



IMMUNOTHERAPIES FOR NONMALIGNANT HEMATOLOGIC DISEASES

Emerging approaches to improve allogeneic hematopoietic cell transplantation outcomes for nonmalignant diseases

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Many congenital or acquired nonmalignant diseases (NMDs) of the hematopoietic system can be potentially cured by allogeneic hematopoietic cell transplantation (HCT) with varying types of donor grafts, degrees of HLA matching, and intensity of conditioning regimens. Unique features that distinguish the use of allogeneic HCT in this population include higher rates of graft failure, immune-mediated cytopenias, and the potential to achieve long-term disease-free survival in a mixed

chimerism state. Additionally, in contrast to patients with hematologic malignancies, a priority is to completely avoid graft-versus-host disease in patients with NMD because there is no theoretical beneficial graft-versus-leukemia effect that can accompany graft-versus-host responses. In this review, we discuss the current approach to each of these clinical issues and how emerging novel therapeutics hold promise to advance transplant care for patients with NMDs.

Introduction

Allogeneic hematopoietic cell transplantation (HCT) is an established curative treatment most commonly used to treat malignant hematologic diseases.¹ To date, allogeneic HCT has also long been the only curative option for patients with many life-threatening, congenital or acquired nonmalignant diseases (NMDs) involving the hematopoietic system.² Robust clinical experience and long-term follow-up supports the use of allogeneic HCT in managing bone marrow (BM) failure syndromes,^{3,4} hemoglobinopathies,^{5,6} primary immunodeficiency diseases,^{7,8} inherited metabolic disorders,⁹ and autoimmune diseases¹⁰ (Table 1). Disease and transplant considerations that can pose unique challenges differentiate the application of allogeneic HCT to NMD compared with the treatment of hematologic malignancies. In this review, we highlight these challenges and describe how recent and future advances hold the potential to overcome current barriers and improve allogeneic HCT outcomes for patients with NMDs.

Transplant considerations for nonmalignant conditions

There are multiple clinical considerations that distinguish the use of allogeneic HCT for NMDs from its use for hematologic malignancies (Table 1). First, higher rates of primary and secondary graft failure have been observed after allogeneic HCT in patients with NMD compared with patients with acute leukemia (relative risk, 3.32; $P < .01$).¹¹ There are multiple factors that possibly contribute to this issue. Reduced intensity conditioning (RIC) and

nonmyeloablative (NMA) conditioning regimens have been used more regularly for select diseases in order to avoid both the short- and long-term toxicity associated with myeloablative conditioning (MAC) regimens, most notably in Fanconi anemia (FA) patients that have a DNA repair defect and hence inherent sensitivity to such.³ However, with lower doses of chemotherapy or radiation, a competent recipient immune system may persist and cause or facilitate graft rejection. In the absence of prior chemotherapy used to treat an underlying hematologic malignancy, for many of the NMDs such as those with inherited metabolic disorders, the host immune system that have graft rejection capabilities and BM hematopoietic and stromal cell compartments that harbor hematopoietic stem and progenitor cells (HSPCs) can compete with the donor graft would be largely unperturbed. Under these conditions, host HSPCs and immune cells may dominate repopulation in the recipient. Furthermore, patients with hemoglobinopathies who have received sufficient numbers of blood transfusions before transplant and have become immunized with donor-specific antibodies may more likely reject their allograft.¹² Finally, BM is considered to be the standard graft source for many NMD transplant indications, given its association with lower rates of GVHD compared with peripheral blood stem cells.¹³ However, BM grafts contain lower CD34⁺ and total nucleated cell doses, which has been shown to be a significant risk factor for graft failure.¹¹

Second, there is no potential benefit associated with the development of graft-versus-host disease (GVHD) in patients with NMDs. A core principle of allogeneic HCT in malignancies is that the curative effect is largely dependent on donor immune

Table 1. Allogeneic HCT for nonmalignant diseases: clinical considerations

| Disease categories | Specific examples | NMD HCT: clinical considerations | NMD HCT: goals to improve outcomes |
|-----------------------------------|---|---|---|
| Bone marrow failure syndromes | <ul style="list-style-type: none"> Severe aplastic anemia Fanconi anemia Congenital amegakaryocytic thrombocytopenia Dyskeratosis congenita Diamond-Blackfan anemia Shwachman-Diamond syndrome Congenital sideroblastic anemia GATA2-associated marrow failure SAMD9 or SMDL9 disorder Paroxysmal nocturnal hemoglobinuria | | |
| Hemoglobinopathies | <ul style="list-style-type: none"> Sickle cell disease Thalassemia | | |
| Primary immunodeficiency diseases | <ul style="list-style-type: none"> SCID Non-SCID combined immunodeficiencies T-cell immunodeficiency, SCID variants Wiskott-Aldrich syndrome Hemophagocytic disorders Severe congenital neutropenia Chronic granulomatous disease Other phagocytic cell disorders X-linked hyper-IgM syndrome IPEX syndrome IFN-γ deficiency | <ol style="list-style-type: none"> Increased risk of graft failure No benefit of any GVHD as no role of GVL Full donor chimerism not necessary for long-term disease control Increased risk for prolonged IMCs Increased risk of atypical infections in certain PIDs | <ol style="list-style-type: none"> Limit graft failure Limit GVHD Limit IMCs |
| Inherited metabolic disorders | <ul style="list-style-type: none"> Mucopolysaccharidoses I (severe; Hurler syndrome) Other mucopolysaccharidoses (II, III, VI) Other lysosomal metabolic diseases Globoid cell leukodystrophy Metachromatic leukodystrophy Cerebral X-linked adrenoleukodystrophy | | |
| Other disorders | <ul style="list-style-type: none"> Juvenile rheumatoid arthritis Osteopetrosis Systemic sclerosis | | |

IFN, interferon; Ig, immunoglobulin; IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked; PID, primary immunodeficiency diseases.

cells that eliminate residual malignant cells that persist after conditioning, conventionally referred to as the graft-versus-leukemia (GVL) effect.¹⁴ Thus, for patients with hematologic malignancy undergoing allogeneic HCT, efforts to minimize GVHD must also have a goal of preserving the GVL effect.¹⁵ Because there is no benefit for GVL in NMDs, more stringent GVHD prevention approaches are required.

Third, immune-mediated cytopenias (IMCs), including hemolytic anemia, thrombocytopenia, and/or neutropenia, are known to occur at a high incidence for patients with inherited metabolic disorders following allogeneic HCT.¹⁶ Although the etiologies of IMCs in patients with NMD have not been fully elucidated, interactions between the donor and recipient immune systems are

thought to trigger these cytopenias, which may be further driven by abnormal immune-mediated reactions because of lysosomal dysfunction that leads to the excessive accumulation of storage disease substrates or end-products that may be seen as “foreign” by donor cells.¹⁷ Even outside of inherited metabolic disorders, a higher incidence of IMCs after allogeneic HCT have been observed with other NMDs compared with hematologic malignancies.¹⁶

Finally, full donor chimerism is not required to achieve therapeutic efficacy in NMDs. Typically, allogeneic HCT recipients experience full donor chimerism (>95% of cells are of donor origin), with low or falling chimerism being indicative of graft failure or relapse.¹⁸ Multiple studies in patients with hematologic

Table 2. Current and emerging approaches to enhance allogeneic HCT for nonmalignant disease

| | Current approaches | Emerging approaches |
|-----------------------|--|--|
| Conditioning regimens | Use of traditional agents in combinations that limit toxicities | Antibody-based conditioning (anti-CD117, anti-CD45) |
| Graft sources | Unmanipulated BM, PBSC, or UCB | Ex vivo UCB expansion (nicotinamide) |
| GVHD prophylaxis | CNI-based prophylactic regimens Addition of ATG PTCy-based prophylactic regimens Traditional T-cell depletion | Wider adoption of PTCy-based regimens Novel targeted agents (CD24, CD26, α 4 β 7 integrin, JAK1 or JAK 1/2, T-cell costimulation) Novel depletion approaches (CD3 ⁺ /CD19 ⁺ , TCR $\alpha\beta$ ⁺ , CD45RA ⁺) Infusion of ex vivo expanded or highly purified regulatory T-cells after CD34 ⁺ selected HCT |

JAK, Janus kinase.

malignancy have suggested the rapid full donor chimerism is associated with lower relapse rates,¹⁹⁻²¹ and interventions such as adjustment of immunosuppression or cellular therapies are often used to achieve such. Rarely, subsets of such patients never reach full donor chimerism but remain as mixed chimerism (5%-95% cells of donor origin). In NMDs, where complete replacement of recipient hematopoiesis is not always necessary to improve the underlying disease state, mixed chimerism may be sufficient to achieve a durable disease response with low rates of GVHD.^{22,23}

Approaches to enhance allogeneic HCT for NMDs

To augment the success of allogeneic HCT for NMDs, novel approaches should be incorporated into transplant platforms that can limit: (1) graft rejection; (2) GVHD; and (3) IMCs. Conditioning regimens, graft sources, and GVHD immune prophylaxis regimens are three essential components of allogeneic HCT that will be prime targets for innovation (Table 2).

