conditions are nonetheless important because they are strongly associated with morbidity and mortality: AFLP, TTP (both inherited and acquired), and C-TMAs, including atypical hemolytic uremic syndrome and catastrophic antiphospholipid antibody syndrome. Figure 4 includes an algorithm for the management of microangiopathic emergencies in pregnancy.

From the hematology perspective, identifying TTP is a priority because treatment must be initiated early to prevent fatality. A markedly decreased ADAMTS13 (under 10% or under 30% with presence of an inhibitor) is specific and diagnostic of TTP; however, this laboratory assay is not widely available and is mostly performed by large academic and reference laboratories.[21] The absence of hypertension and presence of severe thrombocytopenia prior to 20 weeks is helpful in distinguishing TTP from preeclampsia and HELLP.[3] Treatment is typically initiated and directed by the hematology and transfusion medicine teams, with plasma infusion initiated for cTTP or plasma exchange for iTTP.[22] Delivery of the infant is not expected to lead to improvement, and hence TPP should be considered in microangiopathies that persist in the postpartum period.

Identification of catastrophic antiphospholipid antibody syndrome is important in patients with known antiphospholipid antibody syndrome, those with underlying connective tissue disorders, and patients whose presentation includes VTE; unlike arterial events, VTE is not part of the classical presentation of other microangiopathies. Immunosuppression and plasmapheresis[23] in addition to delivery should be considered.

Conclusion

While obstetric microangiopathic emergencies may not be at the forefront of a hematologist's mind, the close collaboration of hematology with the obstetric team in these cases is crucial for optimal patient care. Management of these serious conditions are tremendously complicated, with both a maternal and fetal life often at risk. Starting with a broad differential diagnosis, and using clues provided by clinical features (gestational age, blood pressure, clinical presentation) and laboratory studies (severity of platelet count drop, peripheral smear findings, screening coagulation studies, creatine, liver enzymes), will help clinicians formulate a treatment plan. Decision-making will be a dynamic process, with frequent reevaluation of clinical progress and changes in laboratory parameters. By working together to provide optimal care, hematology and obstetric teams will improve patient outcomes.

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Off-label drug use

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Medical consult: aHUS, TTP? How to distinguish and what to do

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Immune thrombotic thrombocytopenic purpura (iTTP) caused by an autoantibody-mediated deficiency of ADAMTS13 and atypical hemolytic syndrome (aHUS) caused by alternative complement dysregulation are the most common primary thrombotic microangiopathies (TMAs). The evaluation of a patient with TMA is a medical emergency since it is critical to quickly distinguish iTTP and aHUS from other causes of TMA. Untreated iTTP is rapidly fatal, and delays in initiating complement inhibition in aHUS increase the risk of irreversible renal failure. An ADAMTS13 activity level of less than 10% is diagnostic of iTTP in the appropriate clinical setting. In settings where rapid-turnaround ADAMTS13 testing is not available, clinical features and clinical prediction tools are useful to identify patients who should receive emergent plasma exchange. We present an evidence-based approach to the initial (first 24 hours) diagnosis and management of iTTP and review the clinical and laboratory features that can be used to identify patients with aHUS who will benefit from early C5 blockade. We also discuss the potential use of complement blockade to improve outcomes in selected patients with secondary TMA.

LEARNING OBJECTIVES

- Identify patterns of clinical features and use clinical decision tools to distinguish iTTP from aHUS
- Understand the interpretation and application of ADAMTS13 assays for iTTP diagnosis

Introduction

Thrombotic microangiopathies (TMAs) are a group of disorders characterized by thrombocytopenia, microangiopathic hemolytic anemia (MAHA), and ischemic organ injury. The differential diagnosis includes primary TMA syndromes such as immune thrombotic thrombocytopenic purpura (iTTP) and atypical hemolytic uremic syndrome (aHUS, or primary complement-mediated TMA) and multiple causes of "secondary" TMA (Figure 1). While different TMAs have distinct, and sometimes overlapping, pathogenic mechanisms, their clinical presentations are similar and thus present a diagnostic challenge. Rapid and accurate initial diagnosis is critical because untreated iTTP is immediately life-threatening. Delays in initiating complement inhibition for aHUS also increase the risk of permanent renal failure. This review focuses on the critical first 24 hours in managing TMAs and centers on distinguishing iTTP or aHUS from other causes of TMA.

CLINICAL CASE

A 45-year-old woman presented to the emergency department after experiencing headache, malaise, and dark urine for 3 days. Physical examination showed an elevated blood pressure of 174/98 mmHg and mild abdominal tenderness. An initial laboratory evaluation showed a hemoglobin level of 8.5 g/dL, a platelet count of 32×10^9/L, a mean corpuscular volume of 89 fL, a creatinine level of 1.8 mg/dL, and a lactate dehydrogenase (LDH) level of 998 U/L. The peripheral smear showed thrombocytopenia and numerous schistocytes (Figure 2).

Initial evaluation of a patient with suspected TMA

The initial step in the evaluation of a patient with possible TMA is to confirm thrombocytopenia and MAHA (schistocytes). Notably, more than 1% schistocytes (correlating

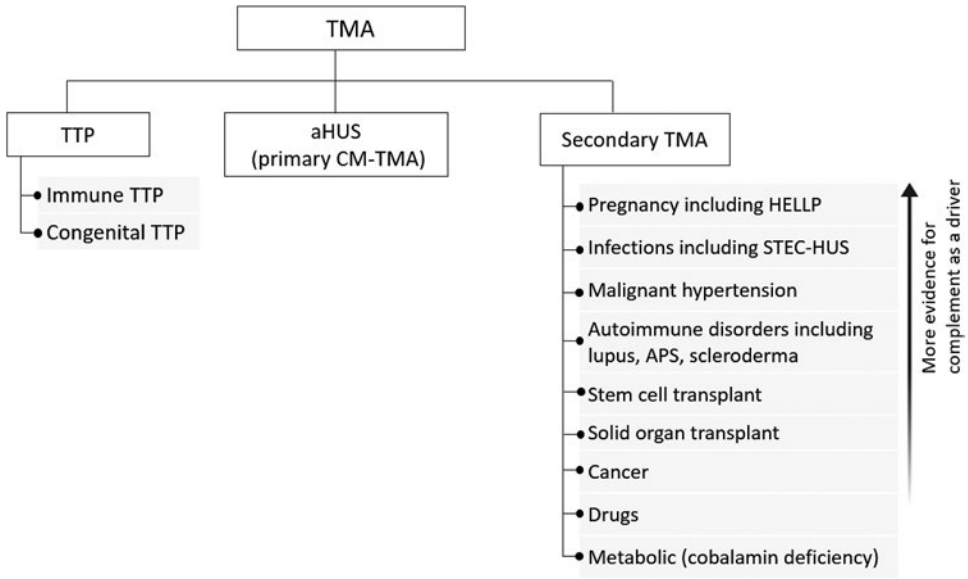

Figure 1. Practical approach to the differential diagnosis of TMA. The initial differential diagnosis of a TMA focuses on differentiating TTP from aHUS, also termed primary complement-mediated TMA, from the multiple causes of secondary TMA since this will impact initial treatment. There is evidence the complement acts as a driver for some secondary TMAs (with and without complement mutations), and complement inhibition may be a useful therapeutic strategy if control of the underlying disorder does not lead to improvement in the TMA and renal function. CM-TMA, complement-mediated TMA.

to >2 per high power field) is considered a robust indicator of TMA. Rarely, schistocytes may not be appreciably increased in patients with early iTTP, and we recommend examining another blood smear 12 to 24 hours later when the clinical picture is highly suspicious for TMA.[1] The next step is to evaluate for causes of secondary TMA or conditions such as disseminated intravascular coagulation or severe cobalamin deficiency that can mimic TMA. Patients with recent diarrheal illness should be evaluated for Shiga toxin–producing *Escherichia coli* (STEC)-HUS.[2] Table 1 summarizes the laboratory workup of a suspected TMA. The most time-sensitive decision is whether therapeutic plasma exchange (TPE) should be initiated emergently for possible iTTP. A secondary goal is to avoid unnecessary risks from central venous catheter insertion and plasma exchange and minimize delays in administering effective therapy with eculizumab or ravulizumab for aHUS.

