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## Prevention of graft-vs.-host disease

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### Abstract

**Introduction**—Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment for many malignant and non-malignant hematologic disorders. However, graft-vs.-host disease (GVHD) remains a major complication of allogeneic HCT and limits the success of this approach.

**Areas covered**—We review recent developments in the prevention of acute and chronic GVHD. In the setting of acute GVHD prevention, we review recent trials of T-cell depletion using Fresenius-ATG as well as studies testing total lymphoid irradiation, mesenchymal stromal cells, rituximab, statins, sirolimus, and other investigational agents. In the setting of chronic GVHD, we review results with Fresenius-ATG as well as B-cell depletion with rituximab, and discuss the potential role of the B-cell regulatory cytokine BAFF in chronic GVHD. Finally, we discuss the emerging role of resident skin and gut bacterial flora—the so-called microbiome—in the pathogenesis of GVHD.

**Expert opinion**—Current methods of acute GVHD prevention are highly successful, and a number of investigational approaches promise to further reduce the risk of this complication. In contrast, chronic GVHD is more poorly understood and more difficult to prevent. Future studies are required to delineate the roles of these approaches and to abrogate GVHD without sacrificing the beneficial immunologic graft-vs.-tumor effect.

### Keywords

Graft-vs.-host disease; hematopoietic cell transplantation; immunologic tolerance

## 1. INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) is an increasingly widely used therapy in a range of malignant and non-malignant hematologic diseases. In allogeneic HCT, the host immune system is replaced by donor hematopoietic cells, with both positive and negative consequences. In malignant disease, the donor immune system can recognize residual tumor cells as foreign and eradicate them by immunologic means—the so-called graft-vs.-tumor (GVT) effect. Unfortunately, donor immune cells may also attack normal host tissue, resulting in the phenomenon of graft-vs.-host disease (GVHD). The occurrence of GVHD remains one of the major barriers to more widespread and successful application of allogeneic HCT.

Graft-vs.-host disease occurs in two distinct forms: acute and chronic. These forms were initially distinguished largely on temporal grounds: GVHD beginning before day +100 after HCT was labeled as “acute”, while GVHD occurring after day +100 was labeled “chronic”. However, it has increasingly been appreciated that acute and chronic GVHD are in fact two separate pathophysiologic entities. Additionally, with the rise of reduced-intensity and non-myeloablative conditioning regimens, the onset of classic “acute” GVHD may occur after day +100. Thus, an arbitrary temporal distinction between acute and chronic GVHD has increasingly been abandoned, and in 2005 the U.S. National Institutes of Health issued consensus statements refining the clinical and diagnostic criteria for these entities [1].

The distinction between acute and chronic GVHD has important implications for prevention. Large strides have been made in the prevention of acute GVHD. In contrast, chronic GVHD remains relatively poorly understood on a pathophysiologic level, and efforts to reduce its incidence have generally been unsuccessful. In this review, we will briefly review standard approaches to the prevention of acute and chronic GVHD, and will focus on recent investigations into novel approaches to GVHD prevention.

## 2. ACUTE GRAFT-VS.-HOST DISEASE

### A. Pathophysiology of acute graft-vs.-host disease

Acute GVHD is a common complication after allogeneic transplantation. Its incidence ranges from approximately 20–60% in the setting of HLA-identical sibling or HLA-matched unrelated-donor transplants. A higher incidence has generally been observed in the setting of HLA-mismatched transplants, while results with umbilical cord blood and HLA-haploidentical transplantation have varied, as reviewed recently by Ballen et al. [2]. Registry data from the Center for International Blood and Marrow Transplant Research, covering allogeneic HCT performed between 1999 and 2005, reported an overall incidence of acute GVHD grades II-IV of 39% with sibling donors and 59% with unrelated donors [3]. It is important to note that the incidence of acute GVHD is subject to diagnostic bias, in which a more aggressive approach to acute GVHD workup may translate into a higher reported incidence of acute GVHD [4]. Distinguishing histologic changes related to GVHD from those related to conditioning-regimen toxicities or other etiologies requires experienced pathologists. As a result, great caution should be exercised in comparing raw incidences of acute GVHD from center to center or cohort to cohort. The best available evidence for prevention of acute GVHD comes from randomized controlled trials. Such trials are few in number, and where they are unavailable, sequential cohort studies from the same institution (using the same diagnostic algorithm) are likely the next best substitute.

Acute GVHD is an inflammatory disease predominantly targeting the skin and gastrointestinal tract. Hepatic involvement, previously a common hallmark of acute GVHD, has virtually disappeared in the past decade, possibly due to the increasing use of less hepatotoxic conditioning regimens and to the advent of ursodiol prophylaxis [5,6]. Major clinical risk factors for acute GVHD include HLA disparity between recipient and donor; the use of unrelated donors; and total body irradiation as a component of the conditioning regimen [7]. The majority of patients with acute GVHD can be successfully treated with corticosteroids, but a minority (approximately 10–30% of patients undergoing HLA-matched allogeneic HCT) develop severe (grade III–IV) acute GVHD [3]. Even in patients who are successfully treated for acute GVHD, exposure to corticosteroids can result in infectious, metabolic, and other adverse effects and increased transplant-related mortality [8]. In most (but not all) studies, acute GVHD has not been found to convey benefit in terms of enhanced graft-vs.-tumor activity [9]. Thus, prevention of acute GVHD holds the promise of reducing transplant-related morbidity and mortality without increasing the risk of malignancy relapse. Additionally, in the setting of non-malignant disease, GVHD has no potential upside and its prevention is central to efforts to expand allogeneic HCT safely.

