Controversies and expectations for the prevention of GVHD: A biological and clinical perspective

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Severe acute and chronic graft versus host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation. Historically, cord blood and matched sibling transplantation has been associated with the lowest rates of GVHD. Newer methods have modified the lymphocyte components to minimize alloimmunity, including: anti-thymocyte globulin, post-transplant cyclophosphamide, alpha/beta T cell depletion, and abatacept. These agents have shown promise in reducing severe GVHD, however, can be associated with increased risks of relapse, graft failure, infections, and delayed immune reconstitution. Nonetheless, these GVHD prophylaxis strategies have permitted expansion of donor sources, especially critical for those of non-Caucasian decent who previously lacked transplant options. This review will focus on the biologic mechanisms driving GVHD, the method by which each agent impacts these activated pathways, and the clinical consequences of these modern prophylaxis approaches. In addition, emerging novel targeted strategies will be described. These GVHD prophylaxis approaches have revolutionized our ability to increase access to transplant and have provided important insights into the biology of GVHD and immune reconstitution.

KEYWORDS
GVHD, GVHD prophylaxis, controversy, GVHD biology, GVHD therapies

Current understanding of the mechanism underlying acute GVHD

While significant advances have been made in our knowledge of the pathogenesis of acute graft versus host disease (aGVHD), our understanding is incomplete. Acute GVHD is thought to primarily be caused by the recognition of self (or host) antigens by donor T cells. Tissue damage is a key component to initiate aGVHD and often begins prior to the introduction of
the graft. This tissue injury is due to inflammation associated with the HCT preparative regimen but may also have occurred as part the underlying disease (e.g., hemophagocytic lymphohistiocytosis) or its treatment (e.g., chemotherapy for hematologic malignancy). Myeloablative and total body irradiation-based regimens associated with greater tissue inflammation have been linked to higher rates of aGVHD (1, 2). Notably, the sites most affected by aGVHD are also the sites of greatest tissue injury after HCT, the skin, gastrointestinal tract, and liver. This tissue damage leads to the activation of antigen presenting cells (APCs) through the release of inflammatory cytokines (TNF, IL-1, and others), pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), and the increased expression of MHC antigens and costimulatory pathways (3–6). This initiates donor T cell activation that target host antigens via host or donor antigen presentation (via MHC) to donor T cells, followed by T cell receptor (TCR) engagement and costimulation in the established proinflammatory milieu (7). This process is augmented in the setting of antigen mismatch and increased T cell dose but can occur even in matched transplants due to the absence of host thymus selection by donor incoming T cells and recognition of minor antigen differences (8, 9). These activated T cells will undergo proliferation and differentiation and produce more proinflammatory cytokines, PAMPs, and DAMPs, leading to an inflammatory cascade that results in the end-organ damage known as aGVHD (3). aGVHD has traditionally been thought to be a donor CD4+ T cell mediated disease, specifically Th1 and Th17 driven. However, donor CD8+ T cells alone can be sufficient to induce aGVHD (10) and Th2 and Th22 cells may play an important role as well (11, 12). Additionally, host tissue-resident T cells have also been implicated in the development of aGVHD (13). Murine studies have suggested that naïve T cells are the primary mediators of aGVHD (14–16), though this has yet to be confirmed clinically. Many risk factors for aGVHD have been identified and include increased HLA disparity, increased intensity of the conditioning regimen, female donors for male recipients, and decreased gut bacterial diversity (17–22). In summary, aGVHD likely stems from tissue injury, inciting a proinflammatory milieu with host antigen presentation, T cell activation that includes many CD4+ subsets, and a vicious cycle of T cell produced proinflammatory cytokines that induce ongoing tissue damage (Figure 1).

**Current understanding of the mechanism underlying chronic GVHD**

The mechanisms underlying chronic GVHD remain incompletely understood. Since acute GVHD is a major risk
factor for the development of cGVHD, there is overlap in these drivers. These include tissue injury, T cell dose, and age of the donor and recipient, and withdrawal of immunosuppression (Figure 2). Tissue injury may be pre-existing, due to pre-transplant preparative regimens such as in aGVHD, or due to second insults, induced by later infections and the impact on the microbiome, or local injury e.g. sun-exposure (23). Similar to aGVHD, tissue injury leads to the production of DAMPS, exposing previously dormant host antigens that can engender alloimmunity (24). Higher T cell dose, HLA mismatch, and use of peripheral blood mobilized stem cells rather than marrow are risk factors for both acute and chronic GVHD (17, 25, 26). This is likely due to presence of higher numbers of alloreactive T cells in the product, and a greater likelihood of alloreactivity due to mismatch. Increasing age of the donor and recipient also increase the likelihood of acute and chronic GVHD, which may be due to greater exposure to host antigens through increased age-related tissue impairment and a more oligoclonal donor T cell populations, linked to decreased tolerance. Advancing age is also linked to impaired thymopoiesis post-HCT, which may explain part of the increased risk of cGVHD, as T cell recovery is then reliant upon peripheral expansion rather than recovery of thymus activity leading to increased diversity and tolerant T cells (27–30). However, strategies to reduce aGVHD have not always decreased cGVHD, and vice versa, suggesting that there are unique biologic pathways that can incite GVHD. Cord blood T cells with a predominance of recent thymic emigrants, and male donors for male recipients have both been associated with less cGVHD, likely due to increased tolerance of the infused lymphoid populations (31–34). Dysregulated T subpopulations have been specifically linked to cGVHD including a preponderance of Th17s, or low Treg/Teff and NKT/Teff ratios (35–39). Miscreant B cells and a paucity of plasmacytoid dendritic cells have similarly been linked to cGVHD propagation (40–46). Alternatively activated macrophages have also been linked to some cGVHD processes (47). Finally, the role of host stromal cells in damaged secondary lymphoid organs may set up pathologic immune responses driving cGVHD, through aberrant interactions with CD4 T cells and B cells (40, 48–50). Collectively, these data support that cGVHD is a dysregulated immunity, often established early in the transplant process, through tissue destruction, alloantigen recognition, and a predominance of activating lymphocytes with few regulator cells to curb the maelstrom that culminates in chronic GVHD.

