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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References

1. Etra A, Gergoudis S, Morales G, et al. Assessment of systemic and gastrointestinal tissue damage biomarkers for GVHD risk stratification. *Blood Adv.* 2022;6(12):3707-3715.
2. Hartwell MJ, Özbek U, Holler E, et al. An early-biomarker algorithm predicts lethal graft-versus-host disease and survival. *JCI Insight.* 2018;3(16):e89798.
3. Gergoudis SC, DeFilipp Z, Özbek U, et al. Biomarker-guided preemption of steroid-refractory graft-versus-host disease with α-1-antitrypsin. *Blood Adv.* 2020;4(24):6098-6105.
4. Breit SN, Wakefield D, Robinson JP, Luckhurst E, Clark P, Penny R. The role of alpha 1-antitrypsin deficiency in the pathogenesis of immune disorders. *Clin Immunol Immunopathol.* 1985;35(3):363-380.
5. Marcondes AM, Hockenbery D, Lesnikova M, et al. Response of steroid-refractory acute GVHD to α1-antitrypsin. *Biol Blood Marrow Transplant.* 2016;22(9):1596-1601.
6. Magenau JM, Goldstein SC, Peltier D, et al. α1-Antitrypsin infusion for treatment of steroid-resistant acute graft-versus-host disease. *Blood.* 2018;131(12):1372-1379.
7. Weissinger EM, Metzger J, Dobbelstein C, et al. Proteomic peptide profiling for preemptive diagnosis of acute graft-versus-host disease after allogeneic stem cell transplantation. *Leukemia.* 2014;28(4):842-852.
8. Weissinger EM, Metzger J, Schleuning M, et al. A multicenter prospective, randomized, placebo-controlled phase II/III trial for preemptive acute graft-versus-host disease therapy. *Leukemia.* 2021;35(6):1763-1772.
9. Sormani MP, Oneto R, Bruno B, et al. A revised day +7 predictive score for transplant-related mortality: serum cholinesterase, total protein, blood urea nitrogen, gamma glutamyl transferase, donor type and cell dose. *Bone Marrow Transplant.* 2003;32(2):205-211.
10. Bacigalupo A, Lamparelli T, Milone G, et al; Gruppo Italiano Trapianto Midollo Osseo (GITMO). Pre-emptive treatment of acute GVHD: a randomized multicenter trial of rabbit anti-thymocyte globulin, given on day +7 after alternative donor transplants. *Bone Marrow Transplant.* 2010;45(2):385-391.
11. Luft T, Benner A, Jodele S, et al. EASIX in patients with acute graft-versus-host disease: a retrospective cohort analysis. *Lancet Haematol.* 2017;4(9):e414-e423.
12. Jiang S, Penack O, Terzer T, et al. Predicting sinusoidal obstruction syndrome after allogeneic stem cell transplantation with the EASIX biomarker panel. *Haematologica.* 2021;106(2):446-453.
13. Peña M, Salas MQ, Mussetti A, et al. Pretransplantation EASIX predicts intensive care unit admission in allogeneic hematopoietic cell transplantation. *Blood Adv.* 2021;5(17):3418-3426.
14. Varma A, Rondon G, Srour SA, et al. Endothelial activation and stress index (EASIX) at admission predicts fluid overload in recipients of allogeneic stem cell transplantation. *Biol Blood Marrow Transplant.* 2020;26(5):1013-1020.
15. Sanchez-Escamilla M, Flynn J, Devlin S, et al. EASIX score predicts inferior survival after allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant.* 2023;58(5):498-505.
16. Mariotti J, Magri F, Giordano L, et al. EASIX predicts non-relapse mortality after haploidentical transplantation with post-transplant cyclophosphamide. *Bone Marrow Transplant.* 2023;58(3):247-256.
17. Luft T, Benner A, Terzer T, et al. EASIX and mortality after allogeneic stem cell transplantation. *Bone Marrow Transpl.* 2020;55(3):553-561.
18. Nawas MT, Sanchez-Escamilla M, Devlin SM, et al. Dynamic EASIX scores closely predict nonrelapse mortality after allogeneic hematopoietic cell transplantation. *Blood Adv.* 2022;6(22):5898-5907.
19. Choi J, Cooper ML, Alahmari B, et al. Pharmacologic blockade of JAK1/JAK2 reduces GvHD and preserves the graft-versus-leukemia effect. *PLoS One.* 2014;9(10):e109799.
20. Ponce DM, Alousi AM, Nakamura R, et al. A phase 2 study of interleukin-22 and systemic corticosteroids as initial treatment for acute GvHD of the lower GI tract. *Blood.* 2023;141(12):1389-1401.
21. Yu X, Ma H, Li B, et al. A novel RIPK1 inhibitor reduces GVHD in mice via a nonimmunosuppressive mechanism that restores intestinal homeostasis. *Blood.* 2023;141(9):1070-1086.
22. Van Lier YF, Vos J, Blom B, Hazenberg MD. Allogeneic hematopoietic cell transplantation, the microbiome, and graft-versus-host disease. *Gut Microbes.* 2023;15(1):2178805.
23. Pasquini MC, Logan B, Jones RJ, et al. Blood and marrow transplant clinical trials network report on the development of novel endpoints and selection of promising approaches for graft-versus-host disease prevention trials. *Biol Blood Marrow Transplant.* 2018;24(6):1274-1280.
24. Cuvelier GDE, Nemecek ER, Wahlstrom JT, et al. Benefits and challenges with diagnosing chronic and late acute GvHD in children using the NIH consensus criteria. *Blood.* 2019;134(3):304-316.
25. Locatelli F, Bauquet A, Palumbo G, Moretta F, Bertaina A. Negative depletion of α/β+ T cells and of CD19+ B lymphocytes: a novel frontier to optimize the effect of innate immunity in HLA-mismatched hematopoietic stem cell transplantation. *Immunol Lett.* 2013;155(1-2):21-23.
26. Bleakley M. Naive T-cell depletion in stem cell transplantation. *Blood Adv.* 2020;4(19):4980.
27. Yu J, Storer BE, Kushekhar K, et al. Biomarker panel for chronic graft-versus-host disease. *J Clin Oncol.* 2016;34(22):2583-2590.
28. Subburaj D, Ng B, Kariminia A, et al. Metabolomic identification of α-ketoglutaric acid elevation in pediatric chronic graft-versus-host disease. *Blood.* 2022;139(2):287-299.
29. Schultz KR, Kariminia A, Ng B, et al. Immune profile differences between chronic GvHD and late acute GvHD: results of the ABLE/PBMTC 1202 studies. *Blood.* 2020;135(15):1287-1298.
30. Paczesny S, Hakim FT, Pidala J, et al. National Institutes of Health Consensus Development Project on criteria for clinical trials in chronic graft-versus-host disease: III. The 2014 Biomarker Working Group Report. *Biol Blood Marrow Transplant.* 2015;21(5):780-792.
31. Cuvelier GDE, Ng B, Abdossamadi S, et al. A diagnostic classifier for pediatric chronic graft-versus-host disease: results of the ABLE/PBMTC 1202 study. *Blood Adv.* 2023;7(14):3612-3623.
32. Cuvelier GDE, Schoettler M, Buxbaum NP, et al. Toward a better understanding of the atypical features of chronic graft-versus-host disease: a report from the 2020 National Institutes of Health Consensus Project Task Force. *Transplant Cell Ther.* 2022;28(8):426-445.
33. Ng B, Cuvelier GDE, Abdossamadi S, Ostroumov E, Kariminia A, Schultz KR. Machine learning-based identification of two distinct immune profiles of chronic GvHD in children with the prospective ABLE 1.0 Study Cohort EBMT meeting April 26, Paris, France, 2023.