The pathophysiology of acute GVHD is complex. A full review is outside the scope of this article, but current insights into the topic were recently reviewed by Ferrara et al. [10] and by Paczesny et al. [11]. Briefly, acute GVHD is mediated primarily by donor T cells and driven by disparities in histocompatibility antigens between donor and recipient. In the setting of HLA-mismatched allogeneic HCT, the HLA disparity is the major driver of GVHD. However, even in the HLA-matched setting, disparities in minor histocompatibility antigens can produce robust, severe, and even fatal acute GVHD [12,13]. However, numerous other pathways have been implicated as modulators of acute GVHD, including

pro-inflammatory cytokines, B-cell and regulatory T-cell activity, T-cell costimulatory pathways, and thymic injury.

The general understanding of acute GVHD can be broken down into several stages. First, acute GVHD is triggered by donor/host disparities in HLA and/or minor histocompatibility antigens. During this initial phase, regimen-related tissue injury may produce a pro-inflammatory cytokine environment which contributes to donor T-cell alloreactivity. While this “cytokine storm” likely contributes to the development of acute GVHD, several lines of evidence suggest that it is not essential. For example, GVHD is seen in F1 hybrid mice receiving parental splenic or marrow cells even in the absence of conditioning [14]; Acute and chronic GVHD occur in patients with severe combined immunodeficiency transplanted without conditioning [15]; and similar rates and severities of acute GVHD are seen after myeloablative and non-myeloablative conditioning, despite the much lower level of tissue injury with non-myeloablative conditioning [16]. In response to histocompatibility disparities and inflammatory cytokines, antigen-presenting cells are activated, which in turn activate donor T cells via MHC and costimulatory signals. Finally, the effector phase of acute GVHD involves target organ damage produced by CD8<sup>+</sup> cytotoxic T lymphocytes and amplified by inflammatory mediators such as IFN- $\gamma$ , TNF- $\alpha$ , IL-1, etc.

## B. Approaches to acute graft-vs.-host disease prevention

The most widely used current approach to acute GVHD prevention in myeloablative allogeneic HCT involves a short course of methotrexate (generally given on days +1, +3, +6, and +11 after HCT) combined with a calcineurin inhibitor (cyclosporine or tacrolimus). This regimen was developed and tested in a series of randomized controlled clinical trials in the early 1980s and remains a standard of care [17,18]. Tacrolimus has been adopted in many centers over cyclosporine in this regimen, based on a randomized controlled clinical trial published in 2000 suggesting greater efficacy in preventing acute GVHD [19]. However, another randomized trial raised the concern that tacrolimus may be associated with a higher risk of relapsed malignancy in patients with advanced disease [20], and a recent retrospective comparison suggested that outcomes with cyclosporine- vs. tacrolimus-based immunosuppression were equivalent [21].

In the setting of non-myeloablative allogeneic HCT, a number of preventive regimens have been used, many of which are based on a calcineurin inhibitor in combination with mycophenolate mofetil (MMF) [22]. HLA-haploidentical transplantation requires aggressive depletion of donor T cells to prevent severe GVHD, given the high degree of HLA disparity. One common approach to GVHD prevention in this setting is the *in vivo* depletion of activated donor T cells using cyclophosphamide, administered at days +3 and +4 after HCT, in combination with other immunosuppressants such as tacrolimus and MMF [23]. All of these approaches are effective and have reduced the rate of severe acute GVHD to 5–20%, but the continued occurrence of severe (and less severe but still clinically meaningful) acute GVHD have stimulated a number of investigations into more effective prophylaxis against acute GVHD.

**B.i. T-cell depletion using monoclonal or polyclonal antibodies**—Given the central role of donor T cells in the pathophysiology of acute GVHD, one of the most heavily studied approaches to acute GVHD prevention is T-cell depletion. Early efforts at *ex vivo* T-cell depletion were largely successful in reducing the risk of acute GVHD, but at the cost of increased risks of graft rejection, poor immune reconstitution, viral reactivation, and relapsed malignancy [24]. With additional refinements and experience, outcomes with *ex vivo* T-cell-depleted allografts have improved substantially, as a recent report of 35 patients from Memorial Sloan-Kettering Cancer Center indicates [25]. Nonetheless, recent efforts

have focused on *in vivo* T-cell depletion using monoclonal antibodies (e.g. alemtuzumab) or polyclonal antisera (e.g. antithymocyte globulin [ATG]), or on selective depletion of donor T-cell subsets thought to be most involved in acute GVHD. Clinical trials continue to test refinements of ex vivo T-cell depletion as well; for example, BMT CTN 0303 is an attempt to standardize T-cell depletion and T-cell dose and determine the effect of this standardization on patient outcomes [26].

A 2011 retrospective registry study by Soiffer et al. found that T-cell depletion using alemtuzumab or ATG resulted in a lower incidence of acute and chronic GVHD, with absolute risk reductions of 15–20%. However, relapse risk was significantly higher after T-cell depletion, and overall and progression-free survival were significantly worse in patients conditioned with alemtuzumab- or ATG-containing regimens [27], highlighting the ongoing concerns with this approach. It has been suggested that T-cell depletion should be reserved for those patients at highest risk of acute GVHD. The Italian GITMO group randomized “high-risk” alternative-donor patients (as identified by clinically available data) to receive thymoglobulin vs. placebo at day +7 after allogeneic HCT, and reported that the thymoglobulin arm had lower incidences of acute and chronic GVHD. However, transplant-related mortality and overall survival did not differ between the two groups [28].

