Chronic GVHD: review advances in prevention, novel endpoints, and targeted strategies

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Allogeneic hematopoietic cell transplantation (allo-HCT) is a curative therapy for many malignant and non-malignant hematologic disorders. Chronic graft-versus-host (cGVHD) disease remains a significant hurdle for long-term survival in patients post allo-HCT, and it remains the leading cause of late non-relapse mortality. The risk factors for development of cGVHD include degree of human leukocyte antigen (HLA) disparity, increasing recipient age, use of peripheral blood stem cells as a source, myeloablative conditioning regimens, prior acute GVHD (aGVHD), and female donor to male recipient. Our biological understanding of cGVHD is mostly derived from transplantation mouse models and patient data. There are three distinct phases in the development of cGVHD. Approaches to prevent GVHD include pharmacologic strategies such as calcineurin inhibitors (cyclosporine, tacrolimus) combined with methotrexate or mTOR inhibitors (sirolimus),and IMP dehydrogenase inhibitors (mycophenolate mofetil). Increasingly, posttransplant cyclophosphamide is emerging as a promising strategy for GVCHD prevention especially in a setting of reduced intensity conditioning. Other approaches include serotherapy (ATG, Campath) and graft manipulation strategies. A significant obstacle to evaluating the response of novel GVHD-directed therapies has been standardized response assessments. This has functioned as a barrier to designing and interpreting clinical trials that are structured around the treatment of cGVHD. Novel endpoints including failure-free survival, Graft-versus-host disease-free, relapse-free survival (GRFS), and current GVHD-free, relapse-free survival (CGRFS) may create a clearer picture for post-HCT outcomes. Targeted therapies including Bruton's tyrosine kinase inhibition, JAK1/2 inhibition, and ROCK2 inhibitors have improved cGVHD therapy, especially in the steroid refractory setting. Continued improvement in prophylactic strategies for cGVHD, identification of accurate cGVHD treatment endpoints, and access to novel therapeutic agents are expected to improve cGVHD outcomes.

LEARNING OBJECTIVES
- Be able to recognize FDA-approved targeted therapies in cGVHD
- Understand the basic strategy of prevention techniques of cGVHD

Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a curative therapy for many malignant and non-malignant hematologic disorders. Chronic graft-versus-host (cGVHD) disease remains a significant hurdle for long-term survival in patients post allo-HCT, and it remains the leading cause of late non-relapse mortality (NRM). The most recent data from the Center for International Blood and Marrow Transplant Research (CIBMTR) suggest that approximately 15% of post-allogeneic stem cell transplant mortality beyond day +100 is related to GVHD.[1] Patients with cGVHD also have increased morbidity, poor quality of life, and increased resource utilization, resulting in higher healthcare costs and patient dissatisfaction rates.[2,3] Despite many advances in HCT, the incidence of cGVHD is predicted to rise, given demographic changes in patients undergoing allo-HCT and the increasing use of peripheral blood stem cell grafts.[4] CIBMTR data suggest that utilization of matched unrelated donors (MUDs) is increasing with time, and a majority of HCTs in adults are now being done using peripheral blood stem cell (PBSC) grafts. Patients above the age of 65 comprise 25% of patients undergoing allo-HCT and are the fastest-growing demographic based on the recent CIBMTR database. Based on the factors described above, the incidence of chronic GVHD will continue to rise as allo-HCT becomes accessible to more patients.[5] The incidence of cGVHD is estimated to be in around 40-70% of patients after undergoing allo-HCT.[6,7] In 2016, the prevalence of cGVHD was predicted to be 14,000 patients based on the Medicare fee-for-service database. In this data set, 40% of

patients developed chronic GVHD within three years of undergoing allo-HCT, with 70% requiring second-line therapy after failing corticosteroids.[6] Chronic GVHD is a multisystem disorder involving multiple organ systems. The most involved organs include the skin, eyes, mouth, gastrointestinal (GI) tract, and liver. Approximately 40% of patients who develop cGVHD have severe disease, and 42% of patients have four or more organs involved. With improvements in supportive care practices and the use of reduced intensity conditioning (RIC) regimens in older patients, there has been a decline in NRM, but with an increase in the incidence of cGVHD as patients are surviving longer post HCT (Odds ratio [OR] 1.19, $P<0.0001$).[4] As our overall population ages and more patients have access to HCT, the incidence and burden of cGVHD will continue to rise. Hence, novel strategies are needed to prevent and treat cGVHD.[7]

The NIH consensus criteria were developed to assess the severity of cGVHD and classified into either mild (one to two organs, each with an organ score of 1), moderate (≥3 organs with a score of 1, or at least one organ with a score of 2), or severe cGVHD (at least one organ with a score of 3 or lung score of 2).[8,9] Involved organs (eyes, mouth, lungs, GI tract, liver, joints fascia, genital tract) in the 2014 NIH grading system are scored for severity (0 to 3) of GVHD manifestations. This system predicts overall survival (OS); for patients diagnosed with moderate or severe cGVHD, the OS and NRM are worse compared to patients classified with mild cGVHD.[10] The NIH classification is a significant advancement in the field because it allows uniform assessment of GVHD target organs and because response to treatment can be accurately evaluated longitudinally in patients, thereby removing interobserver bias. The widespread adoption of NIH consensus criteria has facilitated clinical trial development in cGVHD patients, resulting in the approval of three novel agents for treating patients with cGVHD.