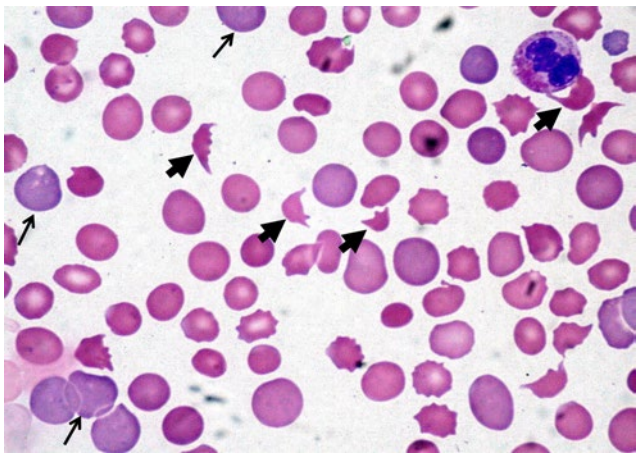

Figure 2. Peripheral blood film showing classic findings of TMA. The image shows features of thrombocytopenia and microangiopathic hemolysis with an increase in schistocytes (***bold arrows***). Reticulocytes (***narrow arrows***) are also increased and indicate increased red cell turnover.

Pathophysiology of iTTP and aHUS

In iTTP, an autoantibody-mediated deficiency of ADAMTS13 causes the accumulation of ultralarge von Willebrand factor (VWF) multimers, leading to platelet aggregation and microvascular thrombi.[3] In contrast, aHUS is caused by dysregulated (uncontrolled) alternative complement pathway activation that triggers inflammation, platelet activation, and direct endothelial injury. Over 50% of patients with primary aHUS have a genetic or acquired defect in complement regulation.[4] aHUS is frequently triggered by a complement-amplifying condition such as infection, pregnancy, or surgery. TMA in the setting of a coexisting condition such as infection, autoimmune disease, cancer, pregnancy, stem cell or solid-organ transplant, or certain medications (tacrolimus, cytotoxic drugs) is often categorized as secondary TMA or secondary aHUS, in which the underlying condition is considered the cause rather than a "trigger." Complement activation is implicated as a driver of some secondary TMAs associated with pregnancy (hemolysis, elevated liver enzymes, and low platelets, or HELLP syndrome), malignant hypertension, autoimmune disorders, and infections.[5] It may be difficult to distinguish whether these conditions are a cause of secondary TMA or a trigger for aHUS.

iTTP vs aHUS: clinical features and clinical prediction tools

Though their clinical presentations are similar, certain features provide clues to the likelihood of iTTP vs aHUS. iTTP

Table 1. Comparison of presenting clinical and laboratory findings in iTTP and aHUS

	Immune TTP[6,7]	aHUS (adult)[8,9,44]
Adult or childhood onset	More common in adults (Congenital TTP more common in children)	Similar prevalence of adults and children
MAHA with thrombocytopenia	100%	~85%
Neurologic findings	Overall, 67%-78% Seizure, 7%-8% Coma, 3%-10% Stroke, 5%-10%	Overall, 8%-43%
Thrombocytopenia	More severe Median: 10–15×10^9/L	Less severe Median: 57–118×10^9/L
Renal dysfunction	Less severe Median serum creatinine: 1.09–1.3 mg/dL	More severe Median serum creatinine: 2.9–4.6 mg/dL
Severe (or malignant) hypertension	N/A, uncommon	Common, 8%-54%
Cardiac involvement	42%	19%
Fever	10%-24%	Not reported
Abdominal symptoms	35%	15%
ADAMTS13 activity (%), median (range)[16]	5 (0–11)	66.5 (12–119)

more often manifests neurologic symptoms and severe thrombocytopenia, but renal impairment is less common.[6,7] In contrast, renal impairment is nearly universal in aHUS, and more than 20% exhibit extrarenal manifestations, including diffuse or focal neurologic events, cardiac injury, and gastrointestinal symptoms including diarrhea, vomiting, and pancreatitis (Table 2).[8,9] New or worsening severe hypertension is a "red flag" for aHUS.[8,9] A recent study reported pathogenic complement variants in over half of patients with malignant hypertension-associated TMA who had poor renal outcomes and did not respond to antihypertensives alone, suggesting that some malignant hypertension may actually be aHUS. While its distinction from malignant hypertension due to other causes is challenging, consider aHUS in young patients without known hypertension or TMA and renal failure that worsens despite blood pressure control.[10]

Table 2. Laboratory evaluation of patients presenting with an acute TMA

Tests	Indication	Utility
CBC, reticulocyte count; LDH; bilirubin (total, indirect), haptoglobin; blood smear	All patients	Establish diagnosis of TMA with thrombocytopenia, hemolytic anemia, and schistocytes.
PT, aPTT, D-dimer, fibrinogen	All patients	Rule out disseminated intravascular coagulation.
ALT, AST; creatinine, urinalysis, cardiac troponin, ECG	All patients	Assess organ damage.
ADAMTS13 activity	All patients	CONFIRM or rule out TTP.
ADAMTS13 inhibitor and antibody	If ADAMTS13<20%	Confirm immune vs congenital TTP. Consider ADAMTS13 sequencing if ADAMTS13 inhibitor and antibody tests are persistently negative and ADAMTS13 activity remains less than 20% in remission.
Stool culture/Shiga toxin	Recent diarrheal illness	Confirm STEC-HUS
Vitamin B_{12}, MMA, homocysteine	Pancytopenia, suggestive blood smear (extreme pleomorphism, macro-ovalocytes, hypersegmented neutrophils	Assess for severe vitamin B12 deficiency that can present as "pseudo-TTP"
Autoimmune screening (eg, antiphospholipid antibodies, lupus anticoagulant, ANA)	aHUS with autoimmune history	Identify secondary TMA vs aHUS trigger
Infectious workup (eg, cultures, HIV)	Based on symptoms and history	Identify secondary TMA vs aHUS trigger
Renal biopsy	If cause of renal injury is unclear	Can distinguish TMA from other cases of renal injury and thrombocytopenia (eg, tubular necrosis from sepsis or medications, posttransplant rejection) but cannot distinguish specific etiology of renal TMA.

ALT, alanine aminotransferase; ANA, antinuclear antibody; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CBC, complete blood count; ECG, electrocardiogram; MMA, methylmalonic acid; PT, prothrombin time.

Severely deficient ADAMTS13 activity is diagnostic of TTP. However, most centers do not perform ADAMTS13 testing in-house, and results of send-out tests can take up to 3 to 7 days. Clinical prediction models, such as the French TTP score and PLASMIC score, use readily available clinical features and laboratory testing to identify patients who should receive emergent TPE (Table 3).[11,12] Importantly, these scores should be applied only to patients with TMA and cannot replace clinical judgment. The French score includes serum creatinine, platelet count, and antinuclear antibody,[11] but the 2-component score with only the platelet count and serum creatinine is most commonly used. A score equal to or greater than 1 has a sensitivity of 98.8% for iTTP and a specificity of 48.1%.[11] Thus, a French score of 0 is useful to rule out iTTP, but a score equal to or greater than 1 does not always rule in iTTP. The PLASMIC score has 7 components and classifies patients as low risk (score <5), intermediate risk (5), and high risk (6-7) for iTTP, and these categories correspond to a 0% to 4%, 5% to 25%, or 62% to 82% probability of iTTP, respectively. An intermediate or higher PLASMIC score (≥5) has 99% sensitivity and 57% specificity for iTTP,[12,13] and this threshold is considered sufficient to start plasma exchange for presumed iTTP. An advantage of the PLASMIC score is that it has been validated in multiple other cohorts and as a meta-analysis of 13 studies.[13] Compared to the French score, it has a higher C statistic (0.93 vs 0.88; *P*=.0032) and classifies fewer patients into the intermediate-risk group compared to the French score.[12] Notably, the sensitivity of both the French and PLASMIC scores decreases to approximately 75% in older adults (>60 years) who are at increased risk of iTTP-related mortality but often present with atypical features such as worse renal function and less profound thrombocytopenia.[14] These tools may also be less reliable in relapsed iTTP, which is often diagnosed early through patients' familiarity with symptoms or laboratory surveillance. Pregnancy-associated TMA represents a particularly high-risk situation, and these scores have not been specifically validated for pregnancy-associated and peripartum TTP. Neither score has been validated in pediatric populations and may require adjustment to incorporate representative measures of renal dysfunction across children of different age groups.