Recently, a randomized clinical trial tested a proprietary formulation of ATG (Fresenius-ATG) vs. placebo in patients undergoing allogeneic HCT from unrelated donors. This trial was well-designed and scrupulously conducted and tested an important clinical question in the prevention of GVHD. The authors reported that ATG reduced the risk of acute GVHD grades II–IV compared to placebo; there were no significant differences in relapse, non-relapse mortality, or overall survival [29,30]. These results were received enthusiastically [31]. However, several important caveats are necessary in interpreting the authors’ findings. The study did not achieve statistical significance for its primary endpoint (a combination of acute GVHD grades III–IV and death before day +100). There was a trend toward a lower incidence of acute GVHD grades III–IV in the ATG arm vs. the placebo arm (11.7% vs. 24.5%,  $p=0.054$ ) and a significant decrease in acute GVHD grades II–IV (33.0% vs. 51.0%,  $p=0.011$ ), but these decreases did not translate into improved 2-year progression-free survival (51.6% in the Fresenius-ATG arm vs. 47.5% in the control group,  $p=0.65$ ).

These findings suggest that ATG prevented predominantly grade II, non-fatal acute GVHD. In this setting, some patients avoid exposure to corticosteroids to treat grade II acute GVHD, but all patients are exposed to ATG, which is similarly immunosuppressive. The authors reported that Fresenius-ATG did not increase the risk of relapsed malignancy. However, these results should be interpreted cautiously, in light of previous studies documenting a higher relapse risk in ATG-treated patients and because of an imbalance in disease risk in the Fresenius-ATG trial. Patients with advanced disease (at higher baseline risk for relapse) made up 39% of the ATG arm, but 56% of the control arm [29]. The over-representation of low-risk patients in the ATG arm may have obscured an effect of ATG on relapse risk, although the authors did attempt to statistically adjust for this factor in their analysis. Interestingly, 13 patients in the ATG arm underwent a second allogeneic HCT to treat relapsed malignancy (as compared to 4 patients in the control arm). Finally, in keeping with the increased risk of viral reactivation with T-cell depletion, patients receiving Fresenius-ATG had a higher incidence of post-transplant lymphoproliferative disorder (PTLD), presumably driven by Epstein-Barr virus. The authors report 5 cases of PTLD in the ATG arm (4 of which were fatal), as compared to 0 in the control arm [29,30].

The ATG-Fresenius trial demonstrated that ATG (at least in the formulation used in the study) could prevent acute GVHD grades II–IV, at the cost of an increased risk of PTLD and without a significant effect on overall or progression-free survival at 2 years. The study did

not show an increased relapse risk with ATG, although the caveat regarding imbalance in disease risk should be borne in mind. The major effect of ATG in this trial was in preventing chronic, rather than acute, GVHD (discussed in more detail below). While the results in acute GVHD are an important addition to the knowledge base in allogeneic transplantation, it is difficult to unreservedly recommend ATG solely on this basis, given the lack of survival benefit and the associated risks (e.g. PTLT). Studies powered to detect differences in overall or progression-free survival would help clarify the issue.

Other approaches to T cell depletion have focused on the role of specific T-cell subsets in producing acute GVHD. In murine models, naïve T cells play an essential role in the pathogenesis of acute GVHD. Depletion of naïve T cells (leaving only memory T cells in the allograft) abrogates acute GVHD in these models [32]. Interestingly, the beneficial graft-vs.-tumor (GVT) effects of allogeneic HCT seem to be mediated by memory effector T cells, at least in murine models [33], suggesting that naïve T-cell depletion might prevent GVHD while preserving GVT effects. Pilot clinical trials of naïve T-cell depletion in human allotransplantation have been initiated to test this hypothesis.

Another interesting approach to *in vivo* selective T-cell depletion is the use of post-transplant cyclophosphamide. Cyclophosphamide has a long history of use as an immunosuppressant and lymphotoxin, and preferentially inhibits expansion of activated lymphocytes. Luznik and colleagues developed an approach in which patients receive bone marrow from an HLA-haploidentical donor, followed by cyclophosphamide at days +3 and +4 after HCT. The three-day delay allows time for donor/host immune interaction, antigen presentation, and T-cell activation. At day +3, host-reactive donor T-cell clones are presumably activated, and are semi-selectively ablated with cyclophosphamide. This approach should result in host-specific donor tolerance while preserving donor-derived immunity against viruses and other pathogens. Cyclophosphamide is relatively inexpensive, readily available, and has a long track record and a well-described safety profile, making it an attractive option for T-cell depletion in this setting. Luznik et al. have reported promising results with this approach in both the HLA-haploidentical and HLA-identical setting, with very low rates of acute and chronic GVHD [23,34,35]. However, this approach has not been compared prospectively to other means of GVHD prophylaxis, and the caveats about comparing GVHD incidence across centers and patient populations described above should be borne in mind. Multi-center trials are currently ongoing to define the role of post-transplant cyclophosphamide in the prevention of acute and chronic GVHD. Ultimately, prospective randomized trials will be necessary to definitively compare this approach to other methods of GVHD prophylaxis.

**B.ii. Total lymphoid irradiation**—On the basis of murine studies, the group at Stanford developed a conditioning regimen of total lymphoid irradiation (TLI) combined with ATG. This regimen is hypothesized to enrich the proportion of host natural killer T cells (NKT), which are more radiation-resistant than conventional T cells. In murine models after TLI, NKT cells come to constitute the majority of the T-cell compartment. These residual host NKT cells are then believed to interact with donor T cells to facilitate tolerance and prevent acute GVHD.

Initial clinical results with this regimen were reported in 2005 [36]. Acute GVHD was reported in 2 of 37 transplanted patients, and extensive chronic GVHD in 7 of 33 at-risk patients. An expanded cohort of 111 patients with longer follow-up was described in a 2009 publication by the same group [37]. The percentage of NKT cells in the peripheral blood increased from a mean of 0.01% before conditioning to 0.5% after conditioning. Full donor chimerism was achieved in 78% of patients, and reported rates of acute GVHD and 1-year non-relapse mortality were again extremely low (6% and 2%, respectively). Extensive

chronic GVHD was seen in 28% of patients at 3 years after HCT. Unfortunately, the low incidences of acute GVHD and non-relapse mortality were offset by a high rate of relapse (60%). Patients who achieved full donor chimerism had a relapse risk of 40%, while those who achieved mixed chimerism uniformly relapsed.