Pathophysiology

The risk factors for the development of cGVHD include the degree of HLA disparity, increasing recipient age, use of PBSC as stem cell source, myeloablative conditioning regimens, prior aGVHD, and female donor to male recipient. Chronic GVHD is primarily thought to be a Th2-mediated T-effector cell response with a relative deficiency of regulatory T cells. Increasingly, the role of B cells, antigen-presenting cells, and macrophages is being understood in the pathogenesis of cGVHD, and therapies targeting these cell types are being tested in clinical trials. Proinflammatory cytokines such as IL-21, IL-17, TGF-β, IL-6, IL-12, IL-1, IFN gamma, TNF, and BAFF are essential mediators for graft-versus-host disease, and these mediators lead to tissue damage and, eventually, fibrosis.

Our biological understanding of cGVHD mainly derives from transplantation mouse models and patient data.[10] There are three distinct phases in the development of cGVHD. The early phase is related to acute inflammation and tissue injury. Conditioning-related tissue damage leads to the activation of donor T cells on contact with antigen-presenting cells, which then upregulate co-stimulatory molecules.[11] Epithelial damage from conditioning stimulates the release of soluble inflammatory mediators that activate antigen-presenting cells that present host antigens to donor T cells.[12] Endothelial damage reduces microvascular density due to intimal arteritis and, subsequently, fibrosis.

In the second phase of cGVHD, hallmarks are chronic inflammation and dysregulated immunity. The tissue injury from phase 1 causes the expansion of alloreactive B and T cells primed by antigen-presenting cells to proliferate into Type-1, Type-2, and Type-17 helper T cells.[13,14] This leads to increased cytokine production by CD4 positive T cells that have previously escaped immune regulation and deletion in the thymus due to thymic injury from conditioning. Within lymphoid follicles, T follicular helper cells produce inflammatory cytokines that lead to the expansion of B cell clones. Thymic epithelial damage due to conditioning leads to loss of regulatory T and B cells and peripheral tolerance.[15]

In the final phase, which is characterized by aberrant tissue repair and fibrosis, platelet-derived growth factor alpha activates fibroblasts, and the production of collagen by transforming growth factor beta secreted by macrophages leads to sclerotic cGVHD.[16]

Prevention

Approaches to prevent GVHD include pharmacologic strategies such as calcineurin inhibitors (cyclosporine, tacrolimus) combined with methotrexate or mTOR inhibitors (sirolimus) and IMP dehydrogenase inhibitors (mycophenolate mofetil). Increasingly, posttransplant cyclophosphamide is emerging as a promising strategy for GVHD prevention, especially in the RIC setting. Other approaches include serotherapy (ATG, Campath) and graft manipulation strategies.

Calcenurin inhibitor (CNI); with four doses of methotrexate (45 mg/m^2) has been the standard of care (SOC); for GVHD prophylaxis in patients undergoing RIC or myeloablative conditioning (MAC), but the field is evolving. Allo-HCT based on studies done at Seattle[17] shows that the combination is more effective in controlling aGVHD than CNI alone. The combination, however, is associated with significant mucositis, delay in engraftment, and interstitial pneumonitis, prompting efforts to look at alternative prophylactic strategies. Sirolimus has immunosuppressive properties by virtue of its FKBP12 binding and mTOR inhibition, which leads to multiple downstream effects and regulatory T cell expansion.[18-20] In combination with CNI and methotrexate (MTX), sirolimus was first used in a phase 1/2 study of alternative donor transplants (mMRD and MUD) in patients receiving TBI-based MAC-allo-HCT. The combination tolerated and effectively controlled acute and chronic GVHD.[21] Subsequent studies showed acceptable GVHD rates with Tacrolimus/sirolimus (T/S) alone in matched related donor (MRD) undergoing MAC[22] and RIC allo-HCTs using Flu/Bu regimen.[23] Based on these promising early results, we were the first group to study the combination of Flu/Mel (n=46) conditioning with Tacrolimus/sirolimus (T/S)-based GVHD prophylaxis in a pilot phase 2 study in 85 patients who received sibling HCT (n=46 received Bu/Cy and n=28 received FTBI/VP16). All patients in this study were engrafted, and the incidence of Gd 2-4 and 3-4 aGVHD was 43% and 19%, respectively, and the two-year incidence of cGVHD was 46%. Higher rates of thrombotic microangiopathy were seen in the Bu/Cy group.[24,25] This regimen has been successfully used in multiple other disease subtypes, including acute lymphoblastic leukemia[26] myelofibrosis,[27] and myelodysplastic syndrome (MDS).[28]

Based on predictable engraftment, low incidence of mucositis, and reasonable control of aGVHD, we have used T/S-based GVHD prophylaxis as our standard for allo-HCTs in patients across multiple disease subtypes since 2005 in both RIC and

MAC setting.[29] A few studies directly compare T/S versus Tac/MTX in the RIC setting. Pidala et al reported in the long-term follow-up of their randomized phase 2 study significantly lower rates of NIH moderate-severe cGVHD in favor of the T/S arm in contrast to Tac/MTX.[30] Another randomized study comparing T/S to CNI/MTX found no differences in key outcomes of aGVHD, NRM, or five-year OS between the two groups.[31] Cutler et al reported clinical outcomes of T/S vs Tac/MTX in acute myeloid leukemia (AML)/MDS patients undergoing allo-HCT after MAC. They did not see any differences between aGVHD or OS between the two groups.[32]

PROGRESS 1 and PROGRESS 2 are two large randomized controlled trials evaluating novel regimens with intriguing results. PROGRESS 1 was a randomized phase 3 study evaluating GVHD prophylaxis interventions with myeloablative conditioning regimens. The three study arms were (1) PTCy from BM graft, (2) control Tac/MTX with BM graft, and (3) CD34 selected T-cell depleted PBSC. Among the 346 patients randomly assigned, the two-year incidence of cGVHD and chronic GVHD, relapse-free survival (CRFS) was no different between the three arms. There was a noted reduction in OS in the CD34 selected PBSC arm (60%; hazard ratio [HR], 1.74; 1.09 to 2.80; P=.02) compared to the control (76.1%) and PTCy (76.2%; HR, 1.02; 0.60 to 1.72; P=.95). CD34 selection was associated with lower moderate to severe cGVHD (HR 0.25; p=0.02).[33] Currently, CNI with methotrexate remains SOC for patients undergoing allo-HCT with MAC regimens.