![FIGURE 2](link-to-image)

**FIGURE 2**
Mechanisms of cGVHD Schema of the contributions to chronic GVHD after hematopoietic cell transplantation. The pink donor and blue recipient represent the gender mismatch (female to male); <8/8 match= not fully HLA matched donor/recipient pair. PBSC= peripheral blood stem cell source; dec. if cord= decreased if cord blood source used, T cell dose= higher T cell doses and B cells are linked to more cGVHD. For the host, the microbiome may potentially play a role in addition to pre-HCT organ injury. The key contributing T cell populations are shown (T Eff- Th, T effector helper cell), Treg, T regulatory cell, RTE, recent thymic emigrants; DAMPS, danger-associated molecular patterns; alt. act. Macrophages, alternatively activated macrophages. The figures were generated by the authors using BioRender.com.

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Strategies to reduce acute and/or chronic GVHD

One of the most important factors in the prevention of GVHD is the selection of a donor. HLA identical matched siblings remains the gold standard for transplantation and is associated with the lowest rates of acute and chronic GVHD. There is ongoing debate over the next best option (unrelated, cord blood, or haploidentical donor) which will be discussed. High resolution HLA typing for unrelated donors is recommended to include HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DPB1 loci with HLA-DQB1 and DRB3/4/5 optional (51). Some studies have shown that mismatches at HLA-DQB1 (52), HDRB3/4/5 (53), and others may increase AGVHD however this has not been validated in larger studies and has not been shown to impact survival. Matching at HLA-A, HLA-B, HLA-C, and HLA-DRB1 should be prioritized and nonpermissive HLA-DPB1 mismatches should be avoided due to its association with increased mortality (52). HLA mismatching at HLA-A, HLA-B, HLA-C, and HLA-DRB1 is associated with increased rates of GVHD and decreased survival but there is no agreed open preference for mismatched loci or allele combination other than prioritizing HLA-C*03:03 over C*03:04 (51). More recently evidence has also suggested that certain HLA-B leader dimorphisms may impact outcomes, with better rates of GVHD and nonrelapse mortality for those mismatched at the threonine leader (TT genotype) compared to either the methionine genotypes (MM or MT) (54).

Other strategies to reduce acute and/or chronic GVHD have focused on preparative regimens and graft manipulation. In addition to the use of immature thymus-derived T cells in cord blood, these have largely included either prioritizing genetically HLA-matched donors and/or graft engineering via ex vivo and in vivo lymphocyte selection, depleting functional malevolent lymphocytes to achieve long term durable tolerance. The potential risks of this approach that curtails the infused lymphocyte activity are increased relapse rates, graft failure, or infections (Figure 3). The application of the GVHD-free, relapse free, survival proportion has enhanced our ability to compare these GVHD-reduction approaches, including these key risks in a single outcome measure, and thus included in the discussions below where possible. However, it is important to note, that the risks of GVHD and relapse may be different in terms of long-term outcomes, so there remains value in reporting rates of relapse and acute and chronic GVHD separately. Herein, we describe the published data for each method of GVHD reduction and then discuss the controversies in the field between the disparate donor and prophylaxis regimen options.

Anti-thymocyte globulin

Anti-thymocyte globulin (ATG) is composed of antibodies to T cells generated in a horse or rabbit and thus work by
antibody mediated clearance of T cells. ATG is typically added to a standard backbone of methotrexate and calcineurin inhibitor though rare studies include other agents (e.g., sirolimus). The equine preparation was generated against human thymus cells. Rabbit ATG is generated by inoculating rabbits with either a human T cell leukemia cell line (ATG-F) or human thymocytes (ATG-T). Because these antibodies are developed in response to different T cell populations, it is not surprising that the polyclonal antibody composition differs. While ATG-F and ATG-T both react to CD2, CD8, CD11a, CD107a, ATG-F has a higher response to CD28 and ATG-T has greater activity against CD3 and CD4 (55). Data have suggested that rabbit ATG may preserve Tregs better than equine ATG, a potential benefit to avert GVHD (56, 57). While equine ATG is still utilized in some preparative regimens, most current practice utilizes rabbit ATG as some larger studies have shown that the rabbit preparation was associated with less acute and chronic GVHD, likely due to better T cell depletion (58, 59). While the effect of rabbit ATG on T cell subpopulations is not fully understood in humans in vivo, one study linked reduced cGVHD to lower naive helper T cells after ATG exposure, and suggested that this was a primary mechanism of action (60). The impact of ATG on immunity is also influenced by the total dose, timing of administration in relation to transplantation, and peri-transplant host lymphocyte count. Higher doses of ATG, timing close to transplantation, and lower host total lymphocyte count can all lead to persistent ATG exposure after the infusion of donor T cells and subsequently to the downstream effects of donor T cell depletion, increasing the potential for relapse, infections, and post-transplant lymphoproliferative disorders (61–66). Thus, these factors must be considered when evaluating outcomes after ATG.

