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Current and future approaches for control of graft-versus-host disease

John Koreth, MBBS, DPhil† and **Joseph H Antin, MD**
Division of Hematologic Malignancies, Dana Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA

Abstract

Graft-versus-host disease (GVHD), both acute and chronic, remains one of the major barriers to improving outcomes after allogeneic stem cell transplantation. The pathophysiology of GVHD is complex and incompletely understood. GVHD is believed to arise from the interaction of: tissue damage and proinflammatory cytokines causing activation of antigen-presenting cells (APCs, donor T-cell activation by APCs and cytokines and host tissue injury by effector T lymphocytes and proinflammatory cytokines. There is also a role for additional lymphocyte subtypes (naive and memory T cells, regulatory T cells, natural killer T cells and B cells) in GVHD pathogenesis. Strategies to improve donor–recipient HLA match, and to minimize conditioning toxicity, cytokine release and APC and effector T-lymphocyte activation, will likely improve prophylaxis of acute (and possibly chronic) GVHD. Therapy of established acute and chronic GVHD is still heavily dependent on corticosteroids, despite their limited efficacy and considerable toxicity. Novel agents (and/or combinations of agents) comprising pharmacologic, biologic and cellular therapies targeting specific steps or subsets involved in immune activation will likely comprise future advances in GVHD control. This article reviews the current state of knowledge regarding the prevention and treatment of acute and chronic GVHD. Novel approaches currently undergoing evaluation are also highlighted.

Keywords

allogeneic stem cell transplantation; graft-versus-host disease

Allogeneic stem cell transplantation (alloSCT) is often the only curative option for patients with various hematologic and/or immune disorders, particularly those with aggressive or advanced hematologic malignancies. However, the toxicity of alloSCT remains a significant barrier to its wider utilization. Graft-versus-host disease (GVHD) remains the most frequent complication after alloSCT.

Clinically, GVHD was categorized as ‘acute’ and ‘chronic’ based on time of presentation. Any GVHD before day 100 was known as ‘acute’, and after day 100 it was known as ‘chronic’. The severity of GVHD was graded: acute GVHD was categorized as grade I–IV by modified Glucksberg criteria (A–D by the International Bone Marrow Transplant Registry index) (Table 1) [1,2]; chronic GVHD was commonly categorized as limited or extensive [3]. Based on these

†Author for correspondence: Division of Hematologic Malignancies, Dana Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA, Tel.: +1 617 632 3470, Fax: +1 617 632 5168, john_koreth@dfci.harvard.edu.

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criteria, grade II–IV acute GVHD is thought to occur in approximately 35% of recipients of matched, related donor transplants, and in up to 50% of unrelated or alternative donor transplant recipients. Chronic GVHD can affect up to 60% of recipients who survive beyond 100 days after matched donor alloSCT.

While the simplicity of the day 100 definition is appealing, it is irrelevant biologically and clinically. For instance, in patients receiving reduced intensity conditioning (RIC) alloSCT, or after donor lymphocyte infusion (DLI), clinical acute GVHD may develop months after the procedure [4]. Hence, there is a current attempt by the National Institutes of Health chronic GVHD consensus project working group to reclassify acute GVHD into classic acute and late-onset acute; and chronic GHVD into classic chronic and overlap syndrome [5]. Classic acute GVHD is characterized by a maculopapular erythematous skin rash, gastrointestinal symptoms or cholestatic hepatic abnormalities occurring within 100 days of alloSCT or DLI, while late acute GVHD presents similarly beyond 100 days after alloSCT or DLI. Classic chronic GVHD consists solely of manifestations ascribable to chronic GVHD (without acute GVHD features) (Table 2), while overlap syndrome has clinical features of both acute and chronic GVHD occurring together.

Risk factors for GVHD

The risk factors for GVHD include:

- Donor–recipient match at the major histocompatibility complex (MHC) loci, for instance, HLA class I (HLA-A, -B and -C) and class II (HLA-DR, -DP and -DQ). Mismatches at HLA-A, -B, -C or -DRB1 (and possibly also -DQ and -DP) increase the risk of GVHD (nonpermissive donor–recipient HLA mismatches may particularly influence GVHD severity) and negatively impact survival [6–10];
- Donor stem cell source: compared with bone marrow stem cells, peripheral blood stem cells (PBSCs) have a higher GVHD risk, while umbilical cord blood cells appear to have a lower risk [11–14];
- T-cell dose: compared with T-cell replete grafts, 2–3 log depletion of $CD3^+$ T lymphocytes of the graft can effectively reduce acute GVHD incidence (although the effect on chronic GVHD is less clear), while less-intensive log reductions of T cells have no significant impact [15,16]. However, the benefit of T-cell depletion is counteracted by increased risks of graft failure, opportunistic infection and disease relapse such that pan-T-cell depletion strategies are not currently favored [17];
- Additional risk factors include donor and recipient age, donor–recipient sex mismatch (female donor to male recipient), donor parity and allosensitization, disease stage and intensity of conditioning (for acute GVHD). Acute GVHD is a powerful predictor of chronic GVHD risk [18].

Measures to reduce GVHD risk would therefore include improvements in donor selection, improved HLA matching, as well as reduced intensity conditioning where possible. However, other trends, such as the increased use of donor PBSCs as a source of stem cells, extending alloSCT to older/sicker patients and the use of alternative donors (haploidentical and HLA-mismatched donors), suggest that GVHD control will remain a significant issue for the foreseeable future.

Etiopathogenesis of GVHD

The etiology of GVHD is complex, but Billingham's criteria still apply [19]. First, the graft must contain immunologically competent cells (T lymphocytes and possibly B lymphocytes). Second, the recipient must be incapable of rejecting the transplanted cells (achieved by

conditioning chemotherapy or radiation). Third, the recipient must express tissue antigens that are not present in the donor (major or minor histocompatibility mismatch).