PROGRESS 2 is a phase 2 multicenter trial in allo-HCT patients who received an RIC regimen and who were randomized to (i) TAC/MMF/PTCy, (ii) TAC/MTX/BOR, (iii) TAC/MTX/Maraviroc and compared to a nonrandomized prospective standard of care cohort TAC/MTX. In all, 273 patients were randomized to the three study arms, and 224 received control, and the composite endpoint GRFS revealed improved outcomes with TAC/MMF/PTCy (HR 0.72; 90% CI 0.54-0.94, p=0.04).[34]

Posttransplant cyclophosphamide (PTCy) in conjunction with tacrolimus and mycophenolate mofetil has been used for many years in haploidentical transplantation with a low incidence of cGVHD,[35] in addition to manageable cGVHD rates in the mismatch unrelated donor (MMUD) setting.[36] There is emerging data showing that patients with HLA-matched unrelated donors using PTCy for GVHD prophylaxis effectively reduce the incidence of GVHD without any substantial changes in relapse and overall survival.[37] PROGRESS 3 is a phase 3 trial evaluating GVHD prophylaxis TAC/MMF/PTCy (experimental) compared to TAC/MTX (standard) in allo-HCT patients receiving either an HLA-matched donor (related or unrelated) or a mismatched (7/8) donor. There were 214 patients in the experimental arm and 217 patients in the standard arm with GRFS as a primary endpoint. The experimental arm had improved GRFS compared to standard prophylaxis (52.7% compared to 34.9%). In addition, there were reduced rates of cGVHD at one year with the experimental prophylaxis group (21.9%) compared to the standard (35.1%).[37]

Abatacept has shown promising results when used as a prophylaxis in combination with Tac/MTx and has been approved for GVHD prophylaxis since December 2021. It is a cytotoxic T-lymphocyte-associated antigen 4 directed monoclonal antibody. In trial ABA2, it was noted that abatacept showed a decrease in D100 grade 3-4 aGVHD rates without any improvement in cGVHD rates.[38] A multicenter phase II randomized controlled trial ABA3 will be performed to evaluate whether an extended abatacept dose compared to a short-term dose can prevent cGVHD (NCT04380740).

Graft manipulation strategies are emerging as promising strategies for preventing GVHD in early clinical trials. These strategies involve the removal of specific T-cell subsets from a PBSC graft or decreasing the inoculum of conventional T cells (Tcons) after infusion with regulatory T cells (Tregs).

Investigators at the University of Perugia pioneered the strategy of using T-cell-depleted stem cell grafts from haploidentical donors in patients with high-risk leukemia. They used a strategy of T-cell depletion by soybean agglutination, E-rosseting, and CD34 positive selection. Using the strategy, they minimized regimen-related toxicity and incidence of graft-versus-host disease.[39] However, relapse-related mortality remained problematic, and this approach was further refined by developing T-cell adoptive immunotherapy wherein patients received myeloablative conditioning followed by co-infusion of regulatory T cells and conventional T cells. This approach achieved complete donor chimerism with a low incidence of acute GVHD and relapse in most patients. The graft versus leukemia effect and low relapse were mainly due to unopposed Tcon alloantigen recognition in the bone marrow.[40,41] Similarly, studies done in patients with high-risk hematologic malignancies using matched donors showed promising results. Patients received HLA-matched Tregs and CD34-selected Hematopoietic progenitor cells (HPC) followed by infusion of equal ratio Tcons after myeloablative conditioning.[42] In matched donor settings, low rates of acute graft-versus-host disease were noted without the use of posttransplant immunosuppression. Thus, the approach of using ex vivo engineered graft after myeloablative conditioning in young patients successfully allows engraftment and is associated with low rates of relapse and known relapse mortality.[43]

Orca-T is a cellular infusion product of purified donor regulatory T cells, and utilization of this product augments alloreactive immune responses. In a phase I/II study with Orca-T patients who received myeloablative conditioning, Orca-T and a single agent prophylaxis of either sirolimus or tacrolimus had low rates of moderate/severe GVHD (6% at one year).[43] Low relapse rates with the loss of control of GVHD have also been shown in haploidentical stem cell transplantation.[40,41]

Naive T cells (CD45RA+) have been shown to cause severe GVHD in murine models. A prospective study evaluated naive T-cell-depleted allo-HCT grafts. The three-year cumulative incidence of mild, moderate, and severe cGVHD were 6%, 1%, and 0%, respectively, cGVHD without any increase in relapse or infections.[44] Studies are currently in progress to evaluate the efficacy of naive T-cell depletion from a PBSC graft in a haploidentical and matched donor setting (NCT03802695)