Studies have consistently shown reduction of acute and chronic GVHD after ATG exposure. Most studies are performed using peripheral blood, many randomized to controls, and most include matched related and unrelated donors (67–76). These studies represent over 1600 adult patients with hematologic malignancies treated with ATG, with the majority receiving ATG-F, with few receiving ATG-T. The median rates of grade II-IV aGVHD were 18% across the studies (ranging from 7-34%), and 28% cGVHD (16-28%) with 12% extensive cGVHD (6-13%), lower than controls in randomized studies (67–76). The relapse rates were not significantly increased in most studies, ranging from 16-37%, with higher rates of relapse often linked to higher exposure of ATG (67–76). In most studies, infections were comparable between ATG treated patients and other GVHD prophylaxis strategies, with severe bacterial infections in 42-45% of patients reported (2 studies), invasive fungal disease in 14% (2 studies), and deaths due to infections ranging from 15-17% of the total (67–76). Notably, the incidence of viral reactivations varied widely, from 7.5-62% of CMV reactivation, with the higher incidence exceeding the controls of this study (68, 70, 71, 75, 76).

In two studies, EBV reactivation was higher than controls, again ranging widely among studies, from 4-71% with the lower incidence possibly linked to the addition of CD20 blockade in the regimens (70–73, 76). Deaths due to infection ranged from 15-17% overall (67, 74). Collectively, these data suggest that infectious immunity was relatively comparable, with the possible exception of CMV and EBV viral reactivations. The rates of graft failure were low (1-3%) with the exception of a single study (21%) (67–75). Overall survival was comparable in most studies, with a median of 64% (55-74%) and GRFS ranging from 38-50% (67–75). Collectively, these data support the use of rabbit ATG for the prevention of acute and chronic GVHD in adults undergoing HCT for hematologic malignancies, especially with peripheral blood donor source, with modest or no impact on GVL, engraftment, and most infections with the exception of viral reactivations.

**Post-transplant cyclophosphamide**

Cyclophosphamide is an alkylating agent that causes DNA strand breakage and replication stress in dividing cells. Its use in the post-transplant setting to prevent GVHD was pioneered at Johns Hopkins University. It has been posited that post-transplant cyclophosphamide (PTCy) works through the preferential killing of alloreactive donor T cells followed by peripheral tolerance induction through clonal deletion and Treg suppression and lastly by central deletion of alloreactive T cell precursors in the thymus (77). However, our understanding of the mechanisms of action of PTCy is evolving and new evidence suggests that alloreactive T cells may not be eliminated but rather have reduced proliferation and impaired function (78). PTCy is typically paired with MMF and tacrolimus that begins following the last dose of cyclophosphamide. Waiting to start tacrolimus and MMF until after cyclophosphamide allows for unchecked alloreactive T cell proliferation in the absence of immunosuppression that is eliminated by the cyclophosphamide.

PTCy was first evaluated in non-myeloablative conditioning regimens for haploidentical transplants utilizing bone marrow grafts (79, 80). A dose of 50mg/kg of cyclophosphamide was given on days 3 and 4 post-transplant followed by MMF and tacrolimus on day +5 (79). The results of the Phase I/II trial revealed grade II-IV and grade III-IV aGVHD rates of 34% and 6% and extensive cGVHD of 5%. Graft rejection occurred in 13% of patients and the relapse rate at 2 years was 58% with a 2-year EFS of 26%. With the encouragingly low rates of GVHD in the initial trial, there have been numerous subsequent trials evaluating PTCy in the haploidentical setting with nonmyeloablative and reduced intensity conditioning. The median rates of grade II-IV aGVHD were 32% across the studies (ranging from 19-34%), 6% grade III-IV aGVHD (0-10%) and 26% cGVHD (13-34%) (79, 81–85). Relapse rates ranged from 31-58% with EFS of 26-55%. There
was one randomized phase 3 study comparing double umbilical cord blood (dUCB) transplant to haploidentical with PTCy with reduced intensity conditioning which showed improved OS with haploidentical transplant but with higher rates of relapse. There was no difference in EFS, aGVHD, or cGVHD (83). With relapse being a concern in reduced intensity and nonmyeloablative haploidentical transplant with PTCy, more intensive conditioning has been evaluated (86–90). The median rates of grade II-IV aGVHD were 24% across the studies (ranging from 16-43%), 7% grade III-IV aGVHD (7-23%) and 30% cGVHD (15-56%). There appeared to be an improvement in relapse (22-44%), EFS (50-79%), and OS (67-85%) with the more intensive conditioning.

PTCy has also been evaluated in the matched related and matched and mismatched unrelated donor setting (81, 91–93). The median rates of grade II-IV aGVHD were 51% across the studies (ranging from 27-77%), 11% grade III-IV aGVHD (0-15%) and 16% cGVHD (9-28%) with the range of relapse, EFS, and OS being 17-28%, 60-69%, and 69-73%. The ACCESS study (NCT04904588) is an ongoing prospective Phase II study evaluating PTCy in HLA-mismatched unrelated donor transplant.

With the success of PTCy in the haploidentical and unrelated donor setting, there has been an increasing debate over whether a matched unrelated or haploidentical donor would be preferred given the lower cost and increased accessibility with a related donor. A recent CIBMTR analysis compared haploidentical and matched unrelated donor (MUD) transplants in which both groups received PTCy GVHD prophylaxis (84). In the reduced intensity setting, lower rates of grade II-IV aGVHD, grade III-IV aGVHD, and non-relapse mortality, while higher DFS and OS were observed in the matched unrelated donor (MUD) setting. No difference in cGVHD was seen. BMT CTN 1703, is a randomized, multicenter, phase III trial comparing PTCy, tacrolimus, and MMF to standard tacrolimus and methotrexate GVHD prophylaxis in patients receiving reduced intensity conditioning with a PBSC graft with hematologic malignancies. Donors will include matched siblings, matched and mismatched unrelated donors. The trial has completed enrollment however, data is not yet available.