Our current understanding of acute GVHD, although incomplete, is better than that of chronic GVHD. In part, this is due to the better availability of mouse models of acute GVHD. Broadly however, both forms of GVHD are believed to be caused by similar alloimmune responses that also underlie the beneficial graft-versus-leukemia (GVL) effect. Maintaining control of GVHD, while enabling the curative GVL response remains the holy grail of allotransplantation.

The development of acute GVHD is frequently divided into three phases (Figure 1):

- Tissue damage, owing to underlying disease, infections and conditioning regimen toxicity, resulting in leakage of bacterial lipopolysaccharides across the damaged gut epithelium and a 'cytokine storm' with the production of inflammatory cytokines, such as TNF-α, and IL-1 by injured cells, resulting in secondary changes in expression of adhesion molecules, MHC antigens and chemokines, which can act as danger signals and activate residual host and donor antigen-presenting cells (APCs) [20–24]. APC activation can occur via both Toll-like receptor (TLR) and non-TLR (e.g., nucleotide-binding oligomerization domain [NOD]) pathways [25,26];
- Donor T-cell activation, cytokine release, proliferation and tissue localization occurs in the context of the proinflammatory post-transplant milieu and after alloantigen presentation and costimulation by APCs (donor or host) [27–30];
- The effector phase of GVHD target organ damage involves a complex interaction of cytokine and cellular effectors. Cytotoxic T lymphocytes (CTLs), both $CD4^+$ and $CD8^+$, are the major cellular effectors of GVHD and cause cell death by a variety of pathways, such as Fas–Fas ligand (FasL), TNF receptor (TNFR)-like death receptors (e.g., TRAIL and TWEAK) and perforin–granzyme [31–36]. Inflammatory cytokines, such as TNF-α and IL-1, synergize with CTLs and can also act directly to promote tissue injury and inflammation in GVHD target organs [37–40].

Based on their cytokine expression pattern, there are at least two types of T helper (Th) effector cells involved in GVHD: Th1 and Th2 cells. Th1 cells generate IL-2, TNF-α and IFN-γ, while Th2 cells produce IL-4 and IL-10. While the 'cytokine storm' phase of GVHD, which is amplified by Th1 cytokines, correlates with the development of acute GVHD, cytokines that polarize donor T cells to Th2 (e.g., granulocyte colony-stimulating factor [G-CSF], IL-4 and IL-18) can reduce acute GVHD [41–44]. However, this model may be an oversimplification, as Th1 and Th2 subsets can each cause injury to distinct GVHD target organs in some mouse models of acute GVHD [45]. Additional complexities involve possible roles for newly identified Th17 cells in GVHD and the interaction between Th17 effector cells and peripheral regulatory T cells (Tregs), given their alternate developmental fates from common naive precursor T cells [46–48].

Additionally, genetic polymorphisms that lead to altered cytokine expression levels (e.g., IL-6, IL-10 and TNF-α) have also been linked to differences in acute and chronic GVHD incidence [49–58]. Furthermore, polymorphisms involving natural killer (NK) cell receptor/ligand complex, collectively termed the killer immunoglobulin-like receptor family (KIR), have been linked to differences in both GVHD and relapse rates after alloSCT [59–61]. Similarly, polymorphisms in the non-TLR (NOD) pathway of adaptive immune activation can impact GVHD risk [62]. Genes involved in drug metabolism have also been linked to toxicity and GVHD after alloSCT [63,64]. Finally, genes with only indirect associations with immune activity have also been linked to GVHD [65–67]. Both donor and recipient polymorphisms are often relevant with regards to GVHD risk, as in the case of IL-10 [68].

There is increasing awareness of the role of additional cellular subsets in GVHD:

- Naive and memory T cells: naive ($CD62L^+$) T cells, but not memory ($CD62L^-$) T cells, are often considered to have alloreactive potential that can result in acute GVHD [69,70]. However, contrasting recent data also suggest a role for alloreactive memory T cells and their precursor stem cells in the development of GVHD [71,72];
- Tregs: $CD4^+CD25^+$ $FoxP3^+$ Tregs from the donor have been shown to suppress the expansion of alloreactive donor T cells and the development of GVHD, without abrogating GVL in this MHC-mismatched murine model [73]. IL-2, initially identified as a lymphocyte growth factor and thought primarily to promote effector T-cell responses *in vivo*, is now identified as a cytokine critical for the development, expansion and activity of Tregs [74,75]. In humans, FoxP3 mRNA levels (considered a relatively specific marker for Tregs) was significantly decreased in patients with GVHD [76,77]. The expression of the cell surface marker CD62L was also found to be critical for the ability of donor Tregs to control GVHD [78,79];
- NKT cells: host NKT cells also have immune suppressive effects that can control GVHD in an IL-4-dependent fashion [80,81]. Human clinical data suggest that enhancing recipient NKT cells by total lymphoid irradiation (TLI) in conjunction with anti-thymocyte globulin-based conditioning similarly promoted Th2 polarization and significantly reduced GVHD [82]. However, it is important to note that NKT cells are heterogeneous and their roles in GVHD are incompletely understood;
- B cells: traditionally, a major role for B cells and humoral immunity in the development of GVHD has not been considered. However, recent work suggests that, in the context of matched sibling PBSC allotransplantation, the concentration of $CD20^+$ B cells in the apheresis product may predict the development of acute GVHD [83]. Additionally, auto- and alloantibodies have been described in chronic GVHD, some of which may play a direct role in disease progression (e.g., activating PDGF receptor antibodies) [84–87]. High circulating levels of B-cell activation factor at 6-months post-transplant were a predictor of subsequent chronic GVHD, further supporting a role for B-cell dysfunction in chronic GVHD [88]. The role of humoral immunity in GVHD remains an area of controversy and further investigation.