Laboratory diagnosis of TTP

ADAMTS13 activity assays

Most ADAMTS13 activity assays are performed by fluorescence resonance energy transfer (FRET) or chromogenic enzyme-linked immunosorbent assay methods detecting cleavage of a VWF peptide (containing the ADAMTS13 cleavage site) by patient plasma.[15] Similar to functional assays for coagulation factors, results are expressed as a percent of normal (pooled plasma) activity. ADAMTS13 activity greater than 10% is diagnostic of TTP, and levels lower than 20% are inconsistent with TTP.[16] Borderline ADAMTS13 activity between 10% and 20% poses a diagnostic dilemma.[17] In a report from the Oklahoma TTP registry, 3 of 78 patients with confirmed iTTP had ADAMTS13 activity of more than 10%, attributed to differences between ADAMTS13 assays or inhibitory anti-ADAMTS13 antibodies that dissociate in vitro, falsely elevating ADAMTS13 activity.[6] ADAMTS13 activity of 10% to 20% may also be seen in iTTP samples collected after plasma transfusion. ADAMTS13 activity between 20% and 60% is common in patients with inflammatory disorders, sepsis, liver disease, and aHUS.[16,18] Some patients with aHUS have ADAMTS13 in the 10% to 20% range.[16,18] Thus, clinical judgment is critical when interpreting borderline ADAMTS13 activity. Patients with ADAMTS13 activity of 10% to 20% and a high probability of iTTP (based on clinical presentation and PLASMIC score) should be treated as having iTTP, especially if they are responding to TPE. Confirming an ADAMTS13 inhibitor or autoantibody supports the diagnosis of iTTP. However, if the clinical picture is atypical for iTTP (renal failure) and there is no improvement after 3 to 5 sessions of TPE, another diagnosis should be considered.

ADAMTS13 inhibitor and autoantibody assays

Most pathogenic anti-ADAMTS13 antibodies are immunoglobulin G, targeting the spacer domain of ADAMTS13, and have an

Table 3. PLASMIC and French scores for iTTP

Clinical prediction tool	Score components (1 point for each)	Probability of severe ADAMTS13 deficiency		
		Low	Intermediate	High
PLASMIC score	Platelet count <30 × 10⁹/L Hemolysis: reticulocytes >2.5%, undetectable haptoglobin, or indirect bilirubin >2 mg/dL No active cancer No history of solid-organ or stem cell transplant MCV <90 fL INR <1.5 Creatinine <2 mg/dL	0–4	5 PLASMIC score ≥5: sensitivity, 99%; specificity; 57%; NPV, 99%; PPV, 56%[13]	6–7 PLASMIC score ≥6: sensitivity, 85%; specificity, 89%; NPV, 92%; PPV, 81%)[13]
French score	Platelet count <30 × 10⁹/L Serum creatinine <2.26 mg/dL Positive ANA[a]	0	1 French score ≥1: sensitivity 98.8%; specificity 48.1%; NPV, 93.3%; PPV, 85%[11]	≥2 French score 3; sensitivity 96.9%; specificity 98.1%; NPV, 38.6%; PPV, 98.7%

[a]The modified French score that includes only platelet count and serum creatinine is more commonly used (since ANA is rarely available at presentation) and has similar performance metrics.

ANA, antinuclear antibody; INR, international normalized ratio; MCV, mean corpuscular volume.

inhibitory or neutralizing function that can be detected in a Bethesda-like assay.[19,20] The ADAMTS13 inhibitor assay may be negative in patients with nonneutralizing antibodies that accelerate clearance.[21] ADAMTS13 antibody testing detects both neutralizing and nonneutralizing antibodies and is more sensitive for iTTP. However, specificity is lower, as nonneutralizing ADAMTS13 antibodies are present in 4% of healthy individuals and 5% to 13% of those with autoimmune disorders.[22]

Rapid ADAMTS13 testing

Assay time, technical expertise, and the low cost-efficiency of running individual samples compromise the ability of most centers to obtain ADAMTS13 results in the time frame required to make clinical decisions (4-8 hours). Several "rapid" ADAMTS13 assays have been developed, including a semiquantitative screening assay (Technoclone) based on flow-through technology and an automated quantitative chemiluminescence-based immunoassay (HemosIL AcuStar).[15] The semiquantitative Technoclone assay has a sensitivity of approximately 90%.[23] While this assay may help with rapid screening, the sensitivity of 90% suggests that rigid adherence to the prescribed cutoff is not advised and that immediate confirmatory testing must be done in all cases, limiting its utility.[23] In comparison, the HemosIL AcuStar assay provides results in approximately 30 minutes, correlates well with the standard FRET assay with a high level of agreement (kappa=0.97) in identifying samples with severe ADAMTS13 deficiency (<10%), and appears to have discriminatory ability even below the limit of detection of the FRET assay.[24,25] Wider adoption of rapid in-house ADAMTS13 testing is potentially the most impactful intervention to reduce health care costs while improving quality of care by rapidly identifying TTP and avoiding potentially harmful interventions.

Congenital TTP

It is usually impossible to distinguish between cTTP and iTTP at initial presentation, and all patients are treated with TPE and immunosuppression. Presentation in early childhood or a family history of iTTP may be clinical clues to cTTP, and the detection of ADAMTS13 inhibitor or autoantibody confirms iTTP. Patients without anti-ADAMTS13 inhibitor/antibody who do not recover ADAMTS13 activity during clinical remission should undergo sequencing to establish the diagnosis of cTTP (Figure 3).

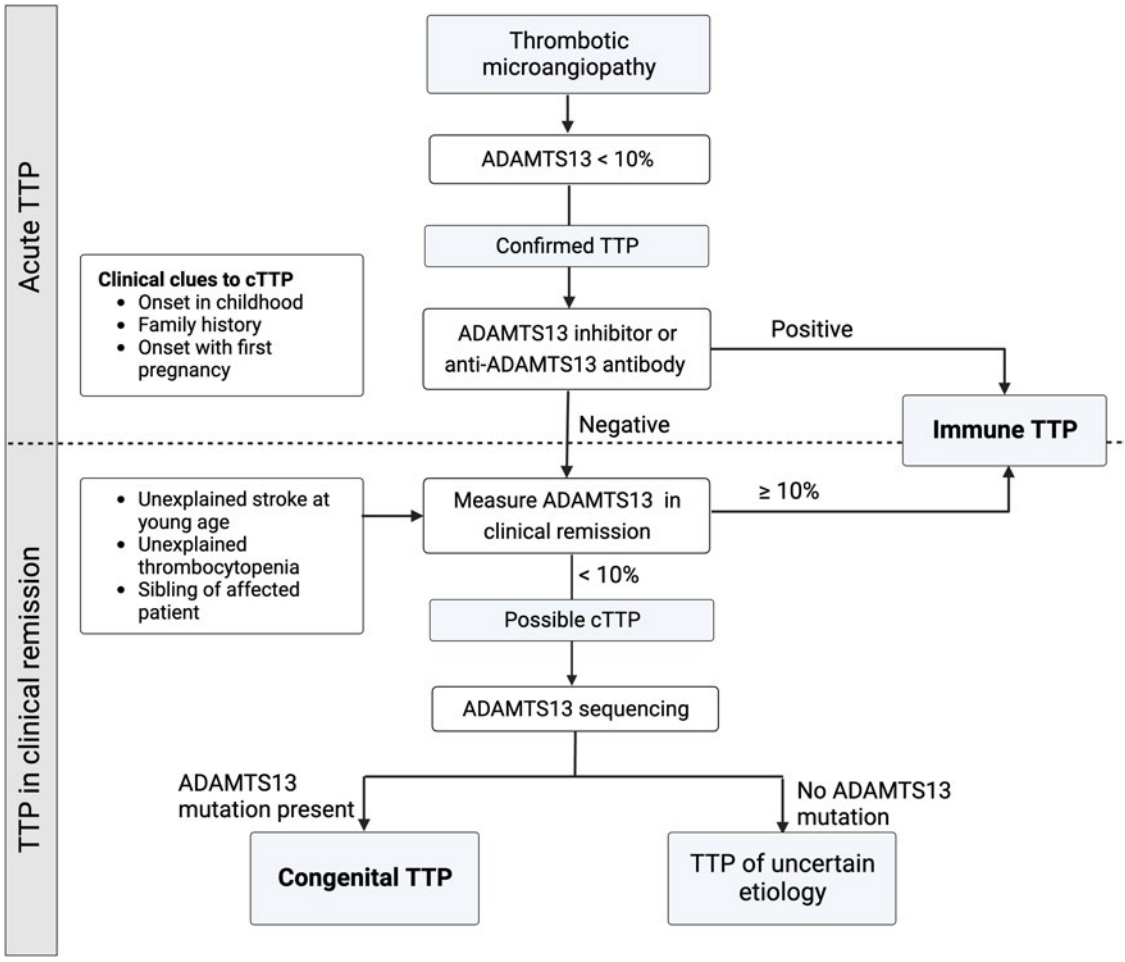

Figure 3. Diagnosing congenital TTP and distinguishing it from iTTP. While some clinical features, such as presentation early in life or a family history of TTP, may support a diagnosis of cTTP, it is virtually indistinguishable from iTTP during an acute episode. The presence of an inhibitor or antibody against ADAMTS13 confirms iTTP. Patients who do not recover ADAMTS13 activity levels over over 10% during clinical remission should undergo ADAMTS13 gene sequencing.