The Italian GITMO group reported their multicenter experience with TLI/ATG conditioning in 2012 [38]. In this study, 45 patients were transplanted using the Stanford TLI/ATG regimen. Again, a low rate of acute GVHD grades II–IV was observed (13.3%), suggesting that this conditioning regimen is effective in preventing acute GVHD (subject to the previously described caveats about comparing raw GVHD incidences between centers and groups). Relapse was again a significant issue, occurring in 44.3% of patients at 3 years despite a relatively low-risk disease mixture containing a number of patients with chronic lymphocytic leukemia and indolent non-Hodgkin lymphoma.

The high rate of relapse observed with this regimen may be related to impairment of GVT effects by ATG in the conditioning regimen, or to aspects of TLI. Based on these results, TLI/ATG appears effective in preventing acute GVHD but is associated with a high risk of relapse. It is important to note that the low rates of GVHD observed in these studies may be partially or wholly attributable to T-cell depletion by ATG, rather than to TLI. Given the high risk of relapse, this approach may be reserved for patients with low-risk disease (for example, indolent non-Hodgkin lymphoma), or may be augmented by additional infusions of manipulated donor lymphocytes to augment the lacking GVT effect, as the Stanford group has proposed [37]. The downside of this latter approach is that, by reinfusing additional donor T lymphocytes, the risk of GVHD increases and the benefit of the conditioning regimen with respect to GVHD may be negated.

**B.iii. Mesenchymal stromal cells**—Interest in bone-marrow-derived mesenchymal stromal cells (MSC) as an approach to GVHD treatment was stimulated by the observation that these cells had immunosuppressive activity *in vitro* and in animal models [39-42]. A 2004 case report described successful treatment of a patient with refractory acute GVHD using donor-derived MSC [43]. Results with MSC in animal models of GVHD have been conflicting; murine studies have reached equivocal results [44,45], while canine studies have failed to demonstrate efficacy in preventing acute GVHD [46]. Nonetheless, on the basis of the initial case report of successful use in human GVHD, a larger series by the same group reported favorable responses in 6 of 8 patients with GVHD treated with MSC [47]. Preliminary results of a randomized, placebo-controlled trial suggested that third-party MSC improved response rates in patients with refractory gut and liver, but not skin, acute GVHD [48]. However, the results of this trial have not been reported in final form at the time of this writing, and results of these randomized trials of MSC in GVHD treatment have generally been viewed as disappointing [49].

The role of MSC in preventing (rather than treating) acute GVHD is unestablished. Cotransplantation of MSC at the time of allogeneic HCT appears safe, and was associated with incidences of acute GVHD grades II–IV of 35% and of extensive chronic GVHD of 65% [50]. Other single-arm studies have described MSC cotransplantation as safe and associated with varying incidences of acute GVHD [51-53]. Of concern, one study suggested an increased risk of relapsed malignancy in patients cotransplanted with MSC [54]. Given the single-arm design of these reports, it is impossible to draw firm conclusions about any prophylactic effect of MSC against acute GVHD. Preclinical and clinical data on the use of MSC in allogeneic HCT were recently summarized in greater detail by Baron & Storb [55].

**B.iv. Rituximab**—While GVHD is recognized as primarily a T-cell-mediated disease, the contributory role of B cells in both acute and chronic GVHD has increasingly been appreciated. Stimulated by reports of successful treatment of refractory chronic GVHD with the anti-CD20 monoclonal antibody rituximab [56-58], B-cell depletion has increasingly been investigated as an approach to the treatment and prevention of GVHD. Current insights into the pathophysiologic role of B cells in GVHD were recently summarized by Shimabukuro-Vornhagen et al [59].

Rituximab has been hypothesized to prevent acute GVHD by depleting B cells, which are capable of presenting antigen to donor T cells. In murine models of allogeneic HCT, depletion of either donor or recipient B cells abrogates acute GVHD [60]. Retrospective registry data supported this hypothesis: a CIBMTR-based study found lower rates of acute GVHD in patients who had received rituximab in the 6 months prior to allogeneic HCT [61]. Similarly, a retrospective study by a French group found that exposure to rituximab before allogeneic HCT was associated with complete elimination of acute GVHD grades III–IV (24% vs. 0% in control vs. rituximab-treated patients) [62].

Data from prospective studies are limited and have been less positive. The M.D. Anderson group, led by Khouri, described the use of high-dose peri-transplant rituximab in patients undergoing allogeneic HCT for indolent non-Hodgkin lymphoma, and observed an incidence of acute GVHD grades II–IV of 11% [63]. The authors hypothesized that this low incidence of acute GVHD might reflect a prophylactic effect of rituximab. However, given that this group previously reported a baseline incidence of acute GVHD grades II–IV of 12% in patients transplanted largely *without* rituximab [64], it seems more likely that the low incidence of acute GVHD in the Khouri study reflects that center's baseline observed rate of acute GVHD rather than a differential prophylactic effect of rituximab. Other groups have reported relatively high incidences of acute GVHD after allogeneic HCT with peri-transplant rituximab; for example, Pidala et al. reported an incidence of acute GVHD grades II–IV of 58% [65]. Thus, it is impossible to conclude on the basis of available prospective data that rituximab has a prophylactic effect against acute GVHD. However, such an effect cannot be definitively ruled out, either, and further prospective trials, particularly randomized controlled trials, would be required to clarify the issue. The role of rituximab in the prevention of acute and chronic GVHD was further summarized in a recent review by Kharfan-Dabaja and Cutler [66].