Prophylaxis of GVHD

Pilot studies omitting GVHD prophylaxis indicated an acute GVHD incidence of nearly 100% [89]. Studies using methotrexate as a single agent for GVHD prophylaxis via inhibition of rapidly dividing alloreactive T cells, indicated an acute grade II–IV GVHD rate of over 50%, even in the setting of HLA-matched sibling donors [90]. The introduction of a calcineurin inhibitor, cyclosporine (and subsequently tacrolimus), represented the next advance in the prevention of GVHD, with improved efficacy in GVHD control compared with methotrexate [91–93]. Cyclosporine and tacrolimus bound to cyclophilin or FK-binding protein 12 (FKBP12), respectively, inhibit calcineurin (a protein phosphatase that is calcium- and calmodulin-dependent) and prevent the dephosphorylation and nuclear translocation of the transcription factor nuclear factor of activated T cells (NFAT). By blocking NFAT, one of the most important regulators of cytokine gene transcription following T-cell activation, calcineurin inhibitors block T-cell activation and proliferation [94,95]. The combination of calcineurin inhibitor (cyclosporine) and methotrexate was more effective than either agent alone, with grade II–IV acute GVHD rates of 20–56% after HLA-matched sibling alloSCT [96,97]. Compared with cyclosporine, tacrolimus has an improved toxicity profile and, more importantly, randomized data indicate improved acute GVHD prophylaxis in both HLA-matched siblings and unrelated donor allotransplants [98,99]. The length of immunosuppressive therapy appears to have no role in improving control of chronic GVHD.

Patients with acute GVHD or biopsy evidence of subclinical acute GVHD were randomly assigned to 6 versus 24 months of cyclosporine therapy. The rates of clinical extensive chronic GVHD were 39 and 51%, respectively, a nonsignificant difference [100]. Similarly, the presence or absence of day 11 methotrexate does not likely impact chronic GVHD rates [101,102].

Corticosteroids, the mainstay of therapy for established acute GVHD, do not have a significant role in GVHD prophylaxis. Various trials compared prednisone and cyclosporine to the three-drug combination of methotrexate, cyclosporine and prednisone. In one large trial, the acute GVHD rate in the cyclosporine and prednisone control arm was 23%, compared with only 9% in the three-drug arm of methotrexate, cyclosporine and prednisone [103]. However, subsequent trials could not demonstrate similarly improved GVHD control, or improved long-term outcomes with the three-drug combination, and, currently, steroids are not routinely used in GVHD prophylaxis [104].

Cyclophosphamide has been used post-transplant since the 1980s for GVHD prevention and act via inhibition of rapidly dividing T cells (in a manner similar to methotrexate) [105]. Stem cells contain high levels of aldehyde dehydrogenase that converts the active metabolite 4-hydroxycyclophosphamide to an inactive nonalkylating metabolite, thus protecting the stem cell from the antiproliferative activity of the agent. Similarly, the gut epithelium has high levels of aldehyde dehydrogenase that is protective against excess mucosal toxicity despite prior intensive conditioning. Used as a single agent after myeloablative conditioning in related and unrelated allotransplants, the grade II–IV acute GVHD rate was 41%, with few late infections, attributed to the brief duration of immune suppressive therapy [106]. It is also currently being evaluated for alternative donor transplants (haploidentical donor) [107].

Mycophenolate mofetil (MMF) is a potent, selective, noncompetitive reversible inhibitor of inosine monophosphate dehydrogenase that inhibits the *de novo* pathway of guanosine nucleotide synthesis. It has potent cytostatic effects on lymphocytes (both T and B) whose proliferation is dependent on *de novo* purine synthesis. With good oral bioavailability, the optimal dosing interval remains uncertain, usually two- to three-times daily. It has been used for GVHD prophylaxis in various combinations (usually with a calcineurin inhibitor ± methotrexate). The incidence of grade II–IV acute GVHD has ranged between 38 and 62% [108,109]. In a single-center randomized study, the combination of cyclosporine plus MMF was associated with faster engraftment and reduced mucositis incidence, but with similar incidence of acute and chronic GVHD and survival comparable to cyclosporine plus methotrexate, possibly affected by limited sample size and follow-up duration for these secondary end points [110]. Longer-term use of cyclosporine in combination with MMF after RIC alloSCT with matched related donors did not impact the rates of acute grade II–IV or chronic GVHD [111].

Sirolimus (also called rapamycin) binds uniquely to FKBP12 and forms a complex with mammalian target of rapamycin (mTOR) that interacts with various upstream pathways including PTEN/PI3 kinase/Akt pathway and the Janus kinase pathway [112,113]. The sirolimus–mTOR complex inhibits several biochemical pathways, resulting in reduction of DNA transcription/translation, protein synthesis and cell cycle progression, which results in T-cell immunosuppression [114,115]. Interestingly, there is apparent differential inhibition of T-cell subsets, possibly involving selective inhibition of Th1 cell responses, and sparing of Th2 and Treg activity [116–120]. Despite theoretical concerns for competition for FKBP binding with calcineurin inhibitors, these agents appear to work synergistically, and sirolimus does not interact with calcineurin or its downstream effectors [112]. In contrast to calcineurin inhibitors, sirolimus may also exert its immunosuppressive effects through suppression of APC activity via a reduction in antigen uptake, cellular processing, intracellular signaling and