Conditioning regimens

We will briefly review the more widely used conditioning regimens for treating patients with representative hematopoietic, immune, or metabolism disorders before discussing new strategies to optimize myelosuppression and immunosuppression without the toxicities of conventional chemotherapies (Figure 1).

BM failure syndromes

RIC for patients with BM failure frequently consist of low-dose cyclophosphamide, fludarabine, and busulfan, and/or low-dose irradiation.²⁴ Patients with FA and dyskeratosis congenita pose a unique challenge because of hypersensitivity to DNA alkylating agents and irradiation,^{25,26} necessitating reduced cyclophosphamide, a DNA alkylating agent, and/or radiation doses to decrease the risk of early toxicity (FA) and late secondary malignancies (both). Although total body irradiation (TBI) doses can be reduced to 300 cGy, further reduction has resulted in an increased incidence of graft rejection.²⁷ Instead, fludarabine and alemtuzumab (anti-CD52 monoclonal antibody [mAb]) are commonly included to suppress graft rejection. A prospective multi-institutional study of alternative donor allogeneic HCT in


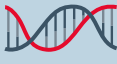
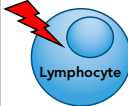
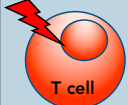
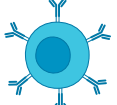
patients with FA using low-dose busulfan, cyclophosphamide, fludarabine, rabbit anti-thymocyte globulin (ATG) and T-cell depleted grafts showed all patients younger than 10 years of age at allogeneic HCT survived and none developed severe acute GVHD.²⁸ A similar trial for patients with dyskeratosis congenita is ongoing (NCT01659606).

Hemoglobinopathies

Stable donor engraftment after RIC or NMA allogeneic HCT can be difficult to obtain in patients with hemoglobinopathies who have an intact immune system and HSPC content. Patients with thalassemia or sickle cell disease (SCD) are at higher risk of graft failure even when MAC containing busulfan or cyclophosphamide are used. Treosulfan has been introduced as a lower toxicity substitute for busulfan; however, a third agent, thiotepa, is often needed to reduce the frequency of mixed chimerism when used in the context of hemoglobinopathies, where there is more active marrow function.^{29,30} Adding thiotepa to the previously tested RIC, consisting of alemtuzumab, fludarabine, and melphalan, has also been used successfully in umbilical cord blood transplantation for patients with SCD.³¹ Furthermore, haploidentical HCT with posttransplantation cyclophosphamide (PTCy) and thiotepa has been shown to improve donor engraftment without significantly increasing morbidity or mortality in patients with SCD.³² A recent study reported that increasing the TBI dose from 200 cGy to 400 cGy reduced graft failure while maintaining the safety of NMA conditioning for haploidentical HCT.³³

Primary immunodeficiency diseases

Severe combined immune deficiency (SCID) is a group of rare disorders that occur as the result of mutations in 1 of more than a dozen known genes that impede the development or function of the immune system. Patients with SCID and the complete absence of T-cell immunity allows allogeneic HCT to be performed without chemotherapy (in select cases) without a high risk of graft failure.³⁴ One exception is adenosine deaminase deficiency in which enzyme replacement by exogenous infusion or in the form of HCT can restore host immune function. In non-SCID primary immunodeficiency diseases, some type of pre-transplant conditioning is always required to achieve sufficient allo-engraftment. Conditioning regimens in this setting often also contain serotherapies such as alemtuzumab or ATG to

| | Category/agents | Benefits | Limitations | HCT Applications | References |
|---|---|--|---|--|------------------|
|  | Radiation | <ul style="list-style-type: none"> • Myelosuppression ++ • Immunosuppression ++ • Prevents graft rejection | <ul style="list-style-type: none"> • Growth impairment • Gonadal dysfunction • Secondary malignancies • Avoid in DNA repair defects | <ul style="list-style-type: none"> • BMFs • Hemoglobinopathies | 27, 33 |
|  | Alkylating agents <ul style="list-style-type: none"> • Busulfan (Bu) • Treosulfan (Treo) • Melphalan (Mel) • Thiotepa (Thio) | <ul style="list-style-type: none"> • Myelosuppression +++ • Bu: specificity for myeloid-erythroid precursors • Treo: less toxicity, does not cross blood brain barrier • Thio: immunosuppressive | <ul style="list-style-type: none"> • Secondary malignancies • Bu: variable bioavailability as an oral agent; VOD • Mel: GI toxicity | <ul style="list-style-type: none"> • Hemoglobinopathies • PIDs • Inherited metabolic disorders | 35, 37-38, 48-49 |
|  | Lymphodepleting agents <ul style="list-style-type: none"> • Fludarabine (Flu) • Cyclophosphamide (Cy) | <ul style="list-style-type: none"> • Immunosuppression +++ • Useful in alloimmunized and multiply transfused pts • Cy: GVHD prevention in haplo-HCT | <ul style="list-style-type: none"> • Cy: cardiac toxicity, cystitis • Flu: lethal neurotoxicity | <ul style="list-style-type: none"> • Important part of alkylator- and radiation-free regimens in DNA repair defects | 28, 32 |
|  | T cell depleting agents <ul style="list-style-type: none"> • Alemtuzumab • ATG | <ul style="list-style-type: none"> • Immunosuppression +++ • Lower risk of GVHD | <ul style="list-style-type: none"> • Increased risk of infection • ATG: Hypersensitivity reactions | <ul style="list-style-type: none"> • PIDs • BMFs • Hemoglobinopathies | 31, 42, 76 |
|  | Targeted conditioning <ul style="list-style-type: none"> • Emapalumab • Anti-CD117 mAbs • Anti-CD45 agents | <ul style="list-style-type: none"> • Non-genotoxic • Reduced off-target toxicity | <ul style="list-style-type: none"> • Limited clinical data available | <ul style="list-style-type: none"> • Emapalumab: HLH • Anti-CD45: Useful in DNA repair defects • CD117: sole conditioning for non-SCID PIDs (preclinical) | 54-56, 58-60 |

Abbreviations: ATG: anti-thymocyte globulin; BMF: bone marrow failure syndrome; CNS: central nervous system; HCT: hematopoietic cell transplantation; HLH: Hemophagocytic lymphohistiocytosis, PID: primary immunodeficiency disease; SCID: severe combined immunodeficiency; VOD: veno-occlusive disease

Figure 1. Current and emerging preparative regimens used in allogeneic HCT for nonmalignant disease.

deplete host graft rejecting cells. Treosulfan and fludarabine can achieve adequate levels of mixed chimerism in children with primary immunodeficiency diseases, including chronic granulomatous disease and Wiskott-Aldrich syndrome.³⁵⁻³⁷ Treosulfan-based regimens are considered less toxic than busulfan-based regimens because of a lower rate of veno-occlusive disease and long-term toxicities including infertility.^{38,39} Treosulfan may have a further advantage over busulfan in young children because it does not cross the blood-brain barrier and may therefore have less neurotoxicity.⁴⁰