The majority of the initial trials evaluating PTCy utilized bone marrow grafts but more recently PBSC grafts have been used. There have been no randomized prospective studies comparing graft sources however multiple retrospective studies have been performed with mixed results (94–97). While most studies have shown higher rates of aGVHD and/or cGVHD with PBSC grafts (95, 96, 98), survival differences have not been observed.

Delayed immune reconstitution and associated increased risk of infections has been observed with haploidentical transplant with PTCy (99–101). A recent CIBMTR analysis found higher rates of CMV reactivation with PTCy in the haploidentical and matched related setting compared to matched related with CNI based immunoprophylaxis (42%, 37%, and 23%, respectively) (99). In multivariate analysis, haploidentical patients receiving PTCy that were CMV-seropositive prior to transplant had significantly lower OS and higher NRM. Higher rates of non-CMV viral infections have also been reported with PTCy in haploidentical transplants (101).

PTCy has been less extensively studied in non-malignant disease and mostly limited to smaller studies. Low rates of GVHD and NRM have been observed however graft failure rates have been high with nonmyeloablative regimens (102–108). Improved engraftment rates have been seen with increased intensity of the conditioning (102). Larger studies evaluating PTCy in nonmalignant diseases are needed.

An additional consideration when using PTCy is the toxicities associated with cyclophosphamide. Cardiotoxicity is a known side effect of cyclophosphamide and tends to occur within the first few weeks following administration (109, 110). A recent study found a significantly higher incidence of early cardiac events with PTCy compared to other GVHD prophylactic regimens (109). Cardiotoxicity may also be exacerbated when using cyclophosphamide in the preparative regimen as higher cumulative doses have been associated with increased frequency of cardiac events (109, 110). Additional studies are needed to evaluate the frequency of cardiac toxicity in patients receiving PTCy.

In summary, PTCy has been evaluated in the haploidentical, matched-related, and the matched and mismatched unrelated donor setting and is associated with low rates of aGVHD and cGVHD. While PTCy has been associated with delayed immune reconstitution and increased risk of viral infections, the nonrelapse mortality rates are low. The impact on relapse and EFS is variable among studies but is improved in myeloablative and more HLA-matched transplants.

**Abatacept**

For T cells to become activated they require both antigen presentation through T cell Receptor (TCR) engagement of the MHC on APCs and costimulation through multiple different costimulatory pathways (7). The best characterized costimulatory signal occurs between CD28 on T cells and CD80/86 on APCs (7). Abatacept is a fusion protein containing the extracellular domain of human CTLA4 and a modified Fc portion of IgG1. Abatacept can bind to CD80/86 and block T-cell costimulatory signaling and inhibit T cell activation (111). Abatacept was originally developed for use in rheumatoid arthritis and recently received FDA approval for the prevention of GVHD after allogeneic transplantation.

Based on encouraging data from an initial pilot study, one large prospective, multi-center, phase II, randomized, placebo-controlled, consortium study has been conducted to use abatacept to prevent aGVHD (112). Abatacept was added to a standard backbone of calcineurin inhibitor and methotrexate (on days -1, +5, +14, and +28) randomizing patients with hematologic malignancies with 8/8 HLA-matched unrelated...
donors to abatacept or placebo, with a single-armed open label stratum of 7/8-HLA-mismated unrelated donors compared to a retrospective registry matched cohort (113). Abatacept exposure was associated with significantly lower grade II-IV aGVHD (43% for 8/8 cohort and 40% for the 7/8 cohort) and grade III-IV in the 7/8 cohort (2%). This reduction translated to improved severe aGVHD-free survival at day 180 (93% for the 8/8 cohort) and (100% for the 7/8 cohort compared to a registry cohort). Notably, this registry cohort of 7/8 patients had not received additional GVHD prophylaxis on the methotrexate and calcineurin inhibitor backbone. When ATG was added to the preparative regimen in an intention to treat analysis, the rate of II-IV aGVHD (40% with abatacept and 42% with ATG) was similar but grade III-IV aGVHD remained significantly higher (3% with abatacept and 22% with ATG) for the 7/8 cohorts. Despite the reduction in aGVHD, rates of cGVHD were similar with abatacept to controls, 62% overall for the 8/8 cohort and similar between abatacept and ATG for the 7/8 62%. The rates of moderate to severe cGVHD were also comparable for abatacept vs controls, 45% and 36% for the 8/8, not available for the 7/8 cohort. Abatacept did not increase relapse rates; 22% of abatacept-treated patients relapsed in the 8/8 cohort and 9% for the 7/8 cohort. The overall survival and event-free survival were similar in all groups, (74% 8/8+ abatacept vs. 80% 7/8 +abatacept, and 66% 8/8+abatacept vs. 80% 7/8+abatacept respectively). The addition of abatacept appeared safe with similar engraftment (100% 7/8) and leukocyte reconstitution compared to controls. No significant increase in CMV or EBV reactivation or end-organ disease was observed, however there was trend towards increased CMV reactivation with abatacept compared to placebo (47% vs 33%). Abatacept has also been evaluated in smaller studies of patients with non-malignant diseases (114) with encouraging results. Larger studies are currently ongoing and include extended dosing of abatacept, (NCT03924401, NCT02867800, NCT04380740). In summary, abatacept effectively reduced rates of acute GVHD, tested in 7/8 and 8/8 HLA-matched donors for children and adults with hematologic malignancies, without impairing leukemia control nor engraftment, not seeming to increase infectious risk, with comparable rates of cGVHD in a large prospective randomized study with more in progress at this time.