induction of apoptosis [121–123]. The combination of sirolimus and tacrolimus appears more effective than sirolimus plus cyclosporine in reducing alloreactive memory T-cell production, abrogation of effector CTL induction and apoptosis induction [124]. Single-institution clinical studies of sirolimus and tacrolimus with and without low-dose methotrexate for GVHD prophylaxis after myeloablative conditioning with cyclophosphamide/total-body irradiation (TBI) indicate excellent efficacy and acceptable toxicity in the matched related and unrelated donor context, with grade II–IV acute GVHD rates of 19 and 23%, respectively [125]. The rates of chronic GVHD, however, were not significantly impacted. Similar efficacy in acute GVHD control was noted despite omitting low-dose methotrexate, and toxicity was further reduced [126]. Similar low-acute GVHD rates were also noted in the context of RIC. Other recent single-institution reports indicate concordant as well as variant estimates of sirolimus efficacy for GVHD prophylaxis in the myeloablative alloSCT context [127,128]. Sirolimus plus tacrolimus is currently being evaluated in a Phase III multi-institution context in comparison to methotrexate plus tacrolimus.

Biologic agents have also been evaluated for GVHD prophylaxis. *In vivo* T-cell depletion with horse- or rabbit-derived polyclonal antithymocyte globulin (ATG) has been evaluated for prevention of GVHD, as initially proposed by Ramsey *et al.* [129]. Such agents administered pre- and peritransplant can simultaneously target host and donor T cells to control both graft rejection and GVHD [130–132]. However, additional cellular components, such as B cells, NK cells and APCs, can also be affected by polyspecific antibodies. Their use does appear to reduce the incidence of chronic GVHD and chronic lung dysfunction, with improved late transplant-related mortality [133]. Whether the reduction in chronic GVHD is also associated with increased disease relapse remains to be determined. Higher doses of rabbit ATG (thymoglobulin) are associated with increased infections that can abrogate its positive impact on GVHD [134]. TLI in conjunction with ATG-based conditioning also significantly reduced GVHD [82].

Monoclonal antibodies, such as alemtuzumab (Campath-1H; anti-CD52), are widely used for *in vivo* GVHD prophylaxis. It has been found to reduce GVHD and nonrelapse mortality after related and unrelated transplants, and can also facilitate engraftment [135]. Monoclonal antibodies targeting the IL-2 receptor (CD25) may also show benefit [136]. However, IL-2 is also critical for Treg development, expansion and activity, hence IL-2 targeting in GVHD may have the unintended consequence of impairing Tregs that are important to control GVHD [74,75]. Low-dose IL-2 is currently being evaluated for GVHD prophylaxis. Some biologic agents that may have activity in established active GVHD, such as IL-1 antagonists and ricin-conjugated CD5 antibody, do not show benefit in the prophylactic setting [137–141]. Interestingly, rituximab, a monoclonal CD20 antibody that depletes B cells, may independently decrease acute GVHD risk [142]. It is also being evaluated for the prophylaxis of chronic GVHD.

In vitro T-cell depletion (TCD) has also been attempted to control GVHD, with some success in controlling acute (and possibly chronic) GVHD. However, in a randomized study comparing GVHD prophylaxis with approximately 1-log TCD (with monoclonal antibody T10B9 targeting the T-cell receptor) plus cyclosporine versus methotrexate and cyclosporine, improved acute GVHD control did not lead to improvement in long-term survival, and disease relapse and infection risk was significantly increased after T-cell depletion [16,143]. In an attempt to circumvent problems associated with global TCD, selective depletion strategies focused on T-cell subsets (e.g., $CD4^+$, $CD6^+$ and $CD8^+$ T cells) have been utilized, with limited success [144–146]. Other studies have combined TCD and scheduled DLI post-alloSCT, to improve relapse rates and outcomes after TCD, with mixed results [147,148]. In an alternative strategy to induce anergy to donor alloantigens, a small trial utilized costimulation blockade

of HLA haploidentical donor T cells by *ex vivo* incubation with CTLA4 antibody and donor APCs with some reported success [149].

Proteasome inhibition may have a role in GVHD control. The transcription factor NF-κB plays an important role in cytokine signaling and the generation of cell-mediated immune responses. In addition, the proteasome has been shown to play a critical role in T-cell activation, proliferation and apoptosis, largely through NF-κB activation [150–152]. In addition to direct cytotoxic effects, the proteasome inhibitor bortezomib demonstrates immunomodulatory effects through NF-κB [153]. It can attenuate TLR4-mediated APC activation, with reduced cytokine production and immunostimulatory activity [154]. Additionally, in the allogeneic setting, bortezomib preferentially and specifically depletes alloreactive T lymphocytes [155]. In murine models of GVHD, bortezomib early after stem cell infusion protected against GVHD without impairing engraftment [156,157]. Phase I and II trials for prevention and treatment of acute GVHD are ongoing, with interesting preliminary results [158].

Additional agents that have efficacy in the treatment of established acute GVHD are also being evaluated for primary GVHD prophylaxis. Examples include pentostatin and etanercept (discussed later). Novel approaches include blocking lymphocyte migration to GVHD target organs using chemokine blockade (although there is significant redundancy in this system, complicating targeting efforts) and the use of extracorporeal photopheresis, which may alter host antigen presentation and enhance Tregs for GVHD control [159–162]. Ursodiol, utilized for control of hepatotoxicity and treatment-related mortality (TRM) peritransplant, was reported to also control severe acute GVHD [163]. However, a meta-analysis confirmed the hepatotoxic and TRM benefit of ursodiol, but did not note improved GVHD control [164]. Thalidomide was evaluated for chronic GVHD prophylaxis in a Phase III trial, with negative impact on mortality and chronic GVHD incidence [165]. Revlimid® or newer congeners may be more useful. Attempts to prevent thymic atrophy and associated chronic GVHD with thymic tissue implants, thymic epithelial cells or thymic hormones have not had positive results [166,167].