The timing of HCT and an aggressive control of hyperinflammation are critical for a good outcome in patients with immune dysregulation diseases, such as primary hemophagocytic lymphohistiocytosis (HLH). Alemtuzumab is efficient at depleting T cells and also CD52-expressing antigen-presenting cells, which may effectively treat residual/smoldering HLH at the time of transplantation. When given with fludarabine and melphalan, proximal (close to graft infusion time) dosing of alemtuzumab is shown to be associated with a high incidence of mixed chimerism, whereas distal (more distant from graft infusion time) dosing is associated with less mixed chimerism but more acute GVHD.⁴¹ In contrast, intermediate dose alemtuzumab (1 mg/kg divided over days -14 to -10) decreases the risk of mixed chimerism and carries a minimal risk of upfront acute GVHD.^{41,42} Several reports have documented high rates of mixed chimerism and graft failure in transplanted patients with HLH, especially when a RIC is used.^{43,44} Innovative treatments are thus needed to improve disease control before HCT. Emapalumab, a neutralizing high affinity mAb that binds to receptor-bound and free interferon- γ present in elevated levels, was US Food and Drug Administration approved for pediatric and adult primary patients with HLH and refractory, recurrent, progressive, or intolerant to

conventional therapy.⁴⁵ Most emapalumab-treated patients proceeded to allogeneic HCT and had outcomes that compared favorably to other MAC or RIC regimens.⁴⁶

Inherited metabolic disorders

Early allogeneic HCT for inherited metabolic disorders primarily used MAC with busulfan and cyclophosphamide and was associated with significant regimen-related toxicity.⁴⁷ Multiple adjustments to busulfan administration (decreased dose, switching from oral to intravenous formulations, pharmacokinetic-directed dosing) and substituting cyclophosphamide with fludarabine to enhance immune suppression have resulted in similar efficacy with reduced toxicity.^{48,49} A less "intensive" regimen of treosulfan or melphalan combined with fludarabine is less toxic, but may be associated with rejection or low-level chimerism requiring the need for retransplantation.⁵⁰

Novel antibody-based conditioning

Targeted agents for conditioning hold promise in being able to enhance intensity of conditioning without adding significant off-target toxicity. Preclinical studies have identified CD117 and CD45 as targets suitable for nongenotoxic antibody-mediated depletion of host hematopoietic cells.^{51,52} CD117 is expressed on HSPCs, early thymocytes, mast cells, and some types of innate lymphoid, digestive tract cells, and tumors, including acute myeloid leukemia and melanoma. This restricted expression pattern is ideally suited for myeloablation. Treatment of mice with anti-CD117 (c-kit) mAbs depletes hematopoietic stem cells (HSCs) with hematopoiesis returning within 2 weeks.⁵³ To achieve >99% elimination of host HSCs and vigorous hematopoietic recovery in immune competent mice, anti-CD47 mAb (blocks the "don't eat me" signal) needed to be added.⁵² Non-human primate studies support the safety and efficacy of

anti-CD117 mAb for HSC depletion.⁵⁴ Early data from a phase 1 dose escalation trial (NCT02963064) showed long-term myeloid chimerism and nascent B- and T-lymphocyte generation without toxicities providing proof of concept for safely replacing and/or augmenting MAC or NMA conditioning.⁵⁵

Anti-CD45 agents including “naked” unconjugated, radio-labeled, and toxin-conjugated mAbs have a broad hematopoietic expression range and are advantageous for combined HSC and immune cell depletion. Radioisotope-labeled anti-CD45 mAbs are in advanced testing in adults with myeloid disorders (NCT02665065), and second-generation agents continue to be developed.⁵⁶ In contrast to unconjugated or radioisotope anti-CD45 antibody, CD45 antibody-drug conjugates (ADCs) are rapidly cleared, avoiding the potential for depleting donor graft hematopoietic and immune cells or requiring an extended period of myelosuppression if more distal administration is required. CD45 ADCs have shown promise in preclinical models of autologous HCT, evidenced by their ability to efficiently deplete HSCs and immune cells in the periphery, thereby enabling donor HSC engraftment and hematopoietic recovery.⁵⁷ For congenital immunodeficiencies, conditioning with lytic anti-CD45 mAbs, alemtuzumab, fludarabine, and low-dose cyclophosphamide was well tolerated and high-level donor chimerism was achieved.⁵⁸ In murine and nonhuman primate studies, a single pretransplant dose of antimurine CD45 ADC as monotherapy permitted high-level allo-engraftment of fully MHC and minor histocompatibility antigen-mismatched donor cells.^{59,60} Human anti-CD45 ADCs have been generated for future trial consideration. Whether pretransplant CD45 ADCs will prove superior in the clinic is unknown and will await such clinical trials in patients with HCT.

Graft sources

BM, peripheral blood stem cells (PBSCs), and umbilical cord blood (UCB) have all been successfully used graft sources for allogeneic HCT in patients with NMD.^{3,61-63} BM has traditionally been the standard graft source for NMD, given its significantly lower rates of chronic GVHD and associated mortality relative to PBSCs. However, in addition to its more laborious harvest procedure, BM can be associated with low total nucleated cell (TNC) counts, resulting in slow count recovery and increased rates of graft failure.⁶⁴ Although not always feasible, higher goal TNC counts for the NMD population may be of benefit. PBSCs are the most frequently used graft source in HCT for malignancy because of ease of collection and robust CD34⁺ cell counts. PBSCs have historically been avoided in NMD given higher rates of chronic GVHD with traditional GVHD prophylaxis, in part because of higher T-cell content.⁶⁵ Given the higher CD34⁺ cell counts, PBSCs may be the preferred graft source if using methods of cellular manipulation. UCB has the advantages of less strict HLA-matching requirements and reduced rates of GVHD because of lower T-cell content. However, its use has been limited by low hematopoietic cell content in a given UCB unit, resulting in impaired engraftment.^{66,67} Slow immune reconstitution and higher risk for infection have been traditionally associated with UCB transplantation, although these events are strongly influenced by the concurrent use of ATG.⁶⁸ In addition to the previously mentioned considerations, the inherited nature of certain NMDs may require screening of familial donors for carrier states, leaving unrelated donor sources (UCB or other) as

the most feasible option. Ongoing advances in allogeneic HCT practice suggest that new strategies via graft manipulation may address these limitations, mainly by (1) limiting GVHD (discussed later) and (2) improving hematopoietic recovery.

Aside from GVHD, poor or slow hematopoietic recovery is a limitation of HCT in NMDs, especially with UCB.⁶⁹ To overcome the delays in engraftment, Eurocord recommends a higher cell dose ($>5 \times 10^7$ TNC/kg) for patients with NMD, including use of double UCB if this dose cannot be achieved with a single unit.⁷⁰ Furthermore, ex vivo UCB expansion approaches have been developed and evaluated clinically.⁷¹ Notably, ex vivo expansion of single UCB units with nicotinamide significantly enhances CD34⁺ cell counts and shortens time to neutrophil and platelet engraftment in the setting of myeloablative HCT for hematologic malignancies.^{72,73} This approach also resulted in rapid donor cell engraftment and improvement in disease manifestation in a phase 1/2 study enrolling pediatric SCD.⁷⁴ In other studies, ex vivo expansion of UCB with an arylhydrocarbon receptor antagonist resulted in a significant increase in CD34⁺ cells, along with shortened time to neutrophil and platelet engraftment compared with unmanipulated grafts.⁷⁵ Novel approaches to graft manipulation hold promise in enhancing clinical outcomes and warrant further investigation in the setting of NMDs.

GVHD prevention Pharmacological approaches

Pharmacological regimens, which are commonly used for GVHD prophylaxis, have traditionally revolved around calcineurin inhibitors (CNIs) paired with another agent such as methotrexate, sirolimus, or mycophenolate. CNI-based regimens using either BM or PBSCs clearly result in unacceptable rates of acute and chronic GVHD⁶⁴ when considering patients with NMD and also have the drawback of prolonged courses of immunosuppressive medications. Historical methods to augment CNI-based regimens have included the addition of polyclonal ATG products, which appears to decrease the incidence of chronic GVHD, albeit at the expense of increased graft rejection and opportunistic infection.^{76,77} Nevertheless, ATG products remain an important standard component of many platforms for NMD HCT involving fully matched donors. Current trials in malignant disease continue to investigate additions of novel agents to CNI-based platforms including agents targeting CD24,⁷⁸ CD26,⁷⁹ $\alpha 4\beta 7$ integrin,⁸⁰ Janus associated kinase-1 (NCT03320642), and T-cell costimulation.⁸¹ Results of these trials will dictate if the addition of any of these novel agents can decrease the risk of GVHD enough to be acceptable for use in patients with NMD.