Novel endpoints

A significant obstacle to evaluating the response of novel GVHD-directed therapies has been standardized response assessments. This has functioned as a barrier to designing and interpreting clinical trials structured around the treatment of cGVHD. Previously, overall survival or survival with the resolution of cGVHD were endpoints used for cGVHD clinical trials, but collection of these data is less than ideal in early phase studies. A consensus criterion was developed in 2005 by the NIH Consensus Conference with quantitative measurements to capture

responses better.[45] A multicenter prospective study of the incidence and prevalence of cGVHD requiring systemic therapy did not show that these response criteria correlated with survival (adjusted HR, 0.6; 95% CI 0.2-1.4; *P*=.20).[46]

Failure-free survival (FFS) is a composite endpoint defined as the absence of treatment change, NRM, and recurrent malignancy during initial systemic therapy.[47] FFS rates were 54% at 12 months at first-line immunosuppression[47] and 45% at second-line.[48] A prospective observational study identified variables associated with lower FFS: higher NIH skin score, higher NIH GI score, worse range of motion score, lower forced vital capacity (%), bronchiolitis obliterans syndrome (BOS), worse health-related quality of life (HRQOL), moderate to severe hepatic dysfunction, absence of treatment for gastric acid, female donor for male recipient, and prior grade II-VI aGVHD.[49] In landmark analyses by the Chronic GVHD Consortium, FFS at one year with a complete response (CR)/partial response (PR) (20%) has been associated with clinical benefit, including lower burden of disease, shorter time to end of the systemic treatment, and better survival.[50] Treatment of cGVHD that incorporates glucocorticoid treatment initially has clinical improvement, but those who have sustained responses and FFS at one year are less than 20%.[51]

The NIH Consensus 2020 Treatment of Chronic GVHD report recommends FFS as a key secondary endpoint to be used in phase 2 cGVHD studies.[51] A few pivotal studies have used FFS as an endpoint.

A large cohort of 745 patients from three observational studies evaluated the effect of initial therapy for cGVHD on FFS. Initial therapies were no prednisone (*n*=137), prednisone alone (*n*=411), or prednisone plus other therapy (*n*=197). There were no associations noted with FFS in regard to the type of initial therapy, the dose of steroids, or the overall cGVHD severity.[52] This may signal that lower doses of prednisone or prednisone-free therapies to treat cGVHD may be on the horizon, but we will need prospective studies to clarify this.

FFS was also utilized as a secondary endpoint in REACH3, a phase 3, open-label, randomized study evaluating ruxolitinib vs best available therapy (BAT) in steroid-refractory/dependent cGVHD, which showed significant improvement in the overall response rate (ORR) ($p<0.0001$), more prolonged FFS ($p<0.0001$), and greater symptom improvement. However, 50% of patients enrolled in RUX discontinued it either because of lack of efficacy (15%), adverse effects (17%), or relapse (5%). FFS was significantly longer in the RUX-treated patients (median FFS not reached vs 5.7 months, HR 0.370; *P*<0.0001).[53]

BMT CTN 0801 evaluated prednisone/sirolimus with or without calcineurin inhibitor calcineurin inhibitor for the treatment of cGVHD and evaluated failure-free survival rates between two and three-drug regimens and failed to show any difference in benefit between a three-drug regimen compared to two-drug regimen.[54]

A phase 2 study evaluated the combination of prednisone and Ofaftumab as initial therapy for cGVHD. This study had 53% FFS at 12 months. This was statistically superior to the landmark Martin et al study.[50] The 12-month FFS with CR/PR compared to the 12-month FFS without CR/PR had a higher likelihood of completely discontinuing steroids by 24 months (OR 8; $p=0.025$).[55] Though the study did not meet its primary endpoint of hypothesized ORR, the secondary endpoint of FFS revealed promising results.

We are still looking for novel therapies that can improve rates of complete/partial responses and failure-free survival. Though FFS can be a helpful endpoint, it does not quantify the extent of organ involvement or the severity of symptoms. Thus, for clinicians, it is unclear how this endpoint may dictate the clinical management of patients.

GVHD-free/relapse-free survival (GRFS) is a composite endpoint that includes grade 3-4 acute GVHD, chronic GVHD requiring systemic therapy, relapse, or death in the first post-HCT year. BMTCTN proposed GRFS as a more effective endpoint in capturing the effectiveness of GVHD prophylaxis. In 907 HCT recipients with tacrolimus and methotrexate as GVHD prophylaxis, one-year GRFS was 31%, with a one-year OS at 63%.[56] These results suggested survival may not completely capture those with suboptimal results. An extensive registry analysis of 5059 HCT recipients with AML evaluated GRFS incidence in MUD and match sibling donor (MSD) recipients. MDS had better GRFS outcomes (HR 1.19, CI 1.07-1.31, $p<0.01$), which may be related to greater extensive cGVHD in MUD recipients (HR 1.42, CI 1.19-1.69, $p<0.01$).[57]

Since mild cGVHD can receive systemic immunosuppression to treat their cGVHD attempts have been made to improve the original GRFS composite endpoint. An extensive European Society for Blood and Marrow Transplantation (EBMT) analysis refined GRFS by replacing cGVHD requiring systemic therapy with the occurrence of severe cGVHD. They analyzed 20 937 patients with AML who received HCT with three-year modified GRFS at 40.1%, with severe cGVHD making up 26%. Of those noted to have severe cGVHD, 86% still had severe cGVHD at the last follow-up, with 14% limited.[58] It has been indicated that moderate to severe cGVHD is associated with inferior survival.[3] Since mild cGVHD can receive systemic immunosuppression to treat their cGVHD. A single institution study attempted to adequately capture the development of NIH-grade moderate to severe cGVHD in a modified GRFS endpoint. The retrospective study evaluated 613 HCT patients after an MRD, MUD, or haplo donor source. It replaced cGVHD requiring systemic immunosuppression in GRFS with the development of NIH-grade moderate or severe cGVHD. One-year modified GRFS was 36% compared to the traditional GRFS of 33%, with moderate/severe cGVHD being the most common (38%) reason for failing at one year.[59] GRFS may not adequately capture the dynamic nature of cGVHD because this endpoint captures GVHD in a binary fashion, and its endpoint does not indicate the resolution of GVHD.