Treatment of established GVHD

In patients with established acute GVHD, the goal of therapy is to achieve rapid control, since the probability of survival depends upon the initial stage of GVHD at presentation and response to therapy [168–170]. Long-term survival of patients with grade 0–I acute GVHD is 50%, while long-term survival of those with grade IV acute GVHD is as low as 11% [168]. Response to therapy is a key predictor of outcome, as mortality in acute grade II–IV GVHD is lowest in those with a complete response to initial therapy [169,171,172]. Corticosteroids are lympholytic and inhibit inflammatory cytokine cascades. Other agents have been utilized as first-line therapy, however none have proven superior to corticosteroids [169,173]. Corticosteroids also remain the primary front-line therapy for chronic GVHD, often in combination with a calcineurin inhibitor, as discussed later.

First-line therapy of GVHD

Corticosteroids (typically prednisone or methylprednisolone) dosed at approximately 2 mg/kg/day are the standard therapy for acute grade II–IV GVHD [174,175]. After single-agent steroid therapy, response rates were approximately 50% [169,171]. Higher doses have not been associated with improved response rates. In a prospective randomized study comparing methylprednisolone at 2 versus 10 mg/kg/day in patients with acute grade II–IV GVHD, response rates, TRM, GVHD progression and overall survival were similar [176]. TRM and long-term survival were significantly improved in patients with early acute GVHD response that permitted steroid taper by day 5 of therapy [172]. In order to reduce the toxicities of prolonged systemic steroids, adjuvant topical steroids have been evaluated. In one randomized

study, prednisone with and without oral nonabsorbable steroids (enteric-coated beclomethasone) were evaluated for therapy of gastrointestinal acute GVHD. Prednisone taper was initiated on day 10 if clinical response occurred. Durable responses and day 200 mortality were improved in the beclomethasone plus prednisone arm [177].

The limited response to systemic steroids alone has prompted evaluation of additional immunosuppressive agents in the initial therapy of GVHD. This strategy has had only limited success, given the increased risks of infection and TRM. ATG is the most widely studied in this setting. Initial studies of upfront therapy with ATG plus steroids reported impressive response rates of 67–80% [178,179]. However, in a randomized study, initial therapy of acute grade II–IV GVHD with prednisone with and without ATG failed to demonstrate an improvement in response rates or survival in the ATG arm. Infectious complications were more common in the combination ATG arm [180].

Other biologic agents have been evaluated in combination with steroids for initial therapy of acute GVHD. One randomized study evaluated systemic steroids with and without the monoclonal antibody daclizumab that targets CD25 (the IL-2 receptor α-chain) present on activated T cells, dosed at 1 mg/kg on days 1 and 4 and weekly thereafter. Overall response rates were similar in the two groups, but survival at 100 days and 1 year was inferior in the daclizumab plus steroid group [181]. Similar lack of benefit was noted in a randomized study evaluating prednisone and cyclosporine with and without another monoclonal antibody targeting the IL-2 receptor (BT563) [182]. CD5, found on the majority of Tcells, acts as a costimulation molecule to regulate signaling via the T-cell receptor. A randomized trial utilizing a CD5-specific immunotoxin or placebo in combination with methylprednisolone found improved early response of acute GVHD in the immunoconjugate arm, but comparable long-term outcomes [140]. The TNF-α inhibitor etanercept in combination with corticosteroids plus tacrolimus demonstrated superior acute GVHD control in comparison to a historical cohort treated with steroids alone, in a small study that was recently updated [183,184].

Currently, none of these agents have displaced corticosteroids as upfront therapy for acute GVHD, but there is significant interest in finding better therapies for initial treatment of acute GVHD. Adjunctive agents that are currently being evaluated in an ongoing randomized trial by the Bone Marrow Transplant Clinical Trials Network include etanercept, denileukin diftitox, mycophenolate mofetil and pentostatin, each in combination with corticosteroids.

Corticosteroids are also the mainstay of therapy for chronic GVHD. Other single agents are associated with a low response rate. Currently, there is no standard second-line therapy for chronic GVHD and therapy typically consists of prolonged administration of a corticosteroid combined with other immunosuppressive medications, such as calcineurin inhibitors (cyclosporine or tacrolimus). In a randomized trial comparing single-agent prednisone versus prednisone plus cyclosporine in patients with extensive chronic GVHD, TRM, survival, relapse, need for salvage therapy and rate of discontinuation of immunosuppressive therapy were similar for the two arms. The median duration of therapy with corticosteroids and cyclosporine was 1.6 years. Owing to increased toxicity in the steroid-only arm, many favor combination therapy [185]. Despite dual immunosuppressive therapy, only 54% of patients were successfully weaned off corticosteroids at 5 years and the mortality directly attributable to chronic GVHD was 17% in the combination therapy arm. Similar lack of benefit with combination therapy was noted in a trial of cyclosporine, prednisone with and without thalidomide as initial therapy of chronic GVHD. Response rates were similar in the two arms, but the complication rate was greater in the thalidomide arm [186]. Additional agents for upfront therapy in chronic GVHD are urgently required. Ongoing studies evaluating the role of mycophenolate mofetil and the proteasome inhibitor bortezomib, each in combination with steroids, are of interest in this regard.

Salvage therapy for GVHD

Acute GVHD patients in whom initial therapy fails are commonly defined as:

- Progression of GVHD after 3 days
- No improvement after 7 days
- Incomplete response after 14 days of systemic methylprednisolone therapy at 2 mg/kg/day or equivalent

These patients are considered for a variety of salvage regimens. There is currently no established second-line regimen for acute GVHD and long-term outcomes remain poor, despite various high initial response rates reported with some second-line agents.