The emergence of PTCy-based GVHD prophylaxis regimens has been a major advance in transplantation, especially when using haploidentical or mismatched unrelated donor transplants.^{82,83} Use of such a platform with haploidentical donors for NMDs has been described in patients with SCD,⁸⁴ other hemoglobinopathies,⁸⁵ and aplastic anemia.⁸⁶⁻⁸⁸ Analyses demonstrate that BM continues to be associated with lower rates of GVHD than PBSCs in this setting as well.⁸⁹ The downsides to PTCy-based regimens include the frequent use of TBI during conditioning, exposure to high-dose alkylating agents, delayed

engraftment compared with conventional CNI-based regimens and concerns regarding CD8 T-cell dysfunction and kinetics of immune reconstitution.⁹⁰ In addition, there are some questions if PTCy is as effective in the very young pediatric population.⁹¹ It remains to be seen if PTCy-based regimens will become a standard platform upon which to build for patients with NMD and if further improvements in GVHD prevention or immune reconstitution can be made. PTCy-based regimens may emerge as an ideal platform for older patients with NMDs given the well documented success with NMA conditioning.⁹²

Ex vivo manipulation

Ex vivo manipulation of donor grafts involves either negative or positive selection of specific cellular subsets in order to decrease the risk of GVHD, commonly referred to as T-cell depletion (TCD). The central question is whether there is sufficient knowledge and technology to modify grafts to achieve lower levels of GVHD, yet to maintain engraftment with minimal rates of opportunistic infection. Inherent to removing donor T cells from the graft is an increased incidence of graft rejection; thus, the vast majority of platforms using ex vivo TCD have involved myeloablative conditioning.

Ex vivo TCD by CD34⁺ positive selection has been used to deplete allo-reactive T lymphocytes and other immune effector cells from PBSC grafts.⁹³ This technique has resulted in successful engraftment and low rates of acute and chronic GVHD in patients with hematologic malignancies, although it has not been widely investigated for NMD.⁹⁴⁻⁹⁶ Presently, the most widely used CD34⁺ cell selection platform is the ClinMACS system (Miltenyi Biotech, Bergisch-Gladbach, Germany) that is Food and Drug Administration approved for patients with acute myeloid leukemia in first remission receiving HCT from a matched sibling donor.⁹⁷ For patients with NMDs, CD34⁺ selected TCD has only been shown to be successful for patients with matched sibling donors or in children with SCID. Fortunately, techniques have evolved from CD34⁺ positive selection that depletes all other cells to more sophisticated manipulation including targeting for depletion CD3⁺/CD19⁺,^{98,99} TCRαβ⁺,¹⁰⁰ or CD45RA⁺ subsets. This approach is thought to better maintain natural killer cells as well as other T-cell subsets (γδ T cells in the case of αβ depletion) with lower GVHD potential to allow better engraftment and potentially improve immune reconstitution after HCT. Multiple clinical trials in pediatric subjects with NMDs, including hemoglobinopathies, primary immunodeficiencies, and FA, have now demonstrated that αβ T-cell graft depletion results in low rates of both acute and chronic GVHD, compared with what would be expected with T-cell replete HCT, in the setting of haploidentical, mismatched related, and matched unrelated donor transplants.¹⁰¹⁻¹⁰⁴

Efforts to improve outcomes after TCD HCT have also involved the infusion of specific cellular products after HCT. Preliminary data have been reported regarding a pilot study in children with NMDs who received infusions of donor T cells transduced with the inducible suicide gene Caspase 9 (iC9) that rapidly eliminates infused donor T cells should GVHD arise.¹⁰⁵ This system is based on a small molecule dimerizing drug, AP1903 (Rimiducid), which homodimerizes a FKBP12 analog that contains modified iC9. Once dimerized, activation of iC9 results in eradication of 99% of iC9-expressing T cells within 2 hours of a single dose of

AP1903 and controls GvHD within 24 to 48 hours.¹⁰⁶ There is a 1000-fold lower affinity of AP1903 to the wild-type FKBP12 because of a single amino acid substitution, thereby providing selectivity for transduced T cells.

Infusion of ex vivo expanded or freshly isolated, highly purified regulatory T cells (Tregs) have been shown to decrease GVHD after UCB or haploidentical HCT.^{107,108} Recently, a novel technology (ORCA Bio) that rapidly sorts and purifies cellular subsets to single-cell precision was shown to have a compellingly low incidence of significant GVHD in adult patients with acute leukemia given CD34⁺ selected HCT with purified Tregs, then followed 2 days later by donor-derived conventional T-cell infusion.^{109,110} The potential applications exist for customized grafts based on factors such as underlying disease as well as specific host-donor combinations.

Current and future trials will determine if progress can be made in GVHD prevention regimens for patients with NMD undergoing HCT. It remains to be seen if any of the novel agents added to standard CNI-based regimens will emerge as a new standard of care. Although PTCy appears to prevent GVHD better than traditional CNI regimens, there are clear drawbacks to PTCy as discussed previously, despite being attractive given its success with RIC. Ex vivo graft manipulation holds much promise and has evolved in recent years. However, expertise, facilities, logistics, and costs of ex vivo manipulations as well as toxicity associated with MAC have limited the widespread adoption of these techniques much beyond academic centers. As discussed, targeted conditioning agents with less nonspecific toxicity may be able to overcome this obstacle in the future. Ultimately, disorders with single gene mutations will likely be better served with genetically modified autologous graft strategies (covered in an accompanying article in this *Blood* review series), which will eliminate any risk of GVHD. Although the observations of single lineage or mixed chimerism can effectively treat specific diseases, it seems unlikely that any platform will be able to reliably produce such a sustainable stable state in different individual patients. Graft engineering technology with the ability to specifically customize graft components with single-cell precision has great potential to ultimately engender successful engraftment and minimize risks of GVHD and opportunistic infection, especially for patients with NMDs, and results of early trials with such technology are eagerly awaited.

Additional considerations

Immune tolerance mechanisms

In an individual patient, a threshold level of donor anti-host alloreactive T cells must be reached to cause GVHD. Immune modulatory mechanisms can elevate this threshold by counterbalancing the efficiency of donor T cells to mediate tissue destruction and GVHD. For illustration, we will discuss mechanisms of PTCy in light of the increasingly widespread use of the GVHD prevention approach. Reduced GVHD rates seen in patients receiving PTCy have been attributed to CD8 T-cell dysfunction that provides a more permissive environment for thymic Treg preservation and peripheral Treg generation.^{111,112} The net result of a diminished donor anti-host T-cell burden coupled with an augmentation in peripheral regulatory mechanisms is a shift in the balance of donor T cells from a dominant state of

alloreactivity to immune regulation and tolerance. Host hematopoietic cells do not appear to contribute to lower GVHD rates because there is no mixed chimerism in PTCy-treated aplastic anemia or SCD.^{33,87} Moreover, hematopoietic cells in these and other NMDs tend to reach >95% engraftment or are rejected.¹¹³ Inclusion of pretransplant ATG and TBI doses of 400 cGy serve to minimize rejection rates,³³ although specific mechanisms associated with lower GVHD rates seen with other approaches may have distinct tolerogenic mechanisms, raising the threshold for donor anti-host alloreactive serves is a common denominator.

Stable mixed chimerism

Many patients with NMD do not require complete donor chimerism to correct the disease phenotype. Stable mixed chimerism has a well-documented ameliorated effect in thalassemia and SCD. In SCD, only 20% donor myeloid chimerism is required to reverse the sickle phenotype.^{114,115} Yet, the greatest concern is that stable donor chimerism will not occur because of transfusion-based allosensitization coupled with a robustly hyperplastic BM.^{116,117} Serial testing can detect decreases in donor chimerism that may herald graft rejection,¹¹⁸ although this must be evaluated carefully in context of mixed chimerism.¹⁸ Although a mixed chimerism state can be managed successfully with a prolonged taper of immune suppression, not all NMDs can be managed with this approach. In Hurler syndrome, full donor chimerism contributed to normal leukocyte IDUA enzymes levels after transplant, which was a predictor of superior long-term outcomes.⁹

Pretransplant therapy or alternative conditioning approaches may enhance donor chimerism after allogeneic HCT. Chimerism data reported in the phase 2/3 trial of emapalumab in HLH are promising¹¹⁹ because complete donor chimerism was observed in all but 1 surviving patient, as opposed to several reports of high rates of mixed and often unstable chimerism in transplanted HLH, especially when RIC regimens are used.^{43,120} In adult patients with severe congenital anemias, an ongoing NMA allogeneic HCT trial with radiation, alemtuzumab, and sirolimus (NCT00061568) has preliminary results showing that stable, mixed chimerism can be achieved.¹²¹