Laboratory diagnosis of aHUS

Complement-directed testing

Available complement testing cannot establish the diagnosis of aHUS, and treatment decisions must be based on clinical presentation and ruling out TTP, STEC-HUS, and secondary TMAs. We limit laboratory evaluation to tests that influence clinical management (Table 1). Serologic testing for complement proteins (eg, C3, C5, soluble C5b-9) or levels of factor H and I is available but has low sensitivity and specificity to identify aHUS.[26] Additionally, variability across assays, effects of preanalytical handling, and significant overlap in levels between different TMAs and healthy individuals limit their use.[27-29] Functional assays that rely on the in vitro deposition of C5b9 on endothelial cells or measure complement-dependent cell killing (modified Ham assay) appear to better discriminate between aHUS and non–complement-mediated TMA.[30,31] However, these assays are not standardized and are currently available only in the research setting.

Sequencing to identify pathogenic variants in complement regulation genes and enzyme-linked immunosorbent assay for factor H autoantibody should be sent for patients with aHUS, but these results are usually not available for several weeks and do not affect the decision to start anticomplement therapy.

Genetic testing does impact long-term management by informing recurrence risk after renal transplant or discontinuing complement inhibition, and aids in counseling first-degree relatives.[2] Complement mutations are found in 50% of aHUS as well as a significant proportion of patients with secondary TMAs, including malignant hypertension, HELLP syndrome, or catastrophic antiphospholipid syndrome.[10] In the future, ultrafast genomic sequencing may aid the management of acute TMA, including the identification of rare, non–complement-mediated genetic disorders (eg, cobalamin C deficiency, *DGKE* mutations) and the confirmation of pathologic variants in complement genes.[32] However, while genetic testing can help confirm a disgnosis of aHUS, it cannot be used to rule out a complement-mediated TMA.

Should I start plasma exchange or eculizumab urgently?

The decision to start TPE for likely iTTP vs up-front therapy with a complement inhibitor for suspected aHUS is influenced by the availability of rapid ADAMTS13 testing (Figure 4). When ADAMTS13 testing with a timely turnaround (4-8 hours) is not available, we recommend starting TPE and high-dose corticosteroids immediately in patients presenting with a TMA at intermediate or high risk for iTTP (PLASMIC score ≥5). If TPE is delayed due to a need for vascular catheter placement or transfer to a TPE-capable facility, we recommend transfusing plasma (10-15 mL/kg) and starting corticosteroids as a temporizing measure. For patients considered "low risk" for iTTP and worsening renal impairment, consider early initiation of anti-C5 therapy. The decision to continue TPE depends on ADAMTS13 results and clinical response. If ADAMTS13 activity is greater than 20%, TPE should be stopped since it does not improve outcomes in non-TTP TMAs, and we suggest starting a terminal complement inhibitor for likely aHUS. If renal function or clinical status worsens on TPE and ADAMTS13 results are not yet available, consider switching to anticomplement therapy for presumed aHUS.

When available, rapid-turnaround ADAMTS13 testing can be used to distinguish between iTTP and aHUS. This reduces unnecessary TPE and delays in initiating effective therapy with eculizumab or ravulizumab in aHUS and facilitates the earlier use of targeted therapies (caplacizumab and rituximab) in iTTP.

Acute iTTP treatment—adjuncts to plasma exchange

In addition to plasma exchange, immunosuppression to suppress the anti-ADAMTS13 antibody is recommended. Most patients receive corticosteroids (usually prednisone at 1 mg/kg/d).[17] Rituximab, an anti-CD20 monoclonal antibody that suppresses the production of anti-ADAMTS13 antibodies and reduces iTTP relapse, is also recommended for de novo and relapsed iTTP but should be started only after ADAMTS13 deficiency is confirmed. Caplacizumab is a nanobody directed against the A1 domain of VWF that inhibits VWF-dependent platelet aggregation and microthrombi formation.[33] In the phase 3 HERCULES trial, caplacizumab along with TPE and immunosuppression led to faster resolution of thrombocytopenia and a 74% reduction in the composite end point of iTTP-related death, recurrence, and major thromboembolic events.[33] Real-world experience also suggests that caplacizumab reduces refractoriness.[34] The International Society on Thrombosis and Haemostasis guidelines on iTTP conditionally suggest using caplacizumab in acute iTTP (new diagnosis and relapsed disease) and starting caplacizumab even while waiting for ADAMTS13 testing since the benefit is likely maximized when started early.[17] However, the rate of serious adverse events, particularly bleeding, is significantly increased with caplacizumab.[17,33,35] Additionally, caplacizumab is not cost-effective based on acute iTTP outcomes at its current list price (approximately $270,000) in the United States.[36] In contrast, a National Institute for Health and Care Excellence analysis from the United Kingdom accepted caplacizumab (with a different pricing agreement) as a cost-effective use of resources for a rare and highly morbid disease.[37] None of these analyses were able to account for the potential impacts of caplacizumab on longer-term neurocognitive outcomes or of newer dosing strategies, which still need to be evaluated.[38] Our practice is to start caplacizumab once ADAMTS13 at less than 10% is confirmed, outpatient availability of medication is assured, and bleeding risk is not increased (recent bleeding or antithrombotic therapy that cannot be stopped while on caplacizumab). For patients with severe neurologic symptoms, we consider starting caplacizumab before ADAMTS13 testing is completed. Platelet transfusions are associated with adverse outcomes, including mortality in iTTPn and should be used only for life-threatening hemorrhage.[39]

CLINICAL CASE (continued)

Our patient had an intermediate PLASMIC score of 5 and was started on TPE. Two days later, the platelet count was 38 × 10⁹/L, but she developed anuric renal failure with a creatinine level of 3.8 mg/dL. TPE was stopped and eculizumab started, with improvement in her platelet count and hemolytic markers and, ultimately, the recovery of renal function. Five days after presentation, ADAMTS13 activity resulted as 56%.

Practical approach to the treatment of aHUS

Treatment with an anti-C5 monoclonal antibody is the standard of care for treating aHUS based on prospective