**CD34+ selection**

In contrast to in vivo approaches to reduce GVHD, ex vivo approaches utilize graft engineering to modify the product prior to infusion. CD34+ positive selection was one of the first such approaches, which the goal of a product highly enriched for CD34+ stem cells with minimal CD3+ lymphocytes, administered on a backbone of ATG (usually rabbit though some studies included equine) without subsequent pharmacologic immunosuppression. The CD34+ selection strategy has largely been used in adult recipients of matched related or unrelated donors for the treatment of hematologic malignancy. CD34+ cell doses are a median of 6-8e6/kg with T cell doses infused of 2-6e6/kg (115–117). Rates of acute GVHD were low, median 16% (range 11-23), with 19% chronic GVHD (3-19%) (74, 115–117). Relapse rates were comparable to other approaches ranging from 22-29% (74, 115–117). While graft failure was only reported in 1 study in 1 patient (of 712 patients) (74, 115–117). Overall survival ranged from 57-70% and only one study reported a GFRS of 59% (74, 115–117). Not surprisingly, infections were commonly reported, observed in 73%-77% of patients, with 57-61% bacterial, 11% invasive fungal, and one study reporting 43% and 18% CMV and EBV reactivations respectively (116, 117). Infections complicated 42-57% of deaths (74, 115). Two small studies have been conducted in pediatrics, totaling 28 patients, approximately half of whom were treated for hematologic malignancies, and all received haploidentical donors (118, 119). While these studies included higher stem cell dose (19 and 21.5 x10e6) and higher T cell doses (1.4 and 4.7 x10e4), the rates of graft failure were high at 21 and 25%, with few patients eligible for relapse (1/10) but low rates of aGVHD and cGVHD, ~16% and 0 and 24% respectively (118, 119). Collectively, these data supported the benefit of CD34+ selection for reduction of acute and chronic GVHD, albeit with high rates of infection for adults with hematological malignancies and graft failure in pediatrics, with no data for alternative donors, minimal for pediatric recipients, and other transplant indications.

**CD45RA depletion**

Murine data initially demonstrated that the alloreactive T cells that engender GVHD are found within the naïve T cell populations (15, 120–122). This led to a graft engineering approach that selectively depleted CD45RA naïve T cells, preserving the CD34+ fraction critical for engraftment and CD45RO memory cells that could maintain T cell activity against infections and tumors (123). Studies in human cells validated that CD8+ T cells reactive to minor H antigens were 5-20 fold higher in the naïve rather than memory fraction (124). Three studies have utilized this approach. In two, which include an initial phase II and then a larger follow-on study (n=138), the preparative regimen for adult recipients of matched sibling transplants for acute leukemia was modified to remove the standard methotrexate and replace it with this targeted approach of naïve T cell reduction. While the incidence of cGVHD was only 7 and 9%, the incidence of grade II-IV aGVHD was 66% and 75%, albeit largely steroid responsive (125, 126). Rates of relapse (21 and 23%) and overall survival (77 and 78%) were similar to other approaches and without graft failure (n=35) (125, 126). Another group employed this approach in haplo-identical transplant for children with acute
leukemia, using sirolimus and MMF for additional GVHD control, CD20 depletion, and adoptive transfer of NK cells post-HCT for disease control (127). In this platform, the rates of grade II-IV aGVHD were modest (18%), but the rates of cGVHD were already 35% with short follow-up (~100 days) (127). Notably, while late outcomes have not been reported in full, a follow-on paper did report a high incidence of HHV6 reactivations with this approach (128). In summary, this is an intriguing approach to selectively modify the T cell fraction of the graft with data to support low rates of cGVHD in the matched donor setting, though with higher rates of aGVHD, largely tested in the pediatric setting.

Alpha/beta T cell/B cell depletion (αβTB depletion)

Another ex-vivo graft engineering approach includes the depletion of alpha/beta T cells and B cells targeting higher CD34+ doses (~10-15x10e6 CD34+ cells/kg) obtained via peripheral blood stem cell collection. Most of these protocols employ antithymocyte globulin and rituximab (CD20+ B cell depletion) as part of the transplant regimen, with the former aimed to decrease the risks of graft rejection and GVHD and the latter to decrease EBV reactivation. Most of these protocols do not employ post-transplant pharmacologic GVHD prophylaxis, testing whether the graft engineering approach is sufficient to avert alloimmunity alone. This αβTB depletion approach was developed to increase donor accessibility with haplo-identical transplantation, removing the cells most likely to induce GVHD, while preserving graft-vs-leukemia and anti-infectious immunity through preservation of natural killer and gamma-delta T cells. Thus, most but not all the studies use this graft engineering approach with related HLA-mismatched donors. This newer therapy has largely been studied in children, comprising 95% of the nearly 700 total patients reported in the literature, and a still small cohort compared to other alternative donor approaches (129–141). More data is needed to evaluate the success of this approach in adult HCT recipients and would be beneficial in pediatric cohorts as well.