**B.v. Statins**—Statins are pharmacologic inhibitors of HMG-CoA reductase, and are widely used to treat dyslipidemia and coronary artery disease. These agents have a wide range of incompletely understood off-target effects, including immunomodulatory effects such as inhibition of antigen-presenting cells [67,68]. In murine models of allogeneic HCT, statin treatment can prevent or ameliorate both acute and chronic GVHD [69,70]. Retrospective human studies have produced interesting results: statin treatment of the hematopoietic cell donor is associated with a dramatically reduced risk of severe acute GVHD in the recipient, but this association holds true only in combination with cyclosporine-based (not tacrolimus-based) post-grafting immunosuppression [71]. Prospective trials are underway to test whether donor statin administration can prevent acute GVHD, and to explore the mechanisms by which statins affect allograft composition and function.

In contrast, *recipient* statin treatment is associated in retrospective studies with a decreased risk of chronic GVHD, but an increased risk of relapsed malignancy [72]. Again, the exact mechanisms by which statins affect GVHD are unclear. Given these retrospective results, recipient statin treatment is under investigation in the setting of allogeneic HCT for non-

malignant disease, where chronic GVHD prevention is a priority and disease relapse is not a concern.

**B.vi. Sirolimus**—Sirolimus is an immunosuppressant with multiple mechanisms of action, including suppression of T-cell responses through the mTOR pathway, inhibition of antigen presentation, and promotion of regulatory T cell ( $T_{reg}$ ) expansion. The Dana-Farber group has reported low rates of acute GVHD with sirolimus-based GVHD prophylaxis [73]. Similarly, the City of Hope reported favorable results with this regimen [74]. A randomized clinical trial (BMT CTN 0402) is currently underway comparing sirolimus/tacrolimus to standard tacrolimus/methotrexate as GVHD prophylaxis.

In contrast, Fred Hutchinson Cancer Research Center studies of sirolimus added to standard GVHD prophylaxis (calcineurin inhibitor plus methotrexate) after myeloablative HCT was stopped early due to a very high rate of acute GVHD (77%) occurring very early after allogeneic HCT (at a median of day +7) [75]. In these studies, sirolimus was also associated with significant toxicity, including 3 cases of dialysis-dependent acute renal failure among 26 treated patients. Sirolimus appeared better tolerated in the setting of non-myeloablative conditioning: in preliminary results from a randomized Phase II trial comparing sirolimus/tacrolimus/MMF to tacrolimus/MMF alone, the addition of sirolimus led to a lower incidence of acute GVHD without evidence of increased toxicity [76]. It is expected that mature results from this randomized Phase II trial, as well as the ongoing BMT CTN Phase III trial, will help clarify the role of sirolimus in GVHD prevention. For further detail on the role of sirolimus in allogeneic HCT, the reader is directed to the recent review by Cutler & Antin [77].

**B.vii. Other investigational approaches**—The range of agents under investigation to prevent acute GVHD is extremely wide. The proteasome inhibitor bortezomib has been found to inhibit NK-kappaB, to deplete alloreactive T cells, and to decrease Th1 cytokine levels in murine models, suggesting potential efficacy against acute GVHD [78,79]. In a single-arm trial, the addition of bortezomib to tacrolimus and methotrexate as GVHD prophylaxis was associated with a 13% incidence of acute GVHD grades II–IV. While definitive conclusions cannot be drawn from this single-arm study, additional investigations into bortezomib in the prevention of acute GVHD are underway.

Regulatory T cells ( $T_{reg}$ ) are capable of suppressing immune reactions and have generated extensive interest as a cellular therapy directed against GVHD. The biology of  $T_{reg}$  in GVHD has been studied extensively in preclinical models, and initial efforts at translation to the clinic are underway [80]. Due to space limitations, the reader is referred to the recent articles by Negrin and by Pidala & Anasetti for detailed reviews of this important topic [81,82].

Palifermin, a recombinant human keratinocyte growth factor, can protect the thymus from damage during allogeneic HCT and has been investigated in the promotion of tolerance and the prevention of acute GVHD. Unfortunately, two randomized placebo-controlled trials and a third matched-control study failed to show any preventive effect of palifermin against acute GVHD [83-85].

Pentostatin, an adenosine deaminase inhibitor and potent immunosuppressant, has been investigated as an adjunct to standard GVHD prophylaxis. A randomized phase I/II study published in 2011 found that pentostatin, added to standard tacrolimus/methotrexate, increased the likelihood that patients would remain alive, engrafted, in remission, and without GVHD at day +100 after allogeneic HCT [86]. Given the relatively short-term



endpoint, larger randomized trials with attention to late infections, immune reconstitution, and disease relapse are warranted to confirm this promising early finding.

Extracorporeal photopheresis (ECP) is a process in which leukocytes are harvested from the peripheral blood via apheresis, incubated *ex vivo* with 8-methoxypsoralen, treated with UV-A light, and then reinfused. The UV-A light exposure induces a conformational change in 8-methoxypsoralen, which then intercalates into DNA and interrupts transcription, inducing apoptosis in the treated leukocytes. ECP has an established role in the treatment of acute and chronic GVHD, and has been explored in a limited fashion as a preventive agent. A 2010 pilot study tested prophylactic ECP in 62 patients undergoing myeloablative allogeneic HCT, and reported rates of acute GVHD grades II–IV of 30–42%, a marginally statistically significant reduction compared to registry controls [87]. ECP is relatively logistically demanding, but has few or no observed adverse effects and may prove amenable to larger controlled studies of acute GVHD prevention.