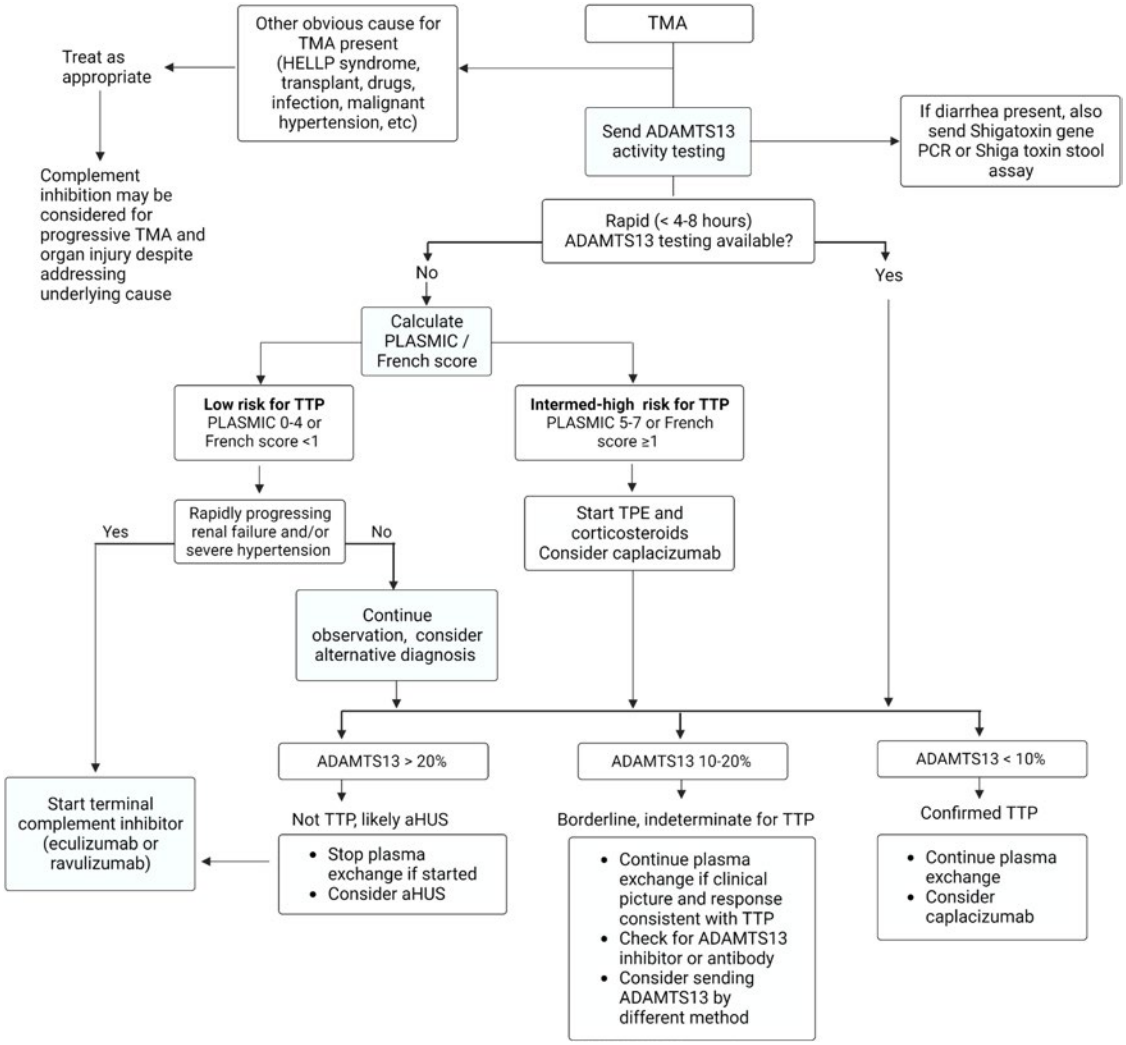

Figure 4. Approach to the initial evaluation and management of a patient with TMA. Early evaluation is focused on distinguishing iTTP or aHUS from secondary TMAs and minimizing delays in treating iTTP that is imminently life-threatening. The diagnostic pathway varied based on the availability of rapid-turnaround ADAMTS13 assays. PCR, polymerase chain reaction.

nonrandomized trials that showed its clear benefit in improving hematologic TMA and renal function.[2,40] The risk of end-stage renal disease and death has been reduced from 50% to 6% to 15% at 1 to 2 years.[40] In the initial studies (trial 1, N=17 and trial 2, N=20) that led to the approval of eculizumab for aHUS, earlier treatment was associated with significantly greater improvement in glomerular filtration rate throughout the treatment period, though the median time to treatment initiation was several months (not days) in both trials.[40] In extension studies, patients in trial 1 who were treated earlier showed ongoing improvement even after 1 year.[41] In a recent pharmacoeconomic analysis, early (≤7 days) initiation of anti-C5 therapy is associated with 3.2 times lower odds of needing dialysis and lower health care utilization and cost.[42] Both eculizumab and the longer-acting ravulizumab are approved for the treatment of aHUS. We prefer eculizumab for the initial treatment of aHUS because the cost of a therapeutic trial is lower in the setting of diagnostic uncertainty, where response to treatment often serves as the final diagnostic tool. Renal function recovery may be faster with eculizumab than ravulizumab, but this comparison is limited by differences in the cohorts in the studies evaluating eculizumab and ravulizumab. Patients in the ravulizumab cohort were older and had a lower rate of complement mutations.[2]

Distinguishing secondary aHUS from aHUS with a trigger is challenging, and the benefit of C5 blockade in most secondary aHUS is not unequivocally established. However, recent research has demonstrated a role of complement as a pathogenic mechanism or shown the efficacy of complement blockage in secondary TMAs due to autoimmune disorders (lupus and antiphospholipid syndrome), pregnancy, and some infections and drugs, including chemotherapeutics.[5] For these secondary TMAs, we suggest eculizumab as a second-line therapy if addressing the underlying condition does not lead to improvement in the TMA and renal function.[2,5] We suggest at least 2 weeks trial of therapy. Improvement in platelet count and LDH are expected within 10 to 14 days, although renal recovery is time dependent and may take months.[40,43]

Conclusion

Early and effective therapy for iTTP and aHUS drastically improves outcomes for these potentially devastating conditions. Currently, early treatment decisions must be made based on clinical features, tools such as the PLASMIC score, and ADAMTS13 testing, when available. Wider adoption of rapid ADAMTS13 testing will mitigate treatment delays, lower costs, and improve outcomes for both iTTP and aHUS. Functional complement assays and ultrarapid gene sequencing are being developed, which may help with diagnosing aHUS and predicting the response to complement inhibition in secondary TMAs. Improved diagnostic assays are critical to optimize the use of novel therapies in iTTP and aHUS.

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Conflict-of-interest disclosure

Charlotte M. Story: no competing financial interests to declare.

Gloria F. Gerber: advisory board: Apellis Pharmaceuticals, Alexion Pharmaceuticals; honorarium: Merck; stockholder (spouse): Pfizer.

Shruti Chaturvedi: advisory board: Alexion, Sanofi, UCB, Sobi, Takeda; consultancy: Alexion, Sanofi, UCB, Sobi, Takeda; honoraria/royalties: UpToDate.com, Dynamed.com.

Off-label drug use

Charlotte M. Story: All authors discuss the-off label use of rituximab for immune TTP, and eculizumab/ravulizumab for secondary TMA.

Gloria F. Gerber: All authors discuss the-off label use of rituximab for immune TTP, and eculizumab/ravulizumab for secondary TMA.

Shruti Chaturvedi: All authors discuss the-off label use of rituximab for immune TTP, and eculizumab/ravulizumab for secondary TMA.

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Consumptive coagulopathy in the ICU

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A consumptive coagulopathy describes a situation where there is a loss of hemostatic factors, which leads to an increased risk of bleeding. Some recent studies have used the term interchangeably with disseminated intravascular coagulation (DIC), but we have reverted to the older definition, which covers a broader range of issues where there is loss of hemostatic factors due to multiple causes, which includes systemic activation of coagulation as seen in DIC. Therefore, the term *consumptive coagulopathy* covers conditions from the hemostatic effects of major hemorrhage to the use of extracorporeal circuits to true DIC. We review the current understanding of the pathophysiology, diagnosis, and management of common consumptive coagulopathy in critical care patients, focusing on recent advances and controversies. Particular emphasis is given to DIC because it is a common and often life-threatening condition in critical care patients and is characterized by the simultaneous occurrence of widespread microvascular thrombosis and bleeding. Second, we focus on the effect of modern medical technology, such as extracorporeal membrane oxygenation, on hemostasis.

LEARNING OBJECTIVES

- To describe the causes and multiple mechanisms contributing to developing consumptive coagulopathy in critical care patients
- To describe the management of consumptive coagulopathies in critically ill patients and consider the potential role of emerging therapies

Introduction

A coagulopathy is a derangement of hemostasis that can result in excessive bleeding and/or thrombosis. A consumptive coagulopathy describes a situation with a loss of hemostatic factors so that a patient is at increased risk of bleeding.[1] Recently, this term has become synonymous with the term *disseminated intravascular coagulation* (DIC). We disagree with this development and have returned to the old definition where a consumptive coagulopathy covers the loss of hemostatic factors due to any cause, not just the uncontrolled activation of coagulation factors seen in DIC. Therefore, it includes patients who have had significant bleeding and/or those using an extracorporeal circuit.

Making a diagnosis of consumptive coagulopathy is a clinicopathologic diagnosis; it requires a clinical evaluation of the patient. We will explore the pathogenesis and management of the common consumptive coagulopathies in critical care through a clinical case. The laboratory changes of common coagulopathies seen in critical care are summarized in Table 1, and the causes of thrombocytopenia are in Table 2.