Because αβTB depletion removes the T cells most closely linked to acute and chronic GVHD and depletes the B cells that can contribute to chronic GVHD, the rates of these complications were anticipated to be lower than that observed after matched donor HCTs despite the increased HLA-mismatch. Overall, this has been observed with median rates of grade II-IV aGVHD of 18% (ranging from 11-28%), and cGVHD of 8% (range 0-30%), with 0-21% experiencing extensive cGVHD (129–144). Notably, most of these studies utilized ATG, which has reduced aGVHD (see above), and not surprisingly, in one study in which ATG was removed, the observed rates of aGVHD were increased (136). The relapse rates ranged from 18-30% for those utilizing αβTB depleted HCT for the treatment of hematologic malignancies, comparable overall to other approaches (129, 135–137, 141, 143, 144). These data are confounded by a difference in the conditioning regimen; most have included myeloablative total body irradiation for all hematologic malignancies, a difference from other regimens that could potentially diminish relapse through greater leukemia clearance (129, 136, 137, 141). One study of αβTB depleted HCT included matched unrelated donors and removed ATG from this preparative regimen due to increased relapsed rates with subsequent associated higher rates of aGVHD, suggesting that this preparative regimen may be best used in the haplo-identical setting (136). This approach has been used for the treatment of relapsed refractory acute myeloid leukemias, without ATG, substituting tocilizumab therapy (anti-IL6), and scheduled CD45ra immature T cell depleted infusions with or without hypomethylating therapy. While small numbers (n=25), an initial complete remission was observed in 95%, and while 42% relapsed, the 3 year event-free survival was 49%, with only 18% aGVHD and 23% cGVHD, suggesting that this approach might have good anti-leukemia control as these numbers exceed historical controls (145). Two studies incorporated zoledronic acid to enhance gamma/delta T cell activity with a trend toward improvement in event-free survival (129, 146). Collectively, these data support that the αβTB depleted haplo-HCT approach may have potent anti-leukemia potential though the optimal additional agents (Tocilizumab vs. ATG or zoledronic acid) have yet to be established. As expected with αβTB cell depletion, the rates of viral reactivation were higher than matched transplant approaches. Those reporting viral reactivations reported consistent rates of 40–65% of patients experiencing reactivations, with 20-65% reactivating CMV, 0-44% EBV, up to 57% adenovirus, 21% HHV6, and 23% BK virus (129–134, 136–142). A large retrospective review showed the incidence of CMV and EBV reactivation were 53% and 33% respectively after αβTB cell depletion and associated with aGVHD (147). While rituximab use pre-transplant does appear to reduce the risk of EBV reactivation, these data show that it can still occur (as most of these group did use rituximab), though the rates of EBV post-transplant lymphoproliferative disease were quite low, at 0.5%, suggesting that this is a treatable problem for most patients (147).

While graft failure was low in patients receiving αβTB depletion for malignant indications (0-3%), the incidence was higher in nonmalignant recipients, ranging from 4-30%, and appears higher than the rates of graft failure rates reported in a large CIBMTR study of similar pediatric populations (129–142, 144). The overall survival in pediatric hematologic malignant patients was 67-90%, 54% for adult, and 84-100% for non-malignant (129–142). The GRFS in pediatric patients was 50%–61% in hematologic malignancy patients and was 69–87% in the pediatric non-malignant cohort (129–142). In summary, this approach is best studied in pediatric HCT recipients with malignant and
nonmalignant diseases, with good anti-leukemia effect, with particular attention paid to the risks of viral reactivations, which should include frequent viral infection surveillance and the plan for preventative or pre-emptive approaches.

**Cord blood**

Umbilical cord blood (UCB) was first identified as a viable alternative donor source in 1989 (148), permitting the use of mismatched transplant with acceptable rates of GVHD. Despite lower total nucleated cell and CD34+ cell counts, UCB has been found to have a higher proportion of hematopoietic progenitor cells than BM and PBSC grafts as well as greater proliferation potential of CD34+ progenitors (149–152). The initial use of UCB grafts was restricted to pediatric patients with related donors due to concerns about low cell doses in adults and the lack of UCB banks (153). However, this has now been overcome with the use of double umbilical cord blood and studies are underway to evaluate other modes of hematopoietic progenitor cell expansion as well (154, 155).

One advantage of UCB transplant has been its success even in the setting of HLA disparity. Due to lower immunogenicity, UCB transplant requires less stringent HLA-matching (out of 6 rather than the 8 typically used for adult donor matching). Greater HLA disparity has been associated with increased rates of graft failure and GVHD (154, 156–158) and studies in nonmalignant disease have shown higher rates of rejection and delayed immune reconstitution which is improved with higher cell doses and better matching (159, 160).

Large studies evaluating single and double UCB transplant have reported median rates of grade II-IV aGVHD of 38% (ranging from 24-45%), 17% grade III-IV aGVHD (9-25%) and 27% cGVHD (14-53%) with the range of relapse, EFS, and OS being 12-45%, 28-70%, and 31-75% respectively (14, 55, 161–170). Some studies comparing single and double UCB transplants have shown increased rates of aGVHD and/or cGVHD (169, 170), while others have found no differences (14, 55, 167, 168). Lower rates of cGVHD have also been observed with UCB when compared to matched related or unrelated donors (162, 164–166). Several studies have also suggested potential for increased graft-versus-leukemia effect and lower relapse with UCB transplant compared to matched related and unrelated BM and PBSC grafts (154, 165, 170–172). The incidence of NRM in UCB transplant is highly variable (6-42%) (14, 24, 123–132) depending on the study. In addition to higher rates of rejection, delayed immune reconstitution and consequently higher infection rates (particularly viral infections) are frequently reported with UCB (164, 173–176). The higher infection rate is thought to be related to the delayed recovery of naïve and memory T cells that is observed with UCB (173) and the absence of passive transference of humoral immunity from the donor. The use of ATG in the conditioning regimen has also been linked to higher NRM (63, 64, 177, 178). The timing of ATG in proximity to the graft infusion may play a role in poorer outcomes (178).

In summary, UCB transplant is associated with tolerable rates of grade II-IV aGVHD and reduced rates of cGVHD while permitting greater HLA disparity, though mismatched donors have been associated with higher GVHD and NRM. Delayed immune reconstitution and higher infection rates and their effect on NRM are a concern with UCB transplant while the graft versus leukemia effect appears to be preserved.