Hypomethylating agents such as 5-azacytidine and decitabine have been shown to expand regulatory T-cell populations both *in vitro* and *in vivo* [88,89]. Because regulatory T cells can suppress alloimmune reactions, these hypomethylating agents have generated interest as potential preventive approaches against GVHD, particularly in patients with myelodysplastic syndromes or acute myeloid leukemia, in whom these agents may also be effective against the underlying hematologic malignancy.

### 3. CHRONIC GRAFT-VS.-HOST DISEASE

#### A. Pathophysiology of chronic GVHD

Chronic GVHD has increasingly been appreciated as a separate clinicopathologic entity from acute GVHD. Chronic GVHD is a multisystem disease involving inflammation and fibrosis, and can affect a wide range of tissues including the eyes, oral mucosa, skin, fascia, lungs, liver, gastrointestinal tract, joints, salivary glands, and genitourinary tract [90]. In some of these organs, clinical and histologic manifestations overlap considerably with those of autoimmune disease (for example, systemic sclerosis or bronchiolitis obliterans). In contrast to acute GVHD, chronic GVHD has remained poorly understood on a pathophysiologic level for a number of reasons: the complexity and heterogeneity of its manifestations, the lack of standardized diagnostic criteria (at least until the promulgation of NIH consensus criteria in 2005 [1]); and the lack of clinically relevant animal models of the disease [91]. Regarding this last limitation, a novel murine model of chronic GVHD was recently reported by Blazar et al. [92]. This model is a significant advance over existing murine models of chronic GVHD, but whether it will lead to translational and clinical advances remains to be seen.

Nonetheless, chronic GVHD remains a major cause of morbidity and mortality after allogeneic HCT and the major determinant of quality of life in transplant survivors [93]. While progress has been seen in decreasing the incidence, severity, and associated mortality of acute GVHD, these successes have not translated into a lower incidence of chronic GVHD [6], underscoring the biological uniqueness of this condition. If anything, the prevalence of chronic GVHD is increasing, as more patients are at risk (by virtue of improved post-transplant survival) and mobilized peripheral blood stem cells have come to be used extensively as an allograft source.

A discussion of the prevention of chronic GVHD is necessarily limited, as the number of interventions shown to reduce the risk of chronic GVHD is extremely small. Standard GVHD prophylaxis with a calcineurin inhibitor plus methotrexate typically results in a reported incidence of chronic GVHD of 25–80% [91]. The reported incidence of GVHD is

even more dependent than that of acute GVHD on the diagnostic approach employed, and the same caveats about comparing center-to-center or cohort-to-cohort incidences apply. The vast majority of investigational agents described above, including those which reduce the risk of acute GVHD, have no demonstrated effect on the risk of chronic GVHD. The two interventions with the best evidence for preventing chronic GVHD are the choice of allograft product (bone marrow vs. mobilized peripheral blood hematopoietic cells) and T-cell depletion.

## B. Approaches to chronic graft-vs.-host disease prevention

**B.i. Source of hematopoietic cells**—G-CSF-mobilized peripheral blood mononuclear cell (G-PBMC) graft products contain 5 to 10 times more donor T cells compared to bone marrow graft products. Several randomized trials and retrospective studies, as well as a 2001 meta-analysis, reported an increased risk and/or severity of chronic GVHD in patients receiving G-PBMC as opposed to bone marrow allografts [94-99]. A large cooperative-group trial, BMT-CTN 0201, tested bone marrow against G-PBMC in a randomized trial involving 551 participants. Although results from this trial have been reported in abstract form only and follow-up is still incomplete, preliminary results suggest that the incidence of chronic GVHD was significantly higher with G-PBMC compared to marrow (53% vs. 40%,  $p=0.02$ ) [100]. In contrast, one randomized trial comparing G-PBMC to bone marrow showed comparable rates of chronic GVHD both at 2 years after HCT and at 10-year follow-up [101,102] (see **TABLE 1**). If one accepts that bone marrow grafts are less likely to produce chronic GVHD, their use in malignant disease is still subject to concerns over slower engraftment and the potential for a higher risk of disease relapse. In contrast, the perceived lower risk of chronic GVHD with bone marrow grafts has led to their widespread use in allogeneic HCT for non-malignant disease, where prevention of chronic GVHD is a high priority and concerns about relapsed malignancy do not apply.

**B.ii. T-cell depletion**—Several studies have suggested that T-cell depletion can reduce the risk of chronic GVHD. Historically, T-cell depletion has been subject to the concerns described above: specifically, higher risks of infection, graft rejection, and disease relapse. The strongest evidence for the use of T-cell depletion to prevent chronic GVHD comes from the Fresenius-ATG study, which was discussed above in reference to prevention of acute GVHD.

The Fresenius-ATG study was a randomized clinical trial in which patients undergoing myeloablative allogeneic HCT from HLA-matched unrelated donors were randomized to receive Fresenius-ATG vs. placebo, in addition to standard cyclosporine/methotrexate post-grafting immunosuppression. At 3 years after HCT, the authors reported a significant reduction in the incidence of chronic GVHD in the Fresenius-ATG arm vs. control (12.2% vs. 45.0%,  $p<0.0001$ ) [30]. The risks of relapse, non-relapse mortality, and overall survival were not significantly different in the two groups, but the Fresenius-ATG group was substantially less likely to require ongoing immunosuppression at 3 years after HCT.