CLINICAL CASE

A 28-year-old man presented to our hospital after a short history of flu-like symptoms and increasing shortness of breath. His electrocardiogram showed atrial tachycardia with a heart rate of 150 bpm. The chest radiograph showed widespread bilateral pulmonary infiltrates. He was hypoxemic (P/F ratio <140/normal >400, <300 indicates acute respiratory failure; the P/F ratio is the ratio of the partial pressure of oxygen in arterial blood (PaO_2) divided by the fraction of inspired oxygen), shocked with a high lactate (8.9 mmol/L/normal <1.5 mmol), oliguric with acute kidney injury, and thrombocytopenic (platelets, 83×10^9/L). The aspartate aminotransferase (AST) was raised at 2600 IU with an activated partial thromboplastin time (aPPT) of 46 seconds. A transthoracic echocardiogram showed reduced

Table 1. Laboratory findings in the coagulopathies of critical care

Condition	PT	aPPT	Fibrinogen levels	D-dimer	Bleeding time	Platelet count	Film comments
Liver failure, early	Prolonged	Unaffected	Unaffected	Unaffected	Unaffected	Unaffected	
Liver failure, end stage	Prolonged	Prolonged	Low	Increased	Prolonged	Decreased	
Uremia	Unaffected	Unaffected	Unaffected	Unaffected	Prolonged	Unaffected	
Disseminated intravascular coagulation	Prolonged	Prolonged	Low	Increased	Prolonged	Decreased	Fragmented red cells
Thrombotic thrombocytopenic purpura	Unaffected	Unaffected	Unaffected	Unaffected	Prolonged	Very low	Fragmented red cells
Hyperfibrinolysis	Prolonged	Prolonged	Low	Very high	Possibly prolonged	Unaffected	

Table 2. The differential diagnosis of thrombocytopenia in critical care

First rule out pseudothrombocytopenia

- Clotted blood sample
- EDTA-dependent antibodies (collect CBC in anticoagulant such as citrate)
- Review medications especially
  - Heparins, including heparin-associated thrombocytopenia
  - IIb/IIIa inhibitors (abciximab, eptifibatide, tirofiban)
  - Adenosine diphosphate receptor antagonists (clopidogrel)
  - Acute alcohol toxicity

Rule out hematinic deficiency, particularly acute vitamin B12 deficiency which has recently been associated with nitrous oxide abuse

Investigation for the consumption of platelets:

- Sepsis
- Major blood loss
- Mechanical fragmentation
  - Post–cardiopulmonary bypass
  - Intra-aortic balloon pump
  - Renal dialysis
  - ECMO
- Immune mediated
  - Immune thrombocytopenic purpura
  - Antiphospholipid syndrome
  - Posttransfusion purpura
- With a microangiopathic hemolytic anemia
  - Disseminated intravascular coagulation
  - Thrombotic thrombocytopenic purpura
  - Hemolytic uremic syndrome
- Hypersplenism
- Other
  - Myelodysplastic syndrome
  - Malignancy
  - Hereditary thrombocytopenia

CBC, complete blood count.

biventricular function. A respiratory virus panel was positive for influenza B; latterly, he was confirmed to have coinfection with a toxin-producing group A streptococcus. He was treated with oseltamivir and benzylpenicillin. He was placed on venovenous extracorporeal membrane oxygenation (ECMO) for severe respiratory failure. A pan-body computed tomography scan immediately postcannulation confirmed almost complete consolidation/opacification of both lungs, satisfactory cannula placement, and a subsegmental pulmonary embolism in the left lower lobe of the lung. He was placed on high-dose renal replacement therapy (35 mL/kg/h). Three hours after cannulation, there was ongoing, continuous ooze of blood at the cannulation sites, and repeat bloods showed the platelet count had fallen to 28×10^9/L, prothrombin time (PT) ratio of 1.8, aPTT of 65 seconds, fibrinogen of 1.6 g/L, and anti-Xa of 0.6 (target range, 0.3–0.5 IU/mL). He was not bleeding but was given a pool of platelets to increment his platelet count to $>50\times10^9$/L according to our local protocol for venovenous ECMO, and his unfractionated heparin infusion was held until the anti-Xa level was <0.5 IU/mL.

Disseminated intravascular coagulation

Patients with DIC may present with bleeding, thrombosis, or a combination of both.[2] Our patient had DIC secondary to overwhelming sepsis and presented with multiple-organ failure. The diagnosis of DIC is based on clinical suspicion, laboratory findings, and the exclusion of other causes of coagulopathy. The International Society on Thrombosis and Haemostasis scoring system includes platelet count, fibrinogen level, PT, and D-dimer levels.[3] A score of 5 or higher is consistent with overt DIC. DIC is characterized by widespread coagulation system activation, resulting in intravascular thrombosis in small vessels and critical organ dysfunction. Hemorrhage occurs due to the loss of hemostatic factors.[3,4] DIC is a complication of many medical conditions, including sepsis, trauma, malignancy, and obstetric complications, but sepsis is the most usual cause within critical care, present in around 30% of cases of severe sepsis. All classes of infectious organisms can cause DIC.

Severe infection induces a complex interplay between the innate immune system, inflammation, endothelial activation, and coagulation.[3] Coagulation is primarily driven by proinflammatory cytokines, such as tumor necrosis factor–α and interleukin 1, promoting tissue factor expression on monocytes. Endothelial activation results in increased permeability and a switch from an anticoagulant to a prothrombotic phenotype.[2,5] Procoagulant microparticles are released, leading to further coagulation activation.[4] Increased levels of plasminogen activator inhibitor 1 inhibits tissue plasminogen activator (t-PA) and thus contributes to the accumulation of microvascular thrombosis.[4]

The role of neutrophil extracellular traps in DIC

Neutrophil extracellular traps (NETs) are a complex web of DNA, nucleosomes, histones, and neutrophil-derived granular proteins ejected from activated neutrophils.[2,5-7] They ensnare and neutralize pathogens, preventing their dissemination away

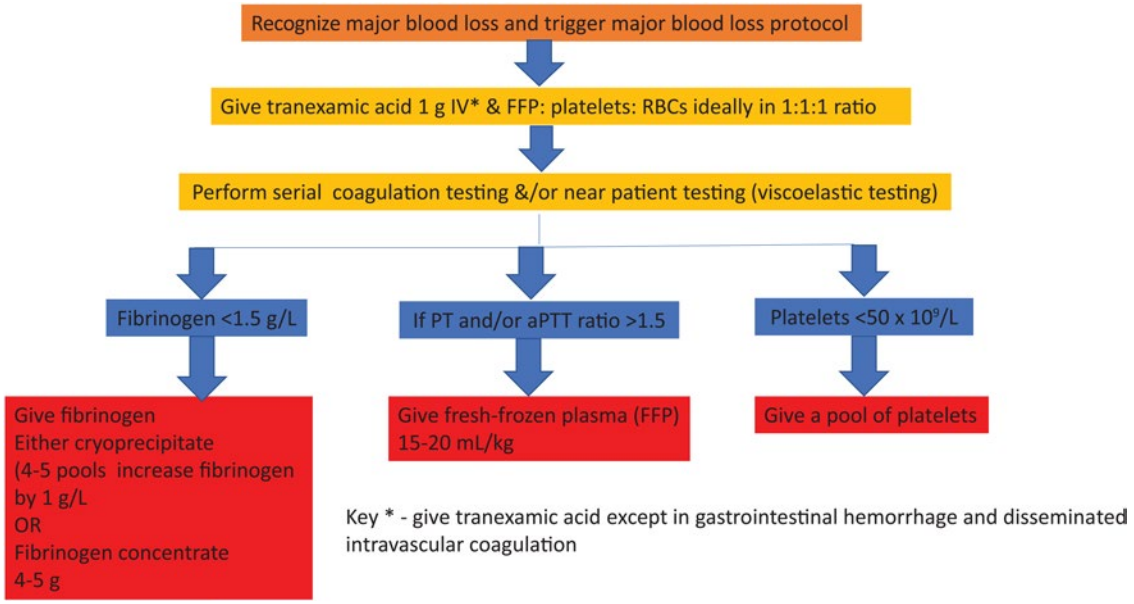


Figure 1. Suggested simple algorithm for the hemostatic management of major bleeding.