34. Ng B, Cuvelier GDE, Abdossamadi S, Ostroumov E, Kariminia A, Schultz KR. Two distinct immune profiles are identified in pediatric chronic GvHD by machine learning in the ABLE Study Cohort EBMT meeting April 25, Paris, France, 2023.
35. Iwasaki M, Kanda J, Arai Y, et al. Establishment of a predictive model for GVHD-free, relapse-free survival after allogeneic HSCT using ensemble learning. *Blood Adv.* 2022;6(8):2618-2627.
36. Arora M, Klein JP, Weisdorf DJ, et al. Chronic GVHD risk score: a center for international blood and marrow transplant research analysis. *Blood.* 2011;117(24):6714-6720.
37. Rayment JH, Sandoval RA, Roden JP, Schultz KR. Multiple breath washout testing to identify pulmonary chronic graft versus host disease in children after haematopoietic stem cell transplantation. *Transplant Cell Ther.* 2022;28(6):328.e1-328.e7.
38. Tamburro RF, Cooke KR, Davies SM, et al; Pulmonary complications of pediatric hematopoietic stem cell transplantation workshop participants. Pulmonary complications of pediatric hematopoietic cell transplantation. A National Institutes of Health Workshop Summary. *Ann Am Thorac Soc.* 2021;18(3):381-394.
39. Alborghetti MR, Correa MEP, Whangbo J, et al. Clinical metabolomics identifies blood serum branched chain amino acids as potential predictive biomarkers for chronic graft vs. host disease. *Front Oncol.* 2019;9:141.
40. Markey KA, Schluter J, Gomes ALC, et al. The microbe-derived short-chain fatty acids butyrate and propionate are associated with protection from chronic GVHD. *Blood.* 2020;136(1):130-136.
41. Arai S, Sahaf B, Narasimhan B, et al. Prophylactic rituximab after allogeneic transplantation decreases B-cell alloimmunity with low chronic GVHD incidence. *Blood.* 2012;119(25):6145-6154.

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