There are no generally accepted, successful interventions for increasing donor-specific engraftment in the setting of mixed chimerism. In certain situations, based upon declining chimerism levels and in consultation with a transplant center with allogeneic HCT expertise in the underlying disorder, donor lymphocyte infusion may be indicated although experience in NMDs has been limited and is associated with a significant risk of GVHD. Repeat allogeneic HCT may be effective in selected patients, particularly those treated later posttransplant after recovering from regimen-related toxicities,¹²² although results for treatment of neutropenic graft failure are much worse than those with non-neutropenic graft failure.¹²³

Immune-mediated cytopenias

Corticosteroids and/or intravenous immunoglobulin along with supportive therapy (eg, use of granulocyte colony-stimulating factor and blood product transfusions) are the mainstays of

treatment of IMCs.^{16,124} Daratumumab, an anti-CD38 mAb that can eliminate CD38 high plasma cells, is now the preferred upfront therapy in moderate to severe IMCs.^{125,126} Agents such as rituximab, which target CD20⁺ B cells, also have shown efficacy in IMCs,^{127,128} suggesting an important role for B cells. More effective B-cell depletion can be achieved with newer agents such as obinutuzumab, a humanized, type II, anti-CD20 mAb, which acts through antibody-dependent cellular cytotoxicity and leads to direct apoptosis of mature B cells.¹²⁹ In a different approach, the proteasome inhibitor, bortezomib, has been used to targets T cells and plasma cells, which produce antibodies directed against 1 or more hematopoietic lineages.¹²⁹ Because the majority of patients develop IMCs while on post-HCT immunosuppression, another important approach is potentially switching the calcineurin inhibitors to sirolimus, which has shown improvement in recurrent and refractory IMCs.¹³⁰

Conclusion

NMDs possess unique challenges as it pertains to the successful implementation of allogeneic HCT. However, the emergence of novel approaches to prevention of graft rejection, GVHD, and IMCs hold promise in overcoming these historical barriers. Because many initial studies are conducted for subjects with hematologic malignancies, carefully planned collaborative clinical trials will need to explore these novel transplant approaches in the NMD population to advance clinical care.

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Footnote

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REFERENCES

- D'Souza A, Lee S, Zhu X, Pasquini M. Current use and trends in hematopoietic cell transplantation in the United States. *Biol Blood Marrow Transplant.* 2017;23(9):1417-1421.
- Kanate AS, Majhail NS, Savani BN, et al. Indications for hematopoietic cell transplantation and immune effector cell therapy: guidelines from the American Society for Transplantation and Cellular Therapy. *Biol Blood Marrow Transplant.* 2020;26(7):1247-1256.
- Peffault de Latour R, Porcher R, Dalle JH, et al; Pediatric Working Party of the European Group for Blood and Marrow Transplantation. Allogeneic hematopoietic stem cell transplantation in Fanconi anemia: the European Group for Blood and Marrow Transplantation experience. *Blood.* 2013;122(26):4279-4286.
- Bacigalupo A, Socié G, Hamladji RM, et al; Aplastic Anemia Working Party of the European Group for Blood Marrow Transplantation. Current outcome of HLA identical sibling versus unrelated donor transplants in severe aplastic anemia: an EBMT analysis. *Haematologica.* 2015;100(5):696-702.
- Baronciani D, Angelucci E, Potschger U, et al. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000-2010. *Bone Marrow Transplant.* 2016;51(4):536-541.
- Eapen M, Brazauskas R, Walters MC, et al. Effect of donor type and conditioning regimen intensity on allogeneic transplantation outcomes in patients with sickle cell disease: a retrospective multicentre, cohort study. *Lancet Haematol.* 2019;6(11):e585-e596.
- Buckley RH, Schiff SE, Schiff RI, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med.* 1999;340(7):508-516.
- Hassan A, Booth C, Brightwell A, et al; Inborn Errors Working Party of the European Group for Blood and Marrow Transplantation and European Society for Immunodeficiency. Outcome of hematopoietic stem cell transplantation for adenosine deaminase-deficient severe combined immunodeficiency. *Blood.* 2012;120(17):3615-3624, quiz 3626.
- Aldenhoven M, Wynn RF, Orchard PJ, et al. Long-term outcome of Hurler syndrome patients after hematopoietic cell transplantation: an international multicenter study. *Blood.* 2015;125(13):2164-2172.
- Greco R, Labopin M, Badoglio M, et al. Allogeneic HSCT for autoimmune diseases: a retrospective study from the EBMT ADWP, IEWP, and PDWP Working Parties. *Front Immunol.* 2019;10:1570.
- Olsson R, Remberger M, Schaffer M, et al. Graft failure in the modern era of allogeneic hematopoietic SCT [published correction appears in *Bone Marrow Transplant.* 2013;48(4):616]. *Bone Marrow Transplant.* 2013;48(4):537-543.
- Mattsson J, Ringdén O, Storb R. Graft failure after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2008;14(1 Suppl 1):165-170.
- Passweg JR, Baldomero H, Basak GW, et al; European Society for Blood and Marrow Transplantation (EBMT). The EBMT activity survey report 2017: a focus on allogeneic HCT for nonmalignant indications and on the use of non-HCT cell therapies. *Bone Marrow Transplant.* 2019;54(10):1575-1585.
- Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. *Blood.* 2008;112(12):4371-4383.
- Chang YJ, Zhao XY, Huang XJ. Strategies for enhancing and preserving anti-leukemia effects without aggravating graft-versus-host disease. *Front Immunol.* 2018;9:3041.
- Gupta AO, Jan Boelens J, Ebens CL, et al. Consensus opinion on immune-mediated cytopenias after hematopoietic cell transplant for inherited metabolic disorders. *Bone Marrow Transplant.* 2021;56(6):1238-1247.
- Rigante D, Cipolla C, Basile U, Gulli F, Savastano MC. Overview of immune abnormalities in lysosomal storage disorders. *Immunol Lett.* 2017;188:79-85.
- Kharfan-Dabaja MA, Kumar A, Ayala E, et al. Standardizing definitions of hematopoietic recovery, graft rejection, graft failure, poor graft function, and donor chimerism in allogeneic hematopoietic cell transplantation: a report on behalf of the American Society for Transplantation and Cellular Therapy. *Transplant Cell Ther.* 2021;27(8):642-649.
- Barrios M, Jiménez-Velasco A, Román-Gómez J, et al. Chimerism status is a useful predictor of relapse after allogeneic stem cell transplantation for acute leukemia. *Haematologica.* 2003;88(7):801-810.
- Koreth J, Kim HT, Nikiforow S, et al. Donor chimerism early after reduced-intensity conditioning hematopoietic stem cell transplantation predicts relapse and survival. *Biol Blood Marrow Transplant.* 2014;20(10):1516-1521.
- Reshef R, Hexner EO, Loren AW, et al. Early donor chimerism levels predict relapse and survival after allogeneic stem cell transplantation with reduced-intensity conditioning. *Biol Blood Marrow Transplant.* 2014;20(11):1758-1766.
- Liesveld JL, Rothberg PG. Mixed chimerism in SCT: conflict or peaceful coexistence? *Bone Marrow Transplant.* 2008;42(5):297-310.
- Stikvoort A, Sundin M, Uzunel M, et al. Long-term stable mixed chimerism after hematopoietic stem cell transplantation in patients with non-malignant disease, shall we be tolerant? *PLoS One.* 2016;11(5):e0154737.
- Alter BP. Inherited bone marrow failure syndromes: considerations pre- and posttransplant. *Blood.* 2017;130(21):2257-2264.
- Auerbach AD, Adler B, O'Reilly RJ, Kirkpatrick D, Chaganti RS. Effect of procarbazine and cyclophosphamide on chromosome breakage in Fanconi anemia cells: relevance to bone marrow transplantation. *Cancer Genet Cytogenet.* 1983;9(1):25-36.
- Gluckman E, Devergie A, Dutreix J. Radiosensitivity in Fanconi anaemia: application to the conditioning regimen for bone marrow transplantation. *Br J Haematol.* 1983;54(3):431-440.
- MacMillan ML, DeFor TE, Young JA, et al. Alternative donor hematopoietic cell transplantation for Fanconi anemia. *Blood.* 2015;125(24):3798-3804.
- Mehta PA, Davies SM, Leemhuis T, et al. Radiation-free, alternative-donor HCT for Fanconi anemia patients: results from a prospective multi-institutional study. *Blood.* 2017;129(16):2308-2315.
- Bernardo ME, Piras E, Vacca A, et al. Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan. *Blood.* 2012;120(2):473-476.
- Strocchio L, Zecca M, Comoli P, et al. Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in children with sickle cell disease. *Br J Haematol.* 2015;169(5):726-736.
- Abraham A, Cluster A, Jacobsohn D, et al. Unrelated umbilical cord blood transplantation for sickle cell disease following reduced-intensity conditioning: results of a phase I trial. *Biol Blood Marrow Transplant.* 2017;23(9):1587-1592.
- de la Fuente J, Dhedin N, Koyama T, et al. Haploidentical bone marrow transplantation with post-transplantation cyclophosphamide plus thiotepa improves donor engraftment in patients with sickle cell anemia: results of an international learning collaborative. *Biol Blood Marrow Transplant.* 2019;25(6):1197-1209.
- Bolaños-Meade J, Cooke KR, Gamper CJ, et al. Effect of increased dose of total body irradiation on graft failure associated with HLA-haploidentical transplantation in patients with severe hemoglobinopathies: a prospective clinical trial. *Lancet Haematol.* 2019;6(4):e183-e193.
- Wahlstrom JT, Dvorak CC, Cowan MJ. Hematopoietic stem cell transplantation for severe combined immunodeficiency. *Curr Pediatr Rep.* 2015;3(1):1-10.
- Slatter MA, Rao K, Amrolia P, et al. Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. *Blood.* 2011;117(16):4367-4375.
- Morillo-Gutierrez B, Beier R, Rao K, et al. Treosulfan-based conditioning for allogeneic HSCT in children with chronic granulomatous disease: a multicenter experience [published correction appears in