from the origin of the infection.[8] During sepsis, the overproduction of NETs and/or the inability to clear them can instigate a vicious cycle of coagulation and inflammation.[7] NETs stimulate coagulation by binding to prothrombin fragment 1 (F1) and fragment 2 (F2) specifically to facilitate FXa cleavage of prothrombin to release active thrombin, unlike FVa, which requires phospholipid surfaces to anchor the classical prothrombinase complex (DOI: 10.1182/blood.2019002973). NETs also stimulate platelets and inactivate tissue factor pathway inhibitor.[2,5] Simultaneously, histones and other NET-associated proteins inflict direct cytotoxic damage on endothelial cells.[9] This endothelial damage fuels the inflammatory response, exacerbating the coagulopathy. Thrombin can also induce NETosis, creating a vicious cycle of inflammation and coagulation,[10] leading to the rapid progression of DIC. Our new understanding of NETs makes them a future therapeutic target. Potential options include the following:

1. **DNase therapy**: DNase is capable of degrading DNA and could potentially dismantle NETs.
2. **Neutrophil elastase and myeloperoxidase inhibitors**: Neutrophil elastase and myeloperoxidase are critical NET components that can propagate inflammation and thrombosis.
3. **PAD4 inhibitors**: PAD4 mediates histone citrullination, which is crucial for NET formation. PAD4 inhibitors decrease NET-associated thrombosis in murine models.[11]
4. **Non-anticoagulant heparin**: Non-anticoagulant forms of heparin bind to histones and can reduce histone-mediated cytotoxicity without increasing the risk of bleeding in a murine model of sepsis.[12]

However, caution is required given the vital role of NETs in innate immunity, and great care will be required in designing trials to prevent harm.

Management of disseminated intravascular coagulation

The cornerstone of managing DIC is the early and aggressive treatment of the underlying condition, such as administering antibiotics for sepsis, as in our case outlined above.[3,4,13] Supportive measures, including fluid resuscitation, blood product transfusion, and organ support, play a crucial role. Algorithm-led transfusion of platelets, fresh-frozen plasma (FFP), and cryoprecipitate are indicated in patients with significant bleeding or at high risk for bleeding due to invasive procedures (see the suggested algorithm in Figure 1, but note not to use tranexamic acid; fibrinolysis is required to break down established thrombi during recovery). However, the optimal thresholds for transfusion and the use of prophylactic transfusion in nonbleeding patients remain controversial due to the lack of clinical trials.[4]

Trials of pharmacologic doses of the physiologic anticoagulants antithrombin, protein C, and activated protein C in sepsis have not been shown to improve clinical outcomes and caused bleeding.[14,11] There have been years of ongoing interest in using low doses of heparin to "switch off" the prothrombotic drive from tissue factor. There is, however, minimal evidence supporting the routine use of heparin in acute DIC, and further research is needed. We would consider, however, using thromboprophylactic dose heparins in selected patients with thrombotic phenotypes of DIC, particularly in sepsis or malignancy.[15]

Recovery from the widespread microvascular thrombosis that is characteristic of DIC is dependent on breakdown by endogenous fibrinolysis. We recommend that antifibrinolytic drugs are not used in DIC because they will inhibit endogenous fibrinolysis and therefore recovery.

The prognosis of DIC is mainly dependent on the cause, the severity of the coagulopathy, and the presence of associated organ dysfunction,[4] and the mortality rates in patients with DIC are high, ranging from 20% to 50%.[15] Factors associated with poor outcomes in DIC include advanced age, high Acute Physiology and Chronic Health Evaluation II scores, and the presence of multiple-organ dysfunction syndrome.

CLINICAL CASE (continued)

Twelve hours after cannulation, the patient's oxygenation had improved, but there was no improvement in the lactate, and he remained profoundly shocked on high doses of vasopressors. Coagulation testing showed a PT ratio of 1.4, aPTT of 52 seconds, fibrinogen of 2.0 g/L, and platelets of 58×10^9/L. An echocardiogram showed that the left ventricular function had deteriorated even further. Therefore, the patient was placed on peripheral venoarterial venous ECMO. Within an hour, however, the left ventricular function had deteriorated to such an extent that the aortic valve was no longer opening. A cardiac Impella was inserted to vent the left ventricle. There was a progressive improvement in organ perfusion, and after that, the inotropes and vasopressors were weaned. The management of the Impella was complicated by significant bleeding from the insertion site in the left groin. His hemoglobin fell to 62 g/L, platelets were 22×10^9/L, PT ratio was 2.1, aPPT ratio was 1.9, and fibrinogen was 0.8 g/L. He was resuscitated with intravenous fluids and transfusion with packed red cells, a pool of platelets, 2 pools of cryoprecipitate, and FFP at an approximate dose of 20 mL/kg. Repeated coagulation testing showed prolonged aPTT and PT ratios >1.5 and fibrinogen <1.5 g/L, so further FFP and cryoprecipitate were given.

Our patient had established DIC secondary to sepsis exacerbated by severe cardiac failure and renal and hepatic dysfunction.

The contribution of liver failure to coagulopathy

Liver failure results in reduced synthesis of coagulation factors and physiologic anticoagulants,[1] but there is a balanced reduction so that while the PT and aPTT may be prolonged, the levels of coagulation factors will be the same as the physiologic anticoagulants: indeed, thrombin generation is preserved.[16] Thrombocytopenia occurs due to splenic sequestration and reduced thrombopoietin production. Last, the failure to clear t-PA results in higher plasma levels, increasing the fibrinolytic potential. Liver failure and DIC produce similar laboratory coagulation changes (see Table 1), emphasizing that any patient with these changing needs clinical review. A history of cirrhosis, excess alcohol consumption, and/or ascites points more toward liver disease. Thrombocytopenia is "relatively" stable in liver disease, and D-dimers are only modestly elevated compared to DIC.[17]

We recommend that critically ill patients receive vitamin K to aid liver synthesis of vitamin K–dependent coagulation factors; usually adequate amounts are present in intravenous and intragastric feeding. If a patient is not being fed, then vitamin K can be given intravenously.

Warkentin and Ning have recently developed an interesting hypothesis as to why some patients with DIC develop a symmetrical peripheral gangrene.[18] They describe that these patients always have severe shock, DIC, and usually acute ischemia of the liver (shock liver), the latter being the cause of physiologic anticoagulant depletion. The time course of symmetrical peripheral gangrene is usually a few days after the start of shock, in keeping with critical depletion of protein C (plasma t1/2 8 hours) and antithrombin (plasma t1/2 of 20 hours). Other risk factors are chronic liver disease and possible transfusion of colloids such as intravenous IgG and albumin. As yet, there are no detailed studies of protein C and antithrombin in this patient group. Warkentin and Ning suggest that treatment would be to replete physiologic anticoagulants by giving plasma or using plasma exchange.[18]

The contribution of renal failure to coagulopathy

Up to 30% of patients in critical care have acute renal failure,[19,20] and this brings an increased risk of bleeding, thrombosis, and mortality.[21] The "hemostatic" lesion is multifactorial and encompasses platelet dysfunction, increased levels of coagulation factors, and impaired fibrinolysis due to increased levels of plasminogen activator inhibitor 1 and anemia. Uremic toxins impair platelet adhesion, activation, and aggregation. Reduced clearance of von Willebrand factor, factor VIII, and fibrinogen and simultaneously reduced synthesis of the physiologic anticoagulants protein C, protein S, and antithrombin are present. Anemia is associated with loss of axial flow, so there is diminished platelet–vessel wall interaction and, thus, a prolonged bleeding time. Recently, the estimated glomerular filtration rate (eGFR) has been shown to be an independent predictor of hypercoagulability and platelet dysfunction.[22]

The hemostatic effects of extracorporeal circuits

Extracorporeal circuits are vital for short-term use in cardiac surgery and hemodialysis or for prolonged life-sustaining periods when ECMO is used. The surfaces of the circuit activate coagulation, and there is a particular fall of levels of plasma fibrinogen, which rapidly binds to and coats the surfaces. Additionally, the mechanical forces from the ECMO pump and the inflexible shape of the circuit result in areas of high sheer stress, which induce the fragmentation of red cells, platelets, and large molecules such as high-molecular-weight von Willebrand's multimers.[23] The latter leads to an acquired von Willebrand syndrome. Management of anticoagulation with extracorporeal circuits is covered in Table 3.

CLINICAL CASE (continued)[4]

The patient remained critically ill, but his peripheral perfusion and coagulation improved (PT ratio 1.2, aPTT 50 seconds, fibrinogen 2.5 f/L, platelets 51×10^9/L) after removing the Impella. He was weaned from ventilation, and the arterial cannula was removed. He was fully liberated from ECMO on day 9 of admission. The patient's course is summarized in Table 4.