Current GVHD-free, relapse-free survival (CGRFS) is a composite endpoint to help address some of the issues associated with GRFS. At any time posttransplant, it is defined as the probability of being alive, in remission, and without clinically significant chronic GVHD, defined as moderate to severe.[60] This is a natural extension of Pidala et al's analysis—a single institution analysis of 422 allo-HCT patients using MRD, MUD, or Haplo donor sources. Solomon et al noted that CGRFS occurrence after one, two, three, and four years was 45%, 46%, 47%, and 49%, respectively. At year 4, less than a quarter of patients were captured by GRFS, but nearly half were captured by CRFS, effectively demonstrating CGRFS as a better endpoint for capturing success without GVHD. In addition, there has been a steady improvement in outcomes over time. The treatment of cGVHD as a dynamic outcome as opposed to a binary one may create a clearer picture of

Table 1. Clinical reports of JAK inhibitor treatment for cGVHD

Reference	JAK inhibitor	Study type	GVHD severity	Patients	Prior treatments, median (range)	Follow-up duration, median (range)	Response	OS (95% CI)
cGVHD								
Khoury et al[67]	Ruxolitinib	Retrospective	Severe	19	NA	18 (6–27) mo	ORR, 89%	NA
Zeiser et al[68–69]	Ruxolitinib	Retrospective	Moderate or severe	41	3 (1–10)	22.4 (3–135) wk	ORR, 85% (CR, 7%)	6 mo, 97% (92%-100%)
						24 (NA) mo	Ongoing, 24%	12 mo, 93% (85%-100%)
Spoerl et al[70]	Ruxolitinib	Pilot	Grade 3	2	4 (3–5)	23.5 (10–37) wk	Response, 100%	NA
Mori et al[71]	Ruxolitinib	Retrospective	Severe	3	2 (1–2)	NA	ORR, 100% (CR, 57%)	NA

post-HCT outcomes. A large number of trials evaluated the endpoints of GRFS/cGRFS at one year, and it is essential to note that though the median time to onset is four to six months after HCT, up to 10% are diagnosed beyond one year with treatment for a median of two to three years.[61] These studies of short duration may under/overestimate the significance of cGVHD in alloHCT in their time-to-event endpoint analysis.

Targeted therapies

Ibrutinib targets Bruton's tyrosine kinase (BTK) pathway in B cells and IL-2 inducible T cell kinase (ITK) in T cells and was the first FDA-approved agent in cGVHD. Its approval was based on a multicenter, open-label, phase 1b/2 study in patients with active cGVHD who were steroid-dependent/refractory. The median follow-up was 14 months, and the overall response rate was 67% (CR 21% and PR 45%), with 71% of responders having a durable response (>20 weeks). Responses were seen in all organs. The update follow-up (median follow-up of 26 months) published two years after the initial publication revealed ORR 69% and CR 31% with sustained responses >44 weeks at 55%. The most common grade 3 adverse effects (AEs) were pneumonia, fatigue, and diarrhea.[62]

JAK1-JAK2 signaling is vital to inflammation and tissue damage in acute and chronic GVHD. Ruxolitinib, a JAK1/2 inhibitor, was evaluated in a phase 3 open-label study in patients with steroid-refractory cGVHD, comparing ruxolitinib 10mg twice daily to investigators' choice (REACH 3). The overall response (CR + PR) at week 24 was 49.7% in the ruxolitinib arm compared to 25.6%. Those randomized to the ruxolitinib arm had longer FFS compared to controls (18.6 vs 5.7 months; $p<0.001$)[63] (see Table 1). A phase I/II study evaluating pacritinib, a novel selective JAK2/IRAK inhibitor in refractory chronic GVHD, is ongoing (NCT05531786).

Belumosodil is an oral selective rho-associated coiled-coil-containing protein kinase 2 (ROCK2) inhibitor. ROCK2 acts on the dysregulated adaptive immune system and fibrosis due to aberrant tissue repair.[64] ROCKstar was a phase 2 multicenter registration study in cGVHD patients who previously received two to five lines of therapy. High response rates (ORR 74% and 77% for 200 mg daily and 200 mg twice daily, respectively) were seen in all organs, including high levels of CR, and responses were seen in all subgroups in addition, including those who previously received ruxolitinib, which was 68%, and ibrutinib, which was 74%. Responses were also generally rapid, with a median response time of five weeks. AEs were seen in 54% of patients.[64] Belumosodil was approved in July 2021.