- Blood*. 2016;128(21):2585]. *Blood*. 2016; 128(3):440-448.
37. Slatter MA, Rao K, Abd Hamid IJ, et al. Treosulfan and fludarabine conditioning for hematopoietic stem cell transplantation in children with primary immunodeficiency: UK experience. *Biol Blood Marrow Transplant*. 2018;24(3):529-536.
 38. Shaw P, Shizuru J, Hoenig M, Veys P; IEWP-EBMT. Conditioning perspectives for primary immunodeficiency stem cell transplants. *Front Pediatr*. 2019;7:434.
 39. Faraci M, Diesch T, Labopin M, et al; Pediatric and Transplant Complications Working Parties of the European Society for Blood and Marrow Transplantation. Gonadal function after busulfan compared with treosulfan in children and adolescents undergoing allogeneic hematopoietic stem cell transplant. *Biol Blood Marrow Transplant*. 2019;25(9):1786-1791.
 40. Wilkinson FL, Sergijenko A, Langford-Smith KJ, Malinowska M, Wynn RF, Bigger BW. Busulfan conditioning enhances engraftment of hematopoietic donor-derived cells in the brain compared with irradiation. *Mol Ther*. 2013;21(4):868-876.
 41. Marsh RA, Vaughn G, Kim MO, et al. Reduced-intensity conditioning significantly improves survival of patients with hemophagocytic lymphohistiocytosis undergoing allogeneic hematopoietic cell transplantation. *Blood*. 2010;116(26):5824-5831.
 42. Marsh RA, Kim MO, Liu C, et al. An intermediate alemtuzumab schedule reduces the incidence of mixed chimerism following reduced-intensity conditioning hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis. *Biol Blood Marrow Transplant*. 2013;19(11):1625-1631.
 43. Allen CE, Marsh R, Dawson P, et al. Reduced-intensity conditioning for hematopoietic cell transplant for HLH and primary immune deficiencies. *Blood*. 2018; 132(13):1438-1451.
 44. Wustrau K, Greil J, Sykora KW, et al. Risk factors for mixed chimerism in children with hemophagocytic lymphohistiocytosis after reduced toxicity conditioning. *Pediatr Blood Cancer*. 2020;67(9):e28523.
 45. Merli P, Jordan MB, Locatelli F. Possible roads to improve hemophagocytic lymphohistiocytosis outcome. *Blood Adv*. 2020;4(24):6127-6129.
 46. Messina C, Zecca M, Fagioli F, et al. Outcomes of children with hemophagocytic lymphohistiocytosis given allogeneic hematopoietic stem cell transplantation in Italy. *Biol Blood Marrow Transplant*. 2018; 24(6):1223-1231.
 47. Gupta A, Downey M, Shanley R, et al. Reduced-toxicity (BuFlu) conditioning is better tolerated but has a higher second transplantation rate compared to myeloablative conditioning (BuCy) in children with inherited metabolic disorders. *Biol Blood Marrow Transplant*. 2020;26(3): 486-492.
 48. Gooptu M, Kim HT, Ho VT, et al. A comparison of the myeloablative conditioning regimen fludarabine/busulfan with cyclophosphamide/total body irradiation, for allogeneic stem cell transplantation in the modern era: a cohort analysis. *Biol Blood Marrow Transplant*. 2018;24(8):1733-1740.
 49. Harris AC, Boelens JJ, Ahn KW, et al. Comparison of pediatric allogeneic transplant outcomes using myeloablative busulfan with cyclophosphamide or fludarabine. *Blood Adv*. 2018;2(11): 1198-1206.
 50. Dinur-Schejter Y, Krauss AC, Erlich O, et al. Bone marrow transplantation for non-malignant diseases using treosulfan-based conditioning. *Pediatr Blood Cancer*. 2015; 62(2):299-304.
 51. Matthews DC, Appelbaum FR, Eary JF, et al. Radiolabeled anti-CD45 monoclonal antibodies target lymphohematopoietic tissue in the macaque. *Blood*. 1991;78(7):1864-1874.
 52. Chhabra A, Ring AM, Weiskopf K, et al. Hematopoietic stem cell transplantation in immunocompetent hosts without radiation or chemotherapy. *Sci Transl Med*. 2016; 8(351):351ra105.
 53. Czechowicz A, Kraft D, Weissman IL, Bhattacharya D. Efficient transplantation via antibody-based clearance of hematopoietic stem cell niches. *Science*. 2007;318(5854): 1296-1299.
 54. Kwon HS, Logan AC, Chhabra A, et al. Anti-human CD117 antibody-mediated bone marrow niche clearance in nonhuman primates and humanized NSG mice. *Blood*. 2019;133(19):2104-2108.
 55. Agarwal R, D'vorak CC, Proshaska S, et al. Toxicity-free hematopoietic stem cell engraftment achieved with anti-CD117 monoclonal antibody conditioning. *Biol Blood Marrow Transplant*. 2019;25(suppl 3): S92.
 56. Gyurkocza B, Nath R, Choe H, et al. Personalized targeted radioimmunotherapy with anti-CD45 iodine (131I) apamistamab [Iomab-B] in patients with active relapsed or refractory acute myeloid leukemia results in successful donor hematopoietic cells engraftment with the timing of engraftment not related to the radiation dose delivered [abstract]. *Blood*. 2020;136(suppl 1). Abstract 193
 57. Palchaudhuri R, Saez B, Hoggatt J, et al. Non-genotoxic conditioning for hematopoietic stem cell transplantation using a hematopoietic-cell-specific internalizing immunotoxin. *Nat Biotechnol*. 2016;34(7):738-745.
 58. Straathof KC, Rao K, Eyrych M, et al. Haemopoietic stem-cell transplantation with antibody-based minimal-intensity conditioning: a phase 1/2 study. *Lancet*. 2009; 374(9693):912-920.
 59. Gillard GO, Proctor JL, Brooks ML, et al. A novel targeted approach to achieve immune system reset: CD45-targeted antibody drug conjugates enable autologous HSCT, ameliorate disease in autoimmune models, potentially kill human immune cells from normal donors and MS patients, and achieve immune depletion in non-human primates (NHP) [abstract]. *Blood*. 2019;134(suppl 1). Abstract 3208.
 60. Hyzy SL, Proctor JL, Gillard GO, et al. Single agent CD45-targeted antibody drug conjugate enables full mismatch allogeneic hematopoietic stem cell transplantation in a murine HSCT model [abstract]. *Blood*. 2020; 136(suppl 1). Abstract 31.
 61. Chu R, Brazauskas R, Kan F, et al. Comparison of outcomes after transplantation of G-CSF-stimulated bone marrow grafts versus bone marrow or peripheral blood grafts from HLA-matched sibling donors for patients with severe aplastic anemia. *Biol Blood Marrow Transplant*. 2011;17(7):1018-1024.
 62. Kumar R, Kimura F, Ahn KW, et al. Comparing outcomes with bone marrow or peripheral blood stem cells as graft source for matched sibling transplants in severe aplastic anemia across different economic regions. *Biol Blood Marrow Transplant*. 2016;22(5):932-940.
 63. Eapen M, Wang T, Veys PA, et al. Allele-level HLA matching for umbilical cord blood transplantation for non-malignant diseases in children: a retrospective analysis. *Lancet Haematol*. 2017;4(7):e325-e333.
 64. Anasetti C, Logan BR, Lee SJ, et al; Blood and Marrow Transplant Clinical Trials Network. Peripheral-blood stem cells versus bone marrow from unrelated donors. *N Engl J Med*. 2012;367(16):1487-1496.
 65. Schmitz N, Dreger P, Suttrop M, et al. Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). *Blood*. 1995;85(6):1666-1672.
 66. Gluckman E, Rocha V, Ionescu I, et al; Eurocord-Netcord and EBMT. Results of unrelated cord blood transplant in fanconi anemia patients: risk factor analysis for engraftment and survival. *Biol Blood Marrow Transplant*. 2007;13(9):1073-1082.
 67. Ruggeri A, Eapen M, Scaravadou A, et al; New York Blood Center. Umbilical cord blood transplantation for children with thalassemia and sickle cell disease. *Biol Blood Marrow Transplant*. 2011;17(9): 1375-1382.
 68. Lindemans CA, Chiesa R, Amrolia PJ, et al. Impact of thymoglobulin prior to pediatric unrelated umbilical cord blood transplantation on immune reconstitution and clinical outcome. *Blood*. 2014;123(1): 126-132.
 69. Ballen KK, Gluckman E, Broxmeyer HE. Umbilical cord blood transplantation: the first 25 years and beyond. *Blood*. 2013; 122(4):491-498.
 70. Ruggeri A. Optimizing cord blood selection. *Hematol Am So Hematol Educ Program*. 2019;2019(1):522-531.
 71. Mehta RS, Rezvani K, Shpall EJ. Cord blood expansion: a clinical advance. *J Clin Oncol*. 2019;37(5):363-366.