The Impella CP device

A vital aspect of this patient's course was the rapid deterioration in left ventricular function. The associated blood stasis can result in thrombosiss in all 4 cardiac chambers and is almost impossible to treat. The Impella device (ABiomed) is a percutaneous left ventricular assist device. It provides temporary support to assist the pumping function of the heart in patients with severe heart failure or undergoing high-risk angiography procedures. It directly pumps blood from the left ventricle to the aorta, bypassing the weakened or failing heart. Impella devices are minimally invasive and are inserted through a catheter-based approach. The catheter is typically introduced into the femoral artery in the groin and advanced up to the left ventricle under

Table 3. Management of anticoagulation and bleeding with extracorporeal circuits

Prior to commencing anticoagulation in ECMO measure	Range	Result
• Hemoglobin	<70 g/L	Y/N
• Platelet count	>50×10⁹/L	Y/N
• PT/INR	PT/INR <2.0	Y/N
• aPTTr	aPTTr <2.0	Y/N
• Anti-Xa level	Anti-Xa level <0.7	Y/N
• Fibrinogen	>1 g/L	Y/N
• Triglycerides	<400 mg/dL	Y/N
• Bilirubin	<6 mg/dL	Y/N
• Active bleeding		Y/N
• No CVA within last 4 weeks		Y/N
Only commence anticoagulation if all the above answers are Y		
• If platelets <50×10⁹/L, transfuse 1 pool of platelets and recheck • If INR >2 or aPTTr >2, transfuse 15 mL/kg FFP • If fibrinogen <1 g/L, transfuse 15 mL/kg		
Starting dose of UFH 18 IU/kg/h using adjusted body weight		
• If total body weight is less than adjusted body weight, use the patient's ACTUAL weight • Dose is capped at 100 kg		
Once 2 consecutive anti-Xa levels are within the therapeutic range, without a pause/interruption to the infusion or a dose adjustment being required, anti-Xa monitoring can be reduced to once daily.		
Target anti-Xa 0.3 to 0.5		
Daily bloods	**Range**	
• Hemoglobin	<70 g/L	• Maintain hemoglobin >70
• Platelet count	>50×10⁹/L	• Maintain platelets >50
• INR	INR <2.0	
• aPTTr	aPTTr <2.0	
• Anti-Xa level	Anti-Xa level <0.7	• If >0.7, hold UFH infusion
• Fibrinogen	>1 g/L	• Maintain >1 g/L
• Triglycerides	<400 mg/dL	• Review sedation
• Bilirubin	<6 mg/dL	• Hemolysis screen
If anti-Xa in range and aPTTr <2, no change to anticoagulation		
If anti-Xa in range and aPTTr >2, investigate for DIC, ensure fibrinogen >1		

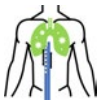
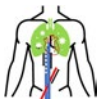
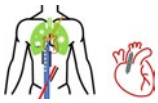
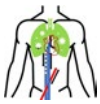
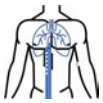
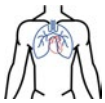
aPTTr, activated partial thromboplastin time ratio; CVA, cerebral vascular accident; INR, international normalised ratio; UFH, unfractionated heparin.

x-ray guidance. Once properly positioned, the Impella device unfolds and deploys its microaxial pump within the left ventricle. It draws blood from the left ventricle through an inlet area and expels it into the ascending aorta through an outlet area, assisting the heart's pumping function. Three types of Impella are available, providing between 2.5 and 5 L of cardiac support each minute. By direct comparison, on average, an intra-aortic balloon pump increases the cardiac output by approximately 0.5 to 1 L. While the Impella can be highly efficacious, its use can be complicated by significant blood loss,[25] the pathogenesis being multifactorial. The Impella contains a purge system where heparin is "purged" through the motor housing to prevent it from ceasing. It is our practice to move to a bicarbonate purge if the Impella is associated with hemorrhage and to provide systemic anticoagulation with heparin as per our routine ECMO anticoagulation protocols. A meta-analysis demonstrated a higher in-hospital mortality in patients who developed bleeding complications following Impella placement when compared to those who did not.[24] Meticulous attention to the device insertion and careful monitoring of hemostasis are essential. Our practice targets an anti-Xa level of 0.3 to 0.5 IU/mL.

The consumptive coagulopathy of hemorrhage

Our current understanding of the pathogenesis of bleeding comes mainly from research on traumatic coagulopathy.[26] Previously, the management of hemorrhage was to restore oxygen-carrying capacity and volume with red cells and intravenous fluids and manage coagulopathy later. However, clinical trials in trauma have shown better outcomes in those treated with the early use of plasma and platelet products,[26] and this principle has been applied to all types of bleeding.[26] Global differences in the management of bleeding reflect the lack of a sound

Table 4. Summary of the clinical case

	The first figure shows extensive consolidation of the lung field secondary to coinfection with influenza and group A streptococcus. The patient was placed on bifemoral venovenous ECMO. The return cannula is placed just at the atrial caval junction. The access cannula for drawing in blood sits approximately 5 to 10cm below the return cannula to prevent the recirculation of freshly oxygenated blood.
	The second figure shows extensive lung consolidation with evolving cardiogenic shock and biventricular failure. An additional 15 Fr arterial return pipe was placed in the right femoral artery. This improved arterial perfusion but increased the afterload and pressure on the failing left ventricle.
	The third figure illustrates the placement of a cardiac Impella* across the aortic valve. The patient's heart was so weak that it could no longer eject against the increased pressure generated by the ECMO arterial return. This is a potentially devastating complication as it leads to blood stasis and can result in thrombi in all 4 cardiac chambers and severe LV dilation and pulmonary edema. The Impella is, in essence, an Archimedes screw, which adds 2.5L to the cardiac output and promotes forward flow, reducing the risk of stasis.
	The fourth figure shows the patient slowly improving. The placement of the Impella was complicated by both hemorrhage and hemolysis. Once the left ventricle had sufficiently recovered, it was removed.
	The fifth figure illustrates the progressive improvement in pneumonia and the patient's return from venoarterial venous to venovenous ECMO for just respiratory support.
	The patient was finally decannulated from ECMO on the ninth day of their ITU. They were liberated from mechanical ventilation on day 12 and fully recovered.

*The image of the Impella was taken and modified from flaticon. www.flaticon.com/free-icons/bolt" title="bolt icons." Bolt icons created by Freepik–Flaticon. ITU, Intensive Therapy Unit; LV, left ventricle.

evidence base. Our suggested simple algorithm is in Figure 1. Hypofibrinogenemia is common in hemorrhage, for the fall in fibrinogen levels is greater than other coagulation factors due to loss in bleeding, consumption in clots, and fibrin(ogen)lysis. FFP lacks enough fibrinogen to achieve a fast rise in levels, so an additional source of fibrinogen is required.[27]

Multiple pragmatic trials have shown reduced mortality with tranexamic acid in traumatic and obstetric hemorrhage, as well as in preventing surgical bleeding.[28,29] A meta-analysis of 216 trials (125 550 patients) showed no increased risk of thrombosis within 8 hours of use.[29]

There are few randomized controlled trials comparing the use of prothrombin complex concentrate (PCC) instead of FFP in the management of major hemorrhages. While its use is safe and recommended in the reversal of vitamin K antagonists, the lack of evidence in major bleeding means it cannot be recommended at this current time. Indeed, a recent trial in trauma—the PROCOAG trial—showed no improvement in clinical outcome but significantly increased thrombotic rates.[30]

Conclusion

Consumptive coagulopathy is common in critically ill patients. Further research is required to improve understanding of hemostatic pathogenesis, refine diagnostic criteria, and develop evidence-based therapeutic interventions.

Conflict-of-interest disclosure

Andrew Retter consults for Volition Diagnostics UK. He has received fees for presentations and reimbursement for travel to conferences. Volition Diagnostics was not involved with this article in any way.

Beverley J. Hunt: no competing financial interests to declare.

Off-label drug use

Andrew Retter: There is no specific off label drug use. DNase therapy, neutrophil elastase and myeloperoxidase inhibitors, and PAD4 inhibitors and non-anticoagulant therapy are suggested as potential future therapies.

Beverley J. Hunt: There is no specific off label drug use. DNase therapy, neutrophil elastase and myeloperoxidase inhibitors, and PAD4 inhibitors and non-anticoagulant therapy are suggested as potential future therapies.

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