Antithymocyte globulin (equine or rabbit polyclonal antibody) is still the most commonly used agent for steroid-refractory acute GVHD [170,187]. This popularity is despite the fact that randomized data did not identify a survival advantage to the ATG arm when patients with acute grade I–IV GVHD nonresponsive to methyprednisolone (2 mg/kg/day) were randomized to higher-dose methylprednisolone (5 mg/kg/day) with and without ATG [172].

Biologic therapies targeting CD25 have been evaluated in the steroid-refractory acute GVHD setting. Daclizumab, the humanized anti-IL2 receptor monoclonal antibody, has been evaluated in three Phase II studies. Overall response rates in the steroid-refractory setting have ranged from 29 to 50% [188–190]. A chimeric human/mouse monoclonal IL-2 receptor antibody, basiliximab, has also been evaluated in steroid-refractory acute GVHD. Phase II studies utilizing various dosing schedules report an overall response rate of 71–83% (complete responses of 18–53%) [191–193]. Denileukin diftitox (Ontak®), a conjugate of human IL-2 fused to the membrane translocation and catalytic domains of diphtheria toxin, targets the high-affinity IL-2 receptor. At the maximum-tolerated dose of 9 μg/kg on days 1, 3, 5, 15, 17 and 19, 71% of steroid-refractory acute GVHD patients had a response to therapy (complete responses of 46%) [194]. Dose-limiting toxicities were transient hepatic transaminitis, infusion reactions and a vascular leak syndrome. Another trial, with a different dose and schedule, reported an overall response rate of 41% with denileukin diftitox [195]. The negative impact on Tregs of targeting CD25 and the IL-2 pathway remains to be determined, but is a concern with regards to long-term GVHD control.

Cytokine blockade has been evaluated for steroid-refractory GVHD therapy. In addition to IL-2 receptor targeting via CD25, TNF-α is also an attractive target. Etanercept, a soluble human TNF receptor fusion protein, was used to treat 13 patients with steroid-refractory acute GVHD, dosed at 25 mg subcutaneously biweekly for 4 weeks. Clinical responses were seen in six patients (46%), including five complete responses [196]. Combination therapy with daclizumab plus etanercept has also been reported, with a 66% overall response rate [197]. However, long-term mortality due to infectious complications and chronic GVHD remained high. In a small retrospective analysis, etanercept in combination with ATG for steroid-refractory visceral acute GVHD may have improved response rate and survival compared with historical controls treated with ATG [198]. Infliximab, the chimeric TNF-α antibody, can neutralize circulating TNF-α and lyse TNF-α-producing cells. Various dose-escalation trials indicate the potential efficacy of the agent, and larger studies document response rates of 59–67%, with a predilection for response in gastrointestinal GVHD [199–201]. Invasive fungal infection rates have been high after infliximab therapy in this population [202].

The monoclonal anti-CD3 antibody OKT3 was initially reported to be an effective salvage therapy for acute GVHD [203]. The toxicity of OKT3 includes cytokine release syndrome (fever, chills, nausea and rash) in 60% of treated patients. In a recent randomized study of 80

patients with steroid-refractory GVHD, comparing OKT3 plus high-dose methylprednisolone (10 mg/kg) versus high-dose methylprednisolone alone, there was no significant difference in overall response rate at day 100 (53 vs 33%; $p = 0.06$) or overall survival at 1 year (45 vs 36%; $p = 0.61$) [204]. To minimize cytokine-release syndrome, a humanized anti-CD3 antibody (that does not bind human Fc receptor), visilizumab (HuM291), was evaluated in Phase I and II studies. In the Phase I study of 11 patients dosed once with visilizumab at 3 mg/m^2, nine patients achieved a response (six with a complete response) [205]. The overall response in the Phase II cohort of 44 steroid-refractory patients (86% with acute grade III–IV GVHD), however was only 32% at 7 weeks (14% complete responses), and survival at 6 months was 32% [206]. The incidence of Epstein–Barr virus (EBV) reactivation in the studies was 40–50%.

In a Phase I trial of the anti-CD147 monoclonal antibody (ABX-CBL) that targets neurothelin, a glycoprotein weakly expressed on leukocytes that is upregulated on activated T and B lymphocytes, monocytes and dendritic cells (APCs), over half the patients with steroid-refractory acute GVHD responded, and overall survival was superior to a historical control group treated with ATG [207]. However, the Phase II/III study comparing ABX-CML versus ATG failed to show a benefit with ABX-CBL [208]. Targeting T cells and APCs with alemtuzumab, a monoclonal antibody against CD52, has had some reported success as therapy of steroid-refractory acute GVHD [209–211]. Owing to simultaneous depletion of B lymphocytes, EBV reactivation and post-transplant lymphoproliferative disorder rates are low, but there is substantial risk of cytomegalovirus (CMV) reactivation and other infections [130,212]. Alefacept (Amevive®) is a novel fusion protein of the extracellular CD2-binding domain of the human leukocyte function antigen-3 (LFA-3) fused to the Fc portion of IgG1, and has selective activity against memory T cells. It has activity in T-cell-mediated autoimmune diseases, and small case series' report on its utility in acute GVHD, with encouraging GVHD responses [213,214]. However, late complications from viral and fungal infections have been noted.