72. Horwitz ME, Wease S, Blackwell B, et al. Phase I/II study of stem-cell transplantation using a single cord blood unit expanded ex vivo with nicotinamide. *J Clin Oncol*. 2019; 37(5):367-374.
73. Horwitz ME, Cutler C, Stiff P, Brunstein CG, Rezvani A, Hanna R. Improved clinical outcomes with omidubicel versus standard myeloablative umbilical cord blood transplantation: results of a phase III randomized, multicenter study. *Transplant Cell Ther*. 2021;27(3):S33-S34.
74. Parikh S, Brochstein JA, Galamidi E, Schwarzbach A, Kurtzberg J. Allogeneic stem cell transplantation with omidubicel in sickle cell disease. *Blood Adv*. 2021;5(3): 843-852.
75. Wagner JE Jr, Brunstein CG, Boitano AE, et al. Phase I/II trial of StemRegenin-1 expanded umbilical cord blood hematopoietic stem cells supports testing as a stand-alone graft. *Cell Stem Cell*. 2016;18(1): 144-155.
76. Finke J, Bethge WA, Schmoor C, et al; ATG-Fresenius Trial Group. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*. 2009; 10(9):855-864.
77. Soiffer RJ, Kim HT, McGuirk J, et al. Prospective, randomized, double-blind, phase III clinical trial of anti-T-lymphocyte globulin to assess impact on chronic graft-versus-host disease-free survival in patients undergoing HLA-matched unrelated myeloablative hematopoietic cell transplantation. *J Clin Oncol*. 2017;35(36):4003-4011.
78. Magenau JM, Jaglowski S, Farag SS, et al. Mitigating damage response with CD34 fusion protein for prevention of acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2020;26(suppl 3):S52-S53.
79. Farag SS, Abu Zaid M, Schwartz JE, et al. Dipeptidyl peptidase 4 inhibition for prophylaxis of acute graft-versus-host disease. *N Engl J Med*. 2021;384(1):11-19.
80. Chen YB, Shah NN, Renteria AS, et al. Vedolizumab for prevention of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Blood Adv*. 2019; 3(23):4136-4146.
81. Watkins B, Qayed M, McCracken C, et al. Phase II trial of costimulation blockade with abatacept for prevention of acute GVHD. *J Clin Oncol*. 2021;39(17):1865-1877.
82. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641-650.
83. Shaw BE, Jimenez-Jimenez AM, Burns LJ, et al. National Marrow Donor Program-sponsored multicenter, phase II Trial of HLA-mismatched unrelated donor bone marrow transplantation using post-transplant cyclophosphamide. *J Clin Oncol*. 2021; 39(18):1971-1982.
84. Bolaños-Meade J, Fuchs EJ, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood*. 2012;120(22):4285-4291.
85. Anurathapan U, Hongeng S, Pakakasama S, et al. Hematopoietic stem cell transplantation for homozygous β -thalassemia and β -thalassemia/hemoglobin E patients from haploidentical donors. *Bone Marrow Transplant*. 2016;51(6): 813-818.
86. Clay J, Kulasekararaj AG, Potter V, et al. Nonmyeloablative peripheral blood haploidentical stem cell transplantation for refractory severe aplastic anemia. *Biol Blood Marrow Transplant*. 2014;20(11):1711-1716.
87. DeZern AE, Zahurak ML, Symons HJ, et al. Haploidentical BMT for severe aplastic anemia with intensive GVHD prophylaxis including posttransplant cyclophosphamide. *Blood Adv*. 2020;4(8):1770-1779.
88. Leick M, Hunter B, DeFilipp Z, et al. Posttransplant cyclophosphamide in allogeneic bone marrow transplantation for the treatment of nonmalignant hematological diseases. *Bone Marrow Transplant*. 2020;55(4):758-762.
89. Bashey A, Zhang MJ, McCurdy SR, et al. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell-replete haploidentical donor transplantation using post-transplant cyclophosphamide [published correction in *J Clin Oncol*. 2019;37(6):528]. *J Clin Oncol*. 2017; 35(26):3002-3009.
90. Massoud R, Gagelmann N, Fritzsche-Friedland U, et al. Comparison of immune reconstitution between anti-T-lymphocyte globulin and post-transplant cyclophosphamide as acute graft-versus-host disease prophylaxis in allogeneic myeloablative peripheral blood stem cell transplantation. *Haematologica*. 2022;107(4):857-867.
91. Jaiswal SR, Chakrabarti A, Chatterjee S, Ray K, Chakrabarti S. Haploidentical transplantation in children with unmanipulated peripheral blood stem cell graft: the need to look beyond post-transplantation cyclophosphamide in younger children. *Pediatr Transplant*. 2016;20(5): 675-682.
92. Imus PH, Tsai HL, Luznik L, et al. Haploidentical transplantation using posttransplant cyclophosphamide as GVHD prophylaxis in patients over age 70. *Blood Adv*. 2019;3(17):2608-2616.
93. Saad A, Lamb LS. Ex vivo T-cell depletion in allogeneic hematopoietic stem cell transplant: past, present and future. *Bone Marrow Transplant*. 2017;52(9):1241-1248.
94. Jakubowski AA, Small TN, Young JW, et al. T cell depleted stem-cell transplantation for adults with hematologic malignancies: sustained engraftment of HLA-matched related donor grafts without the use of antithymocyte globulin. *Blood*. 2007; 110(13):4552-4559.
95. Jakubowski AA, Small TN, Kernan NA, et al. T cell-depleted unrelated donor stem cell transplantation provides favorable disease-free survival for adults with hematologic malignancies. *Biol Blood Marrow Transplant*. 2011;17(9):1335-1342.
96. Devine SM, Carter S, Soiffer RJ, et al. Low risk of chronic graft-versus-host disease and relapse associated with T cell-depleted peripheral blood stem cell transplantation for acute myelogenous leukemia in first remission: results of the blood and marrow transplant clinical trials network protocol 0303. *Biol Blood Marrow Transplant*. 2011; 17(9):1343-1351.
97. Pasquini MC, Devine S, Mendizabal A, et al. Comparative outcomes of donor graft CD34+ selection and immune suppressive therapy as graft-versus-host disease prophylaxis for patients with acute myeloid leukemia in complete remission undergoing HLA-matched sibling allogeneic hematopoietic cell transplantation. *J Clin Oncol*. 2012; 30(26):3194-3201.
98. Bader P, Soerensen J, Jarisch A, et al. Rapid immune recovery and low TRM in haploidentical stem cell transplantation in children and adolescence using CD3/CD19-depleted stem cells. *Best Pract Res Clin Haematol*. 2011;24(3):331-337.
99. Oved JH, Wang Y, Barrett DM, et al. CD3⁺/CD19⁺ depleted matched and mismatched unrelated donor hematopoietic stem cell transplant with targeted T cell addback is associated with excellent outcomes in pediatric patients with nonmalignant hematologic disorders. *Biol Blood Marrow Transplant*. 2019;25(3):549-555.
100. Abdelhakim H, Abdel-Azim H, Saad A. Role of $\alpha\beta$ T cell depletion in prevention of graft versus host disease. *Biomedicine*. 2017; 5(3):E35.
101. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of $\alpha\beta$ T and B cells in children with nonmalignant disorders. *Blood*. 2014; 124(5):822-826.
102. Gaziev J, Isgrò A, Sodani P, et al. Haploidentical HSCT for hemoglobinopathies: improved outcomes with TCR $\alpha\beta$ ⁺/CD19⁺-depleted grafts. *Blood Adv*. 2018;2(3): 263-270.
103. Laberko A, Sultanova E, Gutovskaya E, et al. Mismatched related vs matched unrelated donors in TCR $\alpha\beta$ /CD19-depleted HSCT for primary immunodeficiencies. *Blood*. 2019; 134(20):1755-1763.
104. Strocchio L, Pagliara D, Algeri M, et al. HLA-haploidentical TCR $\alpha\beta$ ⁺/CD19⁺-depleted stem cell transplantation in children and young adults with Fanconi anemia. *Blood Adv*. 2021;5(5):1333-1339.
105. Zhou X, Di Stasi A, Tey SK, et al. Long-term outcome after haploidentical stem cell transplant and infusion of T cells expressing the inducible caspase 9 safety transgene. *Blood*. 2014;123(25):3895-3905.
106. Zhou X, Dotti G, Krance RA, et al. Inducible caspase-9 suicide gene controls adverse effects from alloplete T cells after