As with the reduction in acute GVHD reported in this trial [29], these results were met with considerable enthusiasm, given the lack of effective preventive measures against chronic GVHD and the apparent lack of impact of Fresenius-ATG on relapse risk. As discussed above in the section on acute GVHD prevention, however, several caveats are reasonable. The higher prevalence of low-risk disease in the Fresenius-ATG group may have obscured a negative effect on relapse rate, although the authors statistically adjusted to account for this imbalance. More importantly, 4 fatal cases of PTLD were observed in the Fresenius-ATG arm (vs. 0 in the control arm). For comparison, there were 4 deaths from chronic GVHD in the control arm (vs. 0 in the Fresenius-ATG arm). Thus, one might conclude that the

reduction in chronic-GVHD-related mortality with Fresenius-ATG was negated by an equivalent increase in the risk of PTLD-related death. The authors presented data showing statistically equivalent risks of late bacterial infection in the Fresenius-ATG vs. control groups. Unfortunately, only very limited data about viral infection were presented, as clinical experience suggests that viral reactivation is one of the major causes of morbidity in patients receiving ATG or other means of T-cell depletion.

The endpoint of withdrawal of all immunosuppression is clinically relevant, and was clearly improved in this study by the addition of ATG to the conditioning regimen. However, the lack of improvement in overall survival with Fresenius-ATG and the associated risks of viral reactivation and PTLD temper enthusiasm for this approach. Ideally, confirmatory trials would be powered to detect differences in relapse and non-relapse mortality, but such trials would require a large number of participants and pose a substantial logistical challenge. The results reported in the Fresenius-ATG trial confirm that T-cell depletion with ATG reduces the risk of chronic GVHD, but leave some concerns about PTLD and other ATG-related complications.

**B.iii. Anti-B-cell approaches**—With the increasing recognition of a B-cell contribution to chronic GVHD, rituximab has been studied as both a treatment and as prophylaxis in this setting. The role of rituximab in the prevention of chronic GVHD was recently summarized by Kharfan-Dabaja and Cutler [66]. We agree with their view that there is no definitive evidence of a prophylactic effect of rituximab against chronic GVHD, despite its efficacy in the treatment of some forms of established chronic GVHD. A retrospective review published in 2009 found that rituximab exposure in the 6 months before allogeneic HCT was associated with a lower incidence of extensive chronic GVHD (45.8% vs. 20.1%), although the statistical significance of this finding was borderline ( $p=0.53$ ) [103]. However, two prospective trials incorporating rituximab into the transplant regimen have reported incidences of chronic GVHD of 58 and 60%, in line with baseline rates and arguing against any prophylactic effect of rituximab against chronic GVHD [63,104]. The Stanford group reported in a 2008 meeting abstract that peri-transplant rituximab, combined with TLI/ATG conditioning, resulted in a low rate of chronic GVHD [105]. However, in the context of a low institutional baseline rate of chronic GVHD after TLI/ATG conditioning, evidence for a differential benefit from rituximab is unconvincing.

It has been suggested that abnormal B-cell reconstitution after allogeneic HCT facilitates chronic GVHD. B-cell depletion leads to increased serum levels of BAFF, a B-cell regulatory cytokine. These elevated BAFF levels may then stimulate and sustain alloreactive B-cell clones which contribute to chronic GVHD. Sarantopoulos et al. have made a compelling case for a role of BAFF in chronic GVHD, showing in a series of papers that BAFF levels are elevated in patients who develop chronic GVHD; that these perturbations produced an abnormal B-cell repertoire; and that normalization of BAFF levels was correlated with resolution of chronic GVHD [106-108]. Given the availability of anti-BAFF pharmaceuticals such as belimumab and atacicept, such agents may have a role, either alone or in combination with rituximab, in reducing abnormal BAFF signaling after allogeneic HCT and thus preventing chronic GVHD.

#### 4. NEW DIRECTIONS: GVHD AND THE MICROBIOME

The role of resident bacterial flora (the microbiome) in human autoimmunity is an area of strong current interest. Recent research has implicated the interaction between the host immune system and the microbiome in a range of autoimmune diseases. By extension, the microbiome may also play a key role in the immune interactions which lead to GVHD. The sites targeted by acute GVHD—the skin and gastrointestinal tract—are home to diverse and

complex microbial flora. The heavy use of antimicrobials in the setting of allogeneic HCT presumably causes profound and long-lasting perturbations of these microbiota. The interaction between the microbiome and the innate immune system appears to influence adaptive immune responses. Finally, when immune cells coeducated with the donor microbiome are transplanted and encounter a foreign recipient microbiome, this disparity may play a key role in the development of tolerance vs. alloimmunity.

At present, existing data tying the skin or gut microbiome to GVHD are sparse, but exploratory studies in the area are a high priority for many research groups, including our own. A full review of preliminary data on the microbiome as a contributor to GVHD is outside the scope and space limitations of this article, but the interested reader is referred to recent reviews focusing on this topic [109,110]. If perturbations of the microbiome influence the occurrence of GVHD (through interaction with the innate immune system), then it may be possible to manipulate the microbiome to prevent GVHD. For example, donor microbiota could be transplanted to the recipient before or at the time of transplant to protect the skin and gut from GVHD. Alternately, if specific pathways in the innate immune system are shown to mediate GVHD in response to changes in the microbiome, then specific inhibitors of these pathways could be investigated. Finally, if antibiotic-associated perturbations of the microbiome are linked to GVHD, then the use of antibiotics before, during, and after HCT could be varied to prevent GVHD.

## 5. CONCLUSION

While acute GVHD after allogeneic HCT remains a significant clinical problem, substantial progress has occurred in both the prevention and treatment of this complication [6]. Standard approaches to acute GVHD prophylaxis are effective in many patients, and a large range of investigation approaches hold promise to further reduce the risk of this complication.

Unfortunately, prevention of chronic GVHD has not kept pace, and most efforts to affect its incidence have either failed or been negated by increases in malignancy relapse. Critical barriers include disease heterogeneity and the lack of suitable animal models in which to test and optimize preventive strategies.