Colony-stimulating factor 1 receptor (CSF-1R) dependent macrophages promote inflammation and tissue injury, leading to cGVHD fibrosis. Axatilimab is an IgG4 monoclonal antibody with a high affinity for CSF-1R, leading to impaired CSF-1R signaling via two ligands, CSF-1 and IL-34.[65] A phase I/II open-label study evaluating axatilimab in patients with active cGVHD after two lines of systemic therapy. Among the 22 evaluable patients in phase II, there were high response rates (ORR 50% at cycle 7, day 1, and 82% for the first six cycles) in the phase II cohort. In the entire study population, ORR was 67% (26 of 39), with responses seen in all organs with no differences in outcomes for moderate vs severe cGVHD. Responses were rapid, with a median response time of four weeks. Treatment-related grade ≥3 AEs were in 20% of patients. A phase 2 study evaluating axatilimab in cGVHD at three different dose levels is ongoing (AGAVE-201; NCT04710576).[66]

Conclusion

We anticipate continued improvement of prophylactic strategies for preventing GVHD; the identification of more accurate endpoints for determining the efficacy of treatment for cGVHD; and access to novel therapeutic agents to treat new and refractory cGVHD as well as established cGVHD. We further expect that cGVHD outcomes will continue to improve in allo-HCT recipients.

Authorship

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Conflict-of-interest disclosure

Idoroenyi Amanam: no competing financial interests to declare.
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Off-label drug use

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References

1. Barrett AJ, Battiwalla M. Relapse after allogeneic stem cell transplantation. *Expert Rev Hematol.* 2010;3(4):429-441.
2. Yu J, Lal LS, Anderson A, DuCharme M, Parasuraman S, Weisdorf D. Healthcare resource utilization and costs among patients with steroid-resistant chronic graft-versus-host disease in the United States: a retrospective claims database analysis. *Curr Med Res Opin.* 2021;37(5):755-759.
3. Pidala J, Kurland B, Chai X, et al. Patient-reported quality of life is associated with severity of chronic graft-versus-host disease as measured by NIH criteria: report on baseline data from the Chronic GVHD Consortium. *Blood.* 2011;117(17):4651-4657.
4. Arai S, Arora M, Wang T, et al; Graft-vs-Host Disease Working Committee of the CIBMTR. Increasing incidence of chronic graft-versus-host disease in allogeneic transplantation: a report from the Center for International Blood and Marrow Transplant Research. *Biol Blood Marrow Transplant.* 2015;21(2):266-274.
5. Lin RJ, Artz AS. Allogeneic hematopoietic cell transplantation for older patients. *Hematology Am Soc Hematol Educ Program.* 2021;2021(1):254-263.
6. Bachier CR, Aggarwal SK, Hennegan K, et al. Epidemiology and treatment of chronic graft-versus-host disease post-allogeneic hematopoietic cell transplantation: a US claims analysis. *Transplant Cell Ther.* 2021;27(6):504.e1-504504.e6.
7. Zeiser R, Blazar BR. Pathophysiology of chronic graft-versus-host disease and therapeutic targets. *N Engl J Med.* 2017;377(26):2565-2579.
8. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant.* 2015;21(3):389-401.e1401e1.
9. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant.* 2005;11(12):945-956.
10. Pidala J, Kim J, Anasetti C, et al. The global severity of chronic graft-versus-host disease, determined by National Institutes of Health consensus criteria, is associated with overall survival and non-relapse mortality. *Haematologica.* 2011;96(11):1678-1684.
11. Zhao D, Young JS, Chen Y-H, et al. Alloimmune response results in expansion of autoreactive donor CD4+ T cells in transplants that can mediate chronic graft-versus-host disease. *J Immunol.* 2011;186(2):856-868.
12. Wilhelm K, Ganesan J, Müller T, et al. Graft-versus-host disease is enhanced by extracellular ATP activating P2X7R. *Nat Med.* 2010;16(12):1434-1438.
13. Allen JL, Tata PV, Fore MS, et al. Increased BCR responsiveness in B cells from patients with chronic GVHD. *Blood.* 2014;123(13):2108-2115.
14. Young JS, Wu T, Chen Y, et al. Donor B cells in transplants augment clonal expansion and survival of pathogenic CD4+ T cells that mediate autoimmune-like chronic graft-versus-host disease. *J Immunol.* 2012;189(1):222-233.
15. Sakoda Y, Hashimoto D, Asakura S, et al. Donor-derived thymic-dependent T cells cause chronic graft-versus-host disease. *Blood.* 2007;109(4):1756-1764.
16. Forcade E, Paz K, Flynn R, et al. An activated Th17-prone T cell subset involved in chronic graft-versus-host disease sensitive to pharmacological inhibition. *JCI Insight.* 2017;2(12).
17. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med.* 1986;314(12):729-735.