While chronic GVHD is primarily considered a T-cell disorder, humoral auto- and allo-immune activation has also been documented in this setting. There is evidence of B-cell activation, and alloantibodies (e.g., antibodies to minor histocompatibility Y chromosome antigens in gender-mismatched allotransplants: female donors to male recipients) correlate with chronic GVHD in this setting [85,87]. Rituximab, a humanized monoclonal antibody to CD20 that depletes B cells, has been evaluated in the setting of steroid-refractory chronic GVHD [215–218]. In the largest study, 70% of the 21 chronic GVHD patients treated with rituximab (375 mg/m^2/week for four doses, repeated once if necessary) responded to therapy (primarily skin and musculoskeletal responses) [218]. Antibody titers also fell in the responders. Given the relatively limited toxicity of rituximab, it is an attractive agent for further evaluation in this context.

Chemotherapeutic agents have also been utilized for salvage therapy of steroid-refractory GVHD. Pentostatin, a nucleoside analog, kills lymphocytes by inhibiting adenosine deaminase, and the resulting accumulation of 2′-deoxyadenosine 5′-triphosphate causes apoptosis of dividing lymphocytes. It has modest myeloid toxicity and was well tolerated in GVHD patients with a suggestion of increased CMV and other viral infection risk. Dose adjustment for renal function was necessary [219]. The complete response rate was 63%, but only 26% of patients were alive at the 1-year follow-up. Pentostatin has also been evaluated as salvage therapy in chronic GVHD. In a Phase II study, 32 out of 58 heavily pretreated patients (55%) had an objective response. Infection was the most significant toxicity, with 11 grade 3–4 infectious events. Survival at 1 and 2 years was 78 and 70%, respectively [220].

Mycophenolate mofetil has a reported 65% response rate in steroid-refractory acute GVHD, and over 45% in chronic GVHD, with common toxicities of nausea, diarrhea,

myelosuppression and opportunistic infections [221–224]. The largest study in the chronic GVHD setting reported retrospectively on the use of MMF for *de novo* or refractory chronic GVHD [225]. Response rates were 90% in the *de novo* setting (in conjunction with a calcineurin inhibitor or prednisone) and 75% in the refractory setting. The results of ongoing trials are awaited.

Sirolimus has had limited evaluation in the steroid-refractory acute GVHD setting. One study reported a response to sirolimus in 12 out of 21 steroid-refractory patients, with five complete responses [226]. Toxicity at the targeted serum level of 17–25 ng/ml was high, including thrombotic microangiopathy (TMA), cytopenias and hypertriglyceridemia. The impact of lower target doses of sirolimus remains to be determined in this setting. In steroid-refractory chronic GVHD, sirolimus in combination with tacrolimus and corticosteroids had a response in 22 out of 35 patients (63%), including six patients with a complete response, albeit at the expense of significant renal toxicity and TMA [227]. In another study, 15 out of 16 evaluable patients with steroid-refractory chronic GVHD had a response to sirolimus, but renal toxicity remained a concern [228].

Thalidomide and its analogs (e.g., lenalidomide) have potent immunomodulatory activity and inhibit TNF-α production by lipopolysaccharide-activated monocytes [229,230]. Cytokines such as IL-6, IL-1β and GM-CSF are also inhibited by thalidomide, while lenalidomide can stimulate IL-10 [231]. Thalidomide and its analogs can also reduce leukocyte recruitment and trafficking to inflamed tissues by blockade of TNF-α-induced adhesion molecules (e.g., intercellular adhesion molecule [ICAM]-1, vascular cell adhesion molecule [VCAM]-1 and E-selectin) [232]. Unfortunately, thalidomide did not show benefit in prophylaxis or initial therapy of chronic GVHD [165,186]. Lenalidomide is currently being evaluated in a Phase II setting for second-line therapy of chronic GVHD in patients who have failed a trial of steroids plus calcineurin inhibitors.

Phototherapy, primarily extracorporeal photopheresis (ECP), involving *ex vivo* therapy of T cells plus a psoralen sensitizer, exposed to ultraviolet light (UV-A) and subsequently reinfused, has been utilized to control refractory acute and chronic GVHD. Proposed mechanisms involve alteration of host antigen presentation, effector lymphocyte apoptosis and enhancement of regulatory T cells for improved GVHD control [159–162]. In a Phase I/II study of 59 patients with steroid-refractory acute grade II–IV GVHD, ECP therapy resulted in complete response rates in 70% of patients overall, with 86, 55 and 30% complete responses in grade II, III and IV acute GVHD, respectively, and 82, 61 and 61% complete responses for skin, intestinal and liver GVHD, respectively [233,234]. Complete responders had an overall survival benefit at 4 years of 59% compared with 11% for those who did not obtain a complete response. In the chronic GVHD setting, several retrospective studies have been reported [235–238]. In the largest study involving 71 patients, the overall response rate was 61% (19% complete), primarily responses involving the skin, liver and mucosal surfaces (mouth and eye), and some responses were sustained. However, the cumulative incidence of steroid withdrawal at 1 year was only 22%, with a 53% overall survival at 1 year following initiation of ECP [238]. In a small prospective study of 25 patients with chronic GVHD, skin and/or visceral improvement was noted in 71% of patients and, interestingly, there was no difference in response rates for twice-weekly versus once-weekly ECP [239]. Additional prospective evaluation of ECP in the Phase II and III setting is necessary to confirm these impressive results in both acute and chronic GVHD.