## 6. EXPERT OPINION

The prevention of acute GVHD should be viewed first and foremost as a success story. Systematic randomized clinical trials, based on preclinical animal data, led to the development of standard immunosuppressive approaches which have reduced the incidence of severe acute GVHD to 10% or less in many cases. Further advances have occurred in the past decade [6], most notably in supportive care and the use of ursodiol, which (together with the decreasing use of high-intensity conditioning regimens) have virtually eliminated hepatic acute GVHD.

In some ways, the success of acute GVHD prophylaxis complicates the efforts to make further progress. With a rate of severe acute GVHD of 10% or less with modern prophylactic regimens, massive clinical trials enrolling many hundreds of patients are required to achieve statistical power to show further incremental improvements. Endpoint selection for trials of acute GVHD prevention is a challenge, as others have pointed out [111]. Prevention of less severe (e.g. grade II) acute GVHD is a clinically worthwhile goal, but the toxicity and downstream effects of preventive approaches must be weighed against the expected benefit in morbidity and mortality. The results with Fresenius-ATG, while encouraging in terms of reducing the risk of GVHD, raise the question of trading GVHD for other toxicities such as fatal post-transplant lymphoproliferative disorder. Administration of

statins to the donor may be one safe and relatively inexpensive means of further reducing acute GVHD incidence, although the benefits observed in retrospective studies must be confirmed in prospective clinical trials.

We currently lack the ability to reliably predict, either before allogeneic HCT or at the time of acute GVHD onset, which patients will go on to develop severe, steroid-refractory, or fatal acute GVHD. Our current approaches likely overtreat a substantial fraction of patients with mild GVHD while undertreating the minority who will go on to develop severe or fatal disease. Substantial efforts to personalize and risk-stratify acute GVHD prevention and treatment have yielded a number of potential biomarkers and predictive algorithms, but none have yet entered standard clinical practice. One major barrier is the low incidence of severe acute GVHD (typically approximately 10%). When attempting to detect a low-prevalence condition, even tests with high sensitivity and specificity are likely to have poor positive predictive value and to yield a high percentage of false-positives. This difficulty is often under-appreciated in the literature on acute GVHD biomarkers and must be addressed for these tools to enter routine clinical use.

In the setting of chronic GVHD, the track record in human clinical trials demonstrates that approaches which show initial promise in preclinical and early-phase clinical trials often fail to pan out when tested in a randomized, controlled fashion. In lieu of repeating this pattern, it will be worthwhile to invest greater effort in developing a valid, clinically relevant animal model of chronic GVHD where prevention approaches can be tested and optimized before translation to the clinic. Such animal models exist for acute, but not chronic, GVHD, and this disparity is a major contributor to our success in preventing the former but not the latter condition.

Efforts to prevent chronic GVHD must take into account its association with beneficial graft-vs.-tumor effects. In most studies, chronic GVHD is strongly linked to a lower risk of relapsed malignancy, and in some studies reductions in chronic GVHD incidence have been associated with increases in relapse risk. Further studies aimed at identifying minor histocompatibility antigen targets which mediate chronic GVHD and GVT effects may help disentangle these two entities. In the meantime, approaches which reduce chronic GVHD (such as T-cell depletion) are most suited for patients with non-malignant disease, in whom GVT effects are irrelevant and prevention of chronic GVHD a high priority.

One of the most interesting and novel areas of investigation in the prevention of GVHD is the microbiome. A strong circumstantial case implicates perturbations of the microbiome, and interactions between the donor immune system and recipient microbiome, in the development of alloimmunity. This field of investigation is relatively new, and many questions remain to be answered, but we believe that investments in defining donor/host microbiome and immune interactions will form the basis of major advances over the coming 5 to 10 years in our understanding of and ability to treat GVHD.

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**ARTICLE HIGHLIGHTS**

- Acute and chronic GVHD remain major barriers to successful allogeneic hematopoietic cell transplantation
- The treatment and prevention of acute GVHD have advanced substantially over the past several decades. In contrast, chronic GVHD remains a relatively intractable problem, in part due to the lack of relevant preclinical models.
- A vast range of novel approaches to GVHD prevention are under investigation, from refinements of well-described approaches such as T-cell depletion to novel therapies using statins, anti-B-cell agents, and cellular therapy with regulatory T cells or mesenchymal stromal cells.
- An area of particular current interest is the interaction between the host gut and skin microbiome and the innate immune system, which may trigger or potentiate GVHD.

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**Table 1**

Rates of chronic graft-vs.-host disease with mobilized peripheral blood vs. bone marrow allografts.

Publication	n	PB (%)	Marrow (%)	p value
Storek et al. [95]	74	54	32	0.039
Schmitz et al. [96]	609	61	45	<0.001
Ringden et al. [97]				
AML	1008	46	32	<0.0001
ALL	464	49	40	<0.0001
Anderson et al. [112]	194	37	28	0.7 <sup>*</sup>
Flowers et al. [98]	126	63	52	0.33 <sup>†</sup>
Mohty et al. [99]	101	65	36	0.004
Heldal et al. [113]	57	56	27	Not given
Mielcarek et al. [102]	172	48	37	0.55
Anasetti et al. [100]	551	53	40	0.02

Abbreviations: AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; n, number of patients studied; PB, cytokine-mobilized peripheral blood mononuclear cells.

<sup>\*</sup> Anderson et al. reported similar overall rates of chronic GVHD, but noted that PB recipients had a pattern of “late-onset” chronic GVHD (developing >180 days after HCT) and more frequent late systemic fungal and cytomegalovirus infections than marrow recipients.

<sup>†</sup> While Flowers et al. did not find a difference in overall rates of chronic GVHD, they did report that chronic GVHD in PB recipients was more protracted and less responsive to treatment than chronic GVHD in marrow recipients.