**Experimental approaches**

The prevention of graft versus host disease after allogeneic hematopoietic cell transplantation is a rapidly evolving field. In addition to the strategies listed above, many are currently in development and/or clinical trials. A clinicaltrials.gov search yielded over 50 studies currently active, including engineered grafts that supplement immunomodulatory cells e.g. Treg cells ("Orca-T") NCT03802695 or interferon gamma-primed mesenchymal stromal cells NCT04328714, in vivo depletion with Obinutuzumab (CD20 blockade) NCT02867384, ustekinumab (IL-12 and IL-23 blockade) NCT04572815, tildrakizumab (IL-23 blockade) NCT04112810, or BAFF inhibition with belimumab NCT03207958, or the addition of medications that modulate the immune system, e.g. proteasome inhibition with Ixazomib NCT03225417, histone deacetylase inhibition with vorinostat NCT03842696, JAK1 inhibition with itacitinib NCT04859946, or the addition of alpha-1-antitrypsin NCT03805789.

**Controversies in GVHD prevention strategies**

What regimens might prioritize reduction of acute or chronic GVHD or both?

While aGVHD is a risk factor for cGVHD, not all regimens that reduce one reduce the other. The early data for naïve T cell depletion does not appear to impact rates of aGVHD, especially as this was tested in patients with matched sibling donors and thus those at the lowest risk for GVHD. That said, if one develops highly treatable aGVHD with low rates of cGVHD and relapse, would this be the method of choice for patients with very high-risk hematologic malignancies and a matched sibling donor? Notably, this was tested in young adults and evaluation in pediatrics is underway but unknown. In addition, there is no data for nonmalignant indications, and for these patients neither aGVHD nor cGVHD provides benefit (in contrast to the potential benefit of concurrent graft versus leukemia effect, GVIL). ATG may reduce both but is this needed for all matched sibling transplants? This may be an especially critical
query for young children who incur low rates of chronic GVHD and may have aggressive malignant disease with higher relapse rates. Abatacept has also been tested in children and adults with matched and mismatched unrelated donors and has reduced the rates of severe aGVHD in those with high-risk hematologic malignancies but with the same query for 8/8 matched transplants. Similarly, should CD34 selection be chosen for these patients even if it reduces the rates of acute and chronic GVHD? Should the age of the recipient and/or donor influence the choice of additional prophylaxis? Perhaps, one should consider late teens to older adults a population to consider for these trials in the future.

While this remains an ongoing question in the matched transplant donor setting, historical data clearly justify the use of additional GVHD prophylaxis for alternative donors (<8/8 match), in which unacceptable rates of acute and/or chronic GVHD have been demonstrated. For those without an 8/8 matched donor, the choices typically include a 7/8 matched unrelated donor, a haploidentical donor, and cord blood transplantation. For 7/8 matched unrelated donors, abatacept maintained good antileukemia control, with event free survival rates matching matched cohorts, albeit with some increase in cGVHD. The dose of abatacept is now being extended to evaluate if both acute and chronic GVHD may be averted by this method. However, on balance, good leukemia control may be more valuable with high-risk diseases and merit a higher risk of cGVHD, and this approach did extend the donor pool for many patients by including mismatched unrelated donors. ATG impacted aGVHD and cGVHD in this setting as well, though some studies suggested an impact on graft versus leukemia effects. Alternatively, in the haplo-identical setting, PTCy reduced aGVHD and cGVHD, albeit with some increased in viral reactivations in both approaches and some studies showing higher relapse and nonengraftment. Similarly, in children, the haplo-identical platform has included αβTB depletion, which reduced cGVHD without aGVHD, with similar risks of graft failure and viral reactivations though as yet to be studied in adults. Additionally, cord blood reduces the risk of cGVHD, especially in matched settings, while still incurring a risk of aGVHD but potentially preserving GVL. Abatacept, ATG, cord blood, αβTB depletion, and PTCy markedly increase the donor pool to include mismatched donors without severe acute and/or chronic GVHD, critical as many HCT recipients lack matched donors. Collectively, these data support a personalized approach to the risks of acute and chronic GVHD, including thoughtful evaluation of the risk of relapse, the risk of severe acute or chronic GVHD (e.g., based on age, intensity of preparative regimen), the risk of nonengraftment, and the risks of severe infections.

It is noteworthy that there is large variability in the number of pediatric and adult patients in whom each approach has been tested. The largest data exists for ATG, cord, and PTCy with each of these including some prospective comparison studies with less data for the other (often newer) approaches. In addition, a limitation to comparing approaches is that not all studies report acute and chronic GVHD incidence, infections, graft failure, relapse rates, and GRFS. As we move forward, it would be helpful for each trial to present complete data on these variables to aid in precision medicine of GVHD prophylaxis and to prioritize prospective comparison trials.

What is the best regimen for patients at higher risk for graft failure?

Certain indications for transplant incur higher risks of nonengraftment, due to either decreased fertile stromal milieu (e.g., myelofibrosis) or activated host T cell populations (e.g., autoimmune diseases). When choosing GVHD prophylaxis regimens for these patients, the risks of nonengraftment should be prioritized. Given that PTCy, αβTB depletion, and cord blood may be potentially associated with increased risks of nonengraftment compared to other approaches, these approaches might be deprioritized in these patients. Further, one might also consider the impact of residual ATG on these patients, adopting a pharmacokinetic approach to mitigate the effect on donor T cells that can aid in engraftment. Alternatively, there may be other ways to mitigate this risk, such as testing for donor specific antibodies and using this for donor selection, agents that could enhance the stromal function, or targeting higher stem cell doses either by increased volume at collection or by CD34 stem cell expansion strategies.