- haploidentical stem cell transplantation. *Blood*. 2015;125(26):4103-4113.
107. Martelli MF, Di Ianni M, Ruggeri L, et al. HLA-haploidentical transplantation with regulatory and conventional T-cell adoptive immunotherapy prevents acute leukemia relapse. *Blood*. 2014;124(4):638-644.
 108. Brunstein CG, Miller JS, McKenna DH, et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: kinetics, toxicity profile, and clinical effect. *Blood*. 2016;127(8):1044-1051.
 109. Meyer EH, Laport G, Xie BJ, et al. Transplantation of donor grafts with defined ratio of conventional and regulatory T cells in HLA-matched recipients. *JCI Insight*. 2019;4(10):127244.
 110. Meyer E, Hoeg R, Moroz A, Xie BJ, Hsin-Hsu W, Pawar R. Orca-T, a precision treg-engineered donor product, in myeloablative HLA-matched transplantation prevents acute Gvhd with less immunosuppression in an early multicenter experience. *Transplant Cell Ther*. 2021;27(3):S84-S85.
 111. Kanakry CG, Ganguly S, Zahurak M, et al. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. *Sci Transl Med*. 2013;5(211):211ra157.
 112. Ganguly S, Ross DB, Panoskaltis-Mortari A, et al. Donor CD4⁺ Foxp3⁺ regulatory T cells are necessary for posttransplantation cyclophosphamide-mediated protection against GVHD in mice. *Blood*. 2014;124(13):2131-2141.
 113. McCurdy SR, Luznik L. Post-transplantation cyclophosphamide for chimerism-based tolerance. *Bone Marrow Transplant*. 2019;54(S2 Suppl 2):769-774.
 114. Fitzhugh CD, Cordes S, Taylor T, et al. At least 20% donor myeloid chimerism is necessary to reverse the sickle phenotype after allogeneic HSCT. *Blood*. 2017;130(17):1946-1948.
 115. Abraham A, Hsieh M, Eapen M, et al; Center for International Blood and Marrow Transplant Research. Relationship between mixed donor-recipient chimerism and disease recurrence after hematopoietic cell transplantation for sickle cell disease. *Biol Blood Marrow Transplant*. 2017;23(12):2178-2183.
 116. Andreani M, Manna M, Lucarelli G, et al. Persistence of mixed chimerism in patients transplanted for the treatment of thalassemia. *Blood*. 1996;87(8):3494-3499.
 117. Walters MC, Patience M, Leisenring W, et al; Multicenter Investigation of Bone Marrow Transplantation for Sickle Cell Disease. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. *Biol Blood Marrow Transplant*. 2001;7(12):665-673.
 118. Lawler M, McCann SR, Marsh JC, et al; Severe Aplastic Anaemia Working Party of the European Blood and Marrow Transplant Group. Serial chimerism analyses indicate that mixed haemopoietic chimerism influences the probability of graft rejection and disease recurrence following allogeneic stem cell transplantation (SCT) for severe aplastic anaemia (SAA): indication for routine assessment of chimerism post SCT for SAA. *Br J Haematol*. 2009;144(6):933-945.
 119. Locatelli F, Jordan MB, Allen C, et al. Emapalumab in children with primary hemophagocytic lymphohistiocytosis. *N Engl J Med*. 2020;382(19):1811-1822.
 120. Felber M, Steward CG, Kentouche K, et al. Targeted busulfan-based reduced-intensity conditioning and HLA-matched HSCT cure hemophagocytic lymphohistiocytosis [published correction appears in *Blood Adv*. 2020;4(16):3926]. *Blood Adv*. 2020;4(9):1998-2010.
 121. Shin SH, Park SS, Park S, et al. Non-myeloablative matched sibling stem cell transplantation with the optional reinforced stem cell infusion for patients with hemoglobinopathies. *Eur J Haematol*. 2020;105(4):387-398.
 122. Boelens JJ, Wynn RF, O'Meara A, et al. Outcomes of hematopoietic stem cell transplantation for Hurler's syndrome in Europe: a risk factor analysis for graft failure. *Bone Marrow Transplant*. 2007;40(3):225-233.
 123. Lund TC, Liegel J, Bejanyan N, et al. Second allogeneic hematopoietic cell transplantation for graft failure: poor outcomes for neutropenic graft failure. *Am J Hematol*. 2015;90(10):892-896.
 124. Szanto CL, Langenhorst J, de Koning C, et al. Predictors for autoimmune cytopenias after allogeneic hematopoietic cell transplantation in children. *Biol Blood Marrow Transplant*. 2020;26(1):114-122.
 125. Schuetz C, Hoening M, Moshous D, et al. Daratumumab in life-threatening autoimmune hemolytic anemia following hematopoietic stem cell transplantation. *Blood Adv*. 2018;2(19):2550-2553.
 126. Even-Or E, Naser Eddin A, Shadur B, et al. Successful treatment with daratumumab for post-HSCT refractory hemolytic anemia. *Pediatr Blood Cancer*. 2020;67(1):e28010.
 127. Daikeler T, Labopin M, Ruggeri A, et al. New autoimmune diseases after cord blood transplantation: a retrospective study of EUROCORD and the Autoimmune Disease Working Party of the European Group for Blood and Marrow Transplantation. *Blood*. 2013;121(6):1059-1064.
 128. Kruizinga MD, van Tol MJD, Bekker V, et al. Risk Factors, treatment, and immune dysregulation in autoimmune cytopenia after allogeneic hematopoietic stem cell transplantation in pediatric patients. *Biol Blood Marrow Transplant*. 2018;24(4):772-778.
 129. Pavlasova G, Mraz M. The regulation and function of CD20: an "enigma" of B-cell biology and targeted therapy. *Haematologica*. 2020;105(6):1494-1506.
 130. Bride KL, Vincent T, Smith-Whitley K, et al. Sirolimus is effective in relapsed/refractory autoimmune cytopenias: results of a prospective multi-institutional trial. *Blood*. 2016;127(1):17-28.

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