18. Kirken RA, Wang YL. Molecular actions of sirolimus: sirolimus and mTor. *Transplant Proc.* 2003;35(3):s227-S230.
19. Sehgal SN. Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin Biochem.* 1998;31(5):335-340.
20. Battaglia M, Stabilini A, Migliavacca B, Horejs-Hoeck J, Kaupper T, Roncarolo M-G. Rapamycin promotes expansion of functional CD4+CD25+FOXP3+ regulatory T cells of both healthy subjects and type 1 diabetic patients. *J Immunol.* 2006;177(12):8338-8347.
21. Antin JH, Kim HT, Cutler C, et al. Sirolimus, tacrolimus, and low-dose methotrexate for graft-versus-host disease prophylaxis in mismatched related donor or unrelated donor transplantation. *Blood.* 2003;102(5):1601-1605.
22. Cutler C, Kim HT, Hochberg E, et al. Sirolimus and tacrolimus without methotrexate as graft-versus-host disease prophylaxis after matched related donor peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant.* 2004;10(5):328-336.
23. Ho VT, Aldridge J, Kim HT, et al. Comparison of Tacrolimus and Sirolimus (Tac/Sir) versus Tacrolimus, Sirolimus, and mini-methotrexate (Tac/Sir/MTX) as acute graft-versus-host disease prophylaxis after reduced-intensity conditioning allogeneic peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant.* 2009;15(7):844-850.
24. Rodriguez R, Palmer J, Nakamura R, et al. Tacrolimus and sirolimus as GVHD prophylaxis for HLA-MRD allogeneic HCT conditioned with 3 regimens: toxicity and efficacy in 70 Patients. *Blood.* 2006;108(11):2867-2867.
25. Rodriguez R, Nakamura R, Palmer JM, et al. A phase II pilot study of tacrolimus/sirolimus GVHD prophylaxis for sibling donor hematopoietic stem cell transplantation using 3 conditioning regimens. *Blood.* 2010;115(5):1098-1105.
26. Mei M, Tsai N-C, Mokhtari S, et al. Long-term outcomes of allogeneic hematopoietic cell transplant with fludarabine and melphalan conditioning and tacrolimus/sirolimus as graft-versus-host disease prophylaxis in patients with acute lymphoblastic leukemia. *Biol Blood Marrow Transplant.* 2020;26(8):1425-1432.
27. Ali H, Aldoss I, Yang D, et al. MIPSS70+ v2.0 predicts long-term survival in myelofibrosis after allogeneic HCT with the Flu/Mel conditioning regimen. *Blood Adv.* 2019;3(1):83-95.
28. Nakamura R, Palmer JM, O'Donnell MR, et al. Reduced intensity allogeneic hematopoietic stem cell transplantation for MDS using tacrolimus/sirolimus-based GVHD prophylaxis. *Leuk Res.* 2012;36(9):1152-1156.
29. Salhotra A, Hui S, Yang D, et al. Long-term outcomes of patients with acute myelogenous leukemia treated with myeloablative fractionated total body irradiation TBI-based conditioning with a tacrolimus- and sirolimus-based graft-versus-host disease prophylaxis regimen: 6-year follow-up from a single center. *Biol Blood Marrow Transplant.* 2020;26(2):292-299.
30. Pidala J, Kim J, Alsina M, et al. Prolonged sirolimus administration after allogeneic hematopoietic cell transplantation is associated with decreased risk for moderate-severe chronic graft-versus-host disease. *Haematologica.* 2015;100(7):970-977.
31. Törlén J, Ringdén O, Garming-Legert K, et al. A prospective randomized trial comparing cyclosporine/methotrexate and tacrolimus/sirolimus as graft-versus-host disease prophylaxis after allogeneic hematopoietic stem cell transplantation. *Haematologica.* 2016;101(11):1417-1425.
32. Cutler C, Logan B, Nakamura R, et al. Tacrolimus/sirolimus vs tacrolimus/methotrexate as GVHD prophylaxis after matched, related donor allogeneic HCT. *Blood.* 2014;124(8):1372-1377.
33. Luznik L, Pasquini MC, Logan B, et al. Randomized phase III BMT CTN trial of calcineurin inhibitor–free chronic graft-versus-host disease interventions in myeloablative hematopoietic cell transplantation for hematologic malignancies. *J Clin Oncol.* 2022;40(4):356-368.
34. Bolaños-Meade J, Reshef R, Fraser R, et al. Prevention of graft versus host disease with hematopoietic cell Transplantation with reduced intensity conditioning—a comparison of three prophylaxis regimens (tacrolimus/mycophenolate mofetil/cyclophosphamide, tacrolimus/methotrexate/bortezomib or tacrolimus/methotrexate/maraviroc) versus tacrolimus/methotrexate: a randomised phase 2 trial with a non-randomized contemporaneous control group (BMT CTN 1203). *Lancet Haematology.* 2019;6(3):e132-e143.
35. 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.
36. 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.
37. Bolaños-Meade J, Hamadani M, Wu J, et al; BMT CTN 1703 investigators. Post-transplantation cyclophosphamide-based graft-versus-host disease prophylaxis. *N Engl J Med.* 2023;388(25):2338-2348.
38. Koshy AG, Kim HT, Liegel J, et al. Phase 2 clinical trial evaluating abatacept in patients with steroid-refractory chronic graft-versus-host disease. *Blood.* 2023;141(24):2932-2943.