What is the best regimen for those at highest risk for relapse of malignant disease?

The regimen that best preserves the graft versus leukemia effect remains to be determined. In part, this is because the key lymphocytes and tumor milieu that mediate GVL remain elusive. Data support that functional lymphocytes are key to maintain GVL (179). Thus, GVHD prevention strategies for malignant disease are challenged by competing interests of T cell immunity against infections and the need to maintain active GVL, while desiring less T cell activity to achieve tolerance, especially in the mismatched transplant setting. As relapse remains the primary cause of death after HCT for malignant indications, this is of paramount importance. Several studies have linked cord blood to lower rates of relapse but not all. Higher doses of ATG were linked to relapse in several studies, though lower doses did not appear to increase relapse rates and strategies to optimize timing and dose based on lymphocyte count may be valuable to optimize this strategy. Some studies had higher rates of relapse in other regimens, though not born out by others. Variability of disease status at the time of transplant may have contributed to some of these data, with
some studies permitting residual disease (e.g., for myeloid diseases) and others largely enrolling those without evidence of disease, and de facto, decreasing the risks of relapse.

It is also important to consider other factors that alter the risk of relapse. Reduced preparative regimens increase relapse risk, though are necessary for those with diminished organ function or advanced age. Alternatively, some approaches rely upon total body irradiation which may increase the anti-leukemia benefit by disease reduction in sites with diminished blood flow or high residence in the marrow stromal niches. An increased risk of relapse after HCT may be mitigated by post-transplant disease reduction approaches as well, via pharmacologic interventions (e.g., hypomethylating agents, FLT3 blockade) or via adoptive transfer of donor lymphocytes. These donor lymphocytes infusions have historically been unmanipulated, though newer approaches include removal of CD45RA naïve T cells, or manipulation to expand T cells specific for tumor antigens. Collectively, these considerations suggest that these factors should be carefully considered as the preparative and GVHD prophylaxis regimens are developed, including optimization of initial disease reduction, consideration of the effects of graft versus tumor effect on the GVHD prophylaxis chosen, and the post-transplant options for maintenance of remission.

What are the GVHD prophylaxis considerations for those with nonmalignant disease?

Many patients who undergo transplant for nonmalignant diseases have increased risk for infections (e.g., primary immunodeficiency), non-engraftment, or increased risk for particular organ impairments (e.g., pulmonary and renal dysfunction from sickle cell disease). Those who enter HCT with active viral infections in the setting of pre-existing T cell dysfunction from sickle cell disease). Those who enter HCT may require rapid transplantation which may prioritize the PTCy or αβT depletion, permitting expedited HCT with mismatched related donor, both accessible and likely motivated to proceed.

It is also notable that several groups are developing preparative regimens without cytotoxic chemotherapy. This may shift the landscape for these patients, potentially mitigating aGVHD risk, and permitting prioritization of reduction of cGVHD for these patients.

Finally, there are a dearth of data regarding GVHD prophylaxis to treat nonmalignancy. This is surprising as this population may be the ideal population to test various prophylaxis options. Relapse is not a competing risk and estimates suggest ~25% of patients will develop cGVHD (144).

What is the optimal regimen for immune reconstitution?

The approaches to prevent GVHD, including donor sources, have varying degrees of impact on immune reconstitution. Delayed immune reconstitution is linked to increased infectious complications and associated mortality. Some patients have baseline infectious risk factors necessitating a more rapid immune recovery, e.g., neutrophil recovery for active fungal disease or high-risk bacterial infections or T cell recovery for a history of mycobacterial infections or viral reactivations or active infections. Cord blood has been associated with delayed T cell reconstitution and infections compared to adult donors, attributed to the absence of memory cells to effect viral immunity. Additionally, PBSC grafts have been found to have significantly quicker neutrophil recovery than BM grafts. PTCy has also been linked to delayed T cell reconstitution and increased risk of viral infections, while abatacept showed similar immune reconstitution to controls.

Full T cell reconstitution requires two pathways for recovery. Initially, the infused donor T cells will increase number but not diversity through peripheral expansion. These initial T cells can provide GVL and viral immunosurveillance. Full recovery of diverse T cell reconstitution requires thymopoiesis, an option for children and young adults after hematopoietic cell transplantation. Little is known about the thymic recovery of the different GVHD prophylaxis approaches. However, minimizing the risk of GVHD overall should diminish one risk of thymus impairment, that of direct T cell attack on the thymus tissue. Including studies of thymus-derived T cells, diversity of T cell receptor, evidence of T cell function (e.g., vaccine response), would be beneficial to evaluate the overall effect of each approach on lymphoid immune reconstitution.

Summary

In conclusion, the approach to acute and chronic GVHD prophylaxis should be personalized, factoring in the age of the recipient (and potential donor), the preexisting conditions and disease indication, the intensity of the conditioning regimen, the risk of, and opportunities to mitigate infections or disease relapse/recurrence, and the risks of nonengraftment and delayed immune reconstitution. Recently, there have been many new approaches that have both diminished the rates of severe acute and/or chronic GVHD, that have increased both the
available donor pool and the options for personalized GVHD prophylaxis regimens. Comparisons of alternative donor approaches would potentially be valuable for particular disease indications across the lifetime spectrum of the recipient, to assist practitioners in these weighty decisions. In addition, the optimal GVHD prophylaxis is influenced by other aspects of the HCT, e.g., preparative regimen intensity, enhanced supportive care for the diagnosis and treatment of infections, which may reprioritize the prophylaxis regimens for particular HCT recipients.

**Author contributions**

BW and KW designed, wrote, and edited the manuscript. All authors contributed to the article and approved the submitted version.

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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