Mesenchymal stem cells (MSCs) are bone marrow progenitor cells capable of differentiating into various nonhematopoietic lineages that have immunomodulatory properties and can inhibit T-cell proliferation to mitogens and alloantigens *in vitro* and prolong skin allograft survival *in vivo* [240,241]. In the initial report, infusion of haploidentical (maternal) MSCs

improved the severe treatment-refractory GVHD in a pediatric patient [242]. Subsequent case series have been reported. In the largest Phase II report, 39 out of 55 patients with steroid-refractory severe acute GVHD responded (30 complete responses) after one-to-five infusions of HLA identical, haploidentical or third party MSCs [243]. In a pilot, open-label, randomized trial of HLA-matched sibling allotransplantation with and without MSCs for prophylaxis of GVHD, promising reductions in grade II–IV acute GVHD were noted in the MSC arm (11 vs 53%), but a higher than expected relapse rate in the MSC arm raised concerns [244]. Additional clinical trials evaluating the safety and efficacy of MSCs in acute and chronic GVHD are ongoing.

Supportive care

Supportive care is critical for good outcomes after therapy for GVHD. In the setting of acute GVHD, organ-specific care includes topical emollients and wound care, including burn unit-type topical therapy if necessary. For gut GVHD, bowel rest, hyperalimentation, antimotility agents and antisecretagogues (e.g., octreotide, a somatostatin analog) are also used as necessary. Extensive gut epithelial denudation and bleeding may necessitate transfusion support, and biopsy evaluation is often necessary to confirm the diagnosis and rule out other confounding causes (e.g., viral or bacterial infections).

Infections remain a leading cause of mortality in patients with steroid-refractory acute GVHD, as well as in chronic GVHD. Standard prophylaxis includes trimethoprim–sulfamethoxazole (or equivalent agents) for *Pneumocystis jiroveci*, acyclovir for herpesvirus reactivation, and possibly antifungal agents (azoles or equivalent) for invasive fungal infection prophylaxis (e.g., *Aspergillus* spp.). Antibacterial prophylaxis with agents protective against encapsulated organisms may also be considered. Fungal monitoring with serum β-glucan and galactomannan is often utilized, particularly when prophylactic antifungal agents have not been initiated. Routine monitoring of viruses (e.g., CMV and EBV) with a plan for pre-emptive therapy if results are positive is a standard approach at many stem cell transplant centers. Immunoglobulin-replacement therapy can be useful, especially in patients with serum IgG levels lower than 400 mg/dl and a history of recurrent sinopulmonary infections. Improvements in rapid detection of incipient infections and new agents for therapy of viral, bacterial and fungal processes represent a major advance in ancillary care, and will likely improve clinical outcomes for patients with moderate or severe GVHD.

Expert commentary & five-year view

Despite significant improvements in allotransplantation methodology and supportive care, GVHD remains the major complication. Advances in chronic GVHD prevention and treatment have been especially incremental, owing at least partly to our limited understanding of its pathophysiology. Improvements in HLA typing, minimizing conditioning-related toxicity and advances in acute GVHD prophylaxis (that may supersede the combination of methotrexate plus a calcineurin inhibitor) will offer improved control of acute (and possibly chronic) GVHD, but this may be counterbalanced by the increased use of alternative (HLA-mismatched and haploidentical) donors and the treatment of older and sicker patients. Incorporating additional information predicting GVHD risk during donor and patient selection (e.g., genetic polymorphisms), if feasible in clinical practice, may be an additional way to reduce GVHD incidence.

Corticosteroids (with or without additional agents) remain the standard first-line therapy for both acute and chronic GVHD, even though response rates and patient long-term survival remains suboptimal. Improvements in supportive care can incrementally improve survival, but advances in understanding the basic biology of GVHD will also drive future improvements in

therapy. While broadly immunosuppressive pharmacologic agents will continue to be utilized, it will be important to also look beyond this at approaches designed to sidestep the toxicity of increased nospecific immunosuppresion (i.e., infection and relapse risk).

More targeted therapies, pharmacologic, biologic or cellular agents that can selectively target immune response pathways, optimize the activity or localization of immune cellular subsets and/or modulate cytokine levels in the context of GVHD, are of considerable interest in this regard. The innovative combination of pharmacologic or biologic agents targeting specific immune processes (e.g., inhibiting localization of effector T cells, blockade of specific immune effector pathways and enhancement of Tregs), as well as cellular (e.g., MSCs) and phototherapy-based approaches will be critical to get beyond the limitations of current therapy.

Key issues

- Acute and chronic graft-versus-host disease (GVHD) remain a major complication of allogneic transplantation.
- The pathophysiology of GVHD is incompletely understood, but broadly involves the interplay of factors, including tissue injury from conditioning therapy, release of proinflammatory cytokines, activation of antigen-presenting cells and alloimmune sensitization of effector T lymphocytes, that result in damage to GVHD target organs.
- GVHD prophylaxis involves improved HLA matching of donor and recipient, reduced conditioning regimen toxicity and the use of pharmacologic agents such as methotrexate and calcineurin inhibitors (that may be superseded by alternative agents such as sirolimus, a mammalian target of rapamycin inhibitor).
- Initial therapy of established GVHD, both acute and chronic, remains dependent on the use of corticosteroids, despite their limited efficacy and significant toxicity.
- Standard of care is not well established for therapy of steroid-refractory GVHD. Pharmacologic immunosuppressive agents (e.g., mycophenolate mofetil), biologic agents targeting effector immune cells (e.g., antithymocyte globulin) or proinflammatory cytokines (TNF-α blockade) have shown some efficacy, typically in early-phase studies.
- Supportive care, including symptom control and prophylaxis, early detection and effective therapy of infections remain critical for good outcomes in GVHD patients.
- Novel therapeutic approaches, such as targeting of B cells (e.g., rituximab), enhancement of regulatory T cells (e.g., extracorporeal photopheresis) and cellular therapies (e.g., mesenchymal stem cells) that avoid the toxicity of generalized immunosuppression, will likely play a prominent future role in GVHD therapy.
- Clinical trials testing novel agents (or novel combinations of agents) are critical for future advances in GVHD control.

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