39. Aversa F, Tabilio A, Velardi A, et al. Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Engl J Med*. 1998;339(17):1186-1193.
40. 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.
41. Pierini A, Ruggeri L, Carotti A, et al. Haploidentical age-adapted myeloablative transplant and regulatory and effector T cells for acute myeloid leukemia. *Blood Adv*. 2021;5(5):1199-1208.
42. 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).
43. Oliai C, Hoeg RT, Pavlova A, et al. Precision-engineered cell therapy Orca-T demonstrates high relapse-free survival at 1 year while reducing graft-versus-host disease and toxicity. *Blood*. 2022; 140(suppl 1):654-656.
44. Bleakley M, Sehgal A, Seropian S, et al. Naive T-cell depletion to prevent chronic graft-versus-host disease. *J Clin Oncol*. 2022;40(11):1174-1185.
45. Pavletic SZ, Martin P, Lee SJ, et al; Response Criteria Working Group. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: IV. Response Criteria Working Group report. *Biol Blood Marrow Transplant*. 2006;12(3):252-266.
46. Inamoto Y, Martin PJ, Chai X, et al; Chronic GVHD Consortium. Clinical benefit of response in chronic graft-versus-host disease. *Biol Blood Marrow Transplant*. 2012;18(10):1517-1524.
47. Inamoto Y, Flowers ME, Sandmaier BM, et al. Failure-free survival after initial systemic treatment of chronic graft-versus-host disease. *Blood*. 2014;124(8):1363-1371.
48. Inamoto Y, Storer BE, Lee SJ, et al. Failure-free survival after second-line systemic treatment of chronic graft-versus-host disease. *Blood*. 2013;121(12):2340-2346.
49. Palmer J, Chai X, Martin PJ, et al. Failure-free survival in a prospective cohort of patients with chronic graft-versus-host disease. *Haematologica*. 2015;100(5):690-695.
50. Martin PJ, Storer BE, Inamoto Y, et al. An endpoint associated with clinical benefit after initial treatment of chronic graft-versus-host disease. *Blood*. 2017;130(3):360-367.
51. DeFilipp Z, Couriel DR, Lazaryan A, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: III. The 2020 treatment of chronic GVHD report. *Transplant Cell Ther*. 2021;27(9):729-737.
52. Pidala J, Onstad L, Martin PJ, et al. Initial therapy for chronic graft-versus-host disease: analysis of practice variation and failure-free survival. *Blood Adv*. 2021;5(22):4549-4559.
53. Zeiser R, Polverellia N, Ram R, et al. Ruxolitinib (RUX) vs. best available therapy (BAT) in patients with steroid-refractory/steroid-dependent chronic graft-vs-host disease (cGVHD): primary findings from the phase 3, randomized REACH3 study. *Blood*. 2020;136:22-24.
54. Carpenter PA, Logan BR, Lee SJ, et al; BMT CTN. A phase II/III randomized, multicenter trial of prednisone/sirolimus versus prednisone/ sirolimus/calcineurin inhibitor for the treatment of chronic graft-versus-host disease: BMT CTN 0801. *Haematologica*. 2018;103(11):1915-1924.
55. Lazaryan A, Lee S, Arora M, et al. A phase 2 multicenter trial of ofatumumab and prednisone as initial therapy for chronic graft-versus-host disease. *Blood Adv*. 2022;6(1):259-269.
56. Holtan SG, DeFor TE, Lazaryan A, et al. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. *Blood*. 2015;125(8):1333-1338.
57. Battipaglia G, Ruggeri A, Labopin M, et al. Refined graft-versus-host disease/relapse-free survival in transplant from HLA-identical related or unrelated donors in acute myeloid leukemia. *Bone Marrow Transplant*. 2018;53(10):1295-1303.
58. Sangiolo D, Storb R, Deeg HJ, et al. Outcome of allogeneic hematopoietic cell transplantation from HLA-identical siblings for severe aplastic anemia in patients over 40 years of age. *Biol Blood Marrow Transplant*. 2010;16(10):1411-1418.
59. Solh M, Zhang X, Connor K, et al. Donor type and disease risk predict the success of allogeneic hematopoietic cell transplantation: a single-center analysis of 613 adult hematopoietic cell transplantation recipients using a modified composite endpoint. *Biol Blood Marrow Transplant*. 2017;23(12):2192-2198.
60. Solomon SR, Sizemore C, Zhang X, et al. Current graft-versus-host disease–free, relapse-free survival: a dynamic endpoint to better define efficacy after allogeneic transplant. *Biol Blood Marrow Transplant*. 2017;23(7):1208-1214.
61. Lee SJ, Flowers ME. Recognizing and managing chronic graft-versus-host disease. *Hematology*. 2008;2008(1):134-141.
62. Miklos D, Cutler CS, Arora M, et al. Ibrutinib for chronic graft-versus-host disease after failure of prior therapy. *Blood*. 2017;130(21):2243-2250.
63. Zeiser R, Polverelli N, Ram R, et al; REACH3 Investigators. Ruxolitinib for glucocorticoid-refractory chronic graft-versus-host disease. *N Engl J Med*. 2021;385(3):228-238.
64. Cutler C, Lee SJ, Arai S, et al. Belumosudil for chronic graft-versus-host disease after 2 or more prior lines of therapy: the ROCKstar Study. *Blood*. 2021;138(22):2278-2289.
65. Arora M, Jagasia M, Stasi AD, et al. Phase 1 study of axatilimab (SNDX-6352), a CSF-1R humanized antibody, for chronic graft-versus-host disease after 2 or more lines of systemic treatment. *Blood*. 2020;136(Supplement 1): 1-2.
66. Kitko CL, Arora M, DeFilipp Z, et al. Axatilimab for chronic graft-versus-host disease after Failure of at least two prior systemic therapies: results of a phase I/II study. *J Clin Oncol*. 2023;41(10):1864-1875.
67. Khoury HJ, Langston AA, Kota VK, et al. Ruxolitinib as sparing agent for steroid-dependent chronic graft-versus-host disease (cGVHD). *Blood*. 2015;126(23):1938-1938. doi: 10.1038/s41409-017-0081-5.
68. Zeiser R, Burchert A, Lengerke C, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multicenter survey. *Leukemia*. 2015;29(10):2062-2068.
69. Zeiser R, Burchert A, Lengerke C, et al. Long-term follow-up of patients with corticosteroid-refractory graft-versus-host disease treated with ruxolitinib. *Blood*. 2016;128(22):4561-4561.
70. Spoerl S, Mathew NR, Bscheider M, et al. Activity of therapeutic JAK1/2 blockade in graft-versus-host disease. *Blood*. 2014;123(24):3832-3842.
71. Mori Y, Ikeda K, Inomata T, et al. Ruxolitinib treatment for GvHD in patients with myelofibrosis. *Bone Marrow Transplant*. 2016;51(12):1584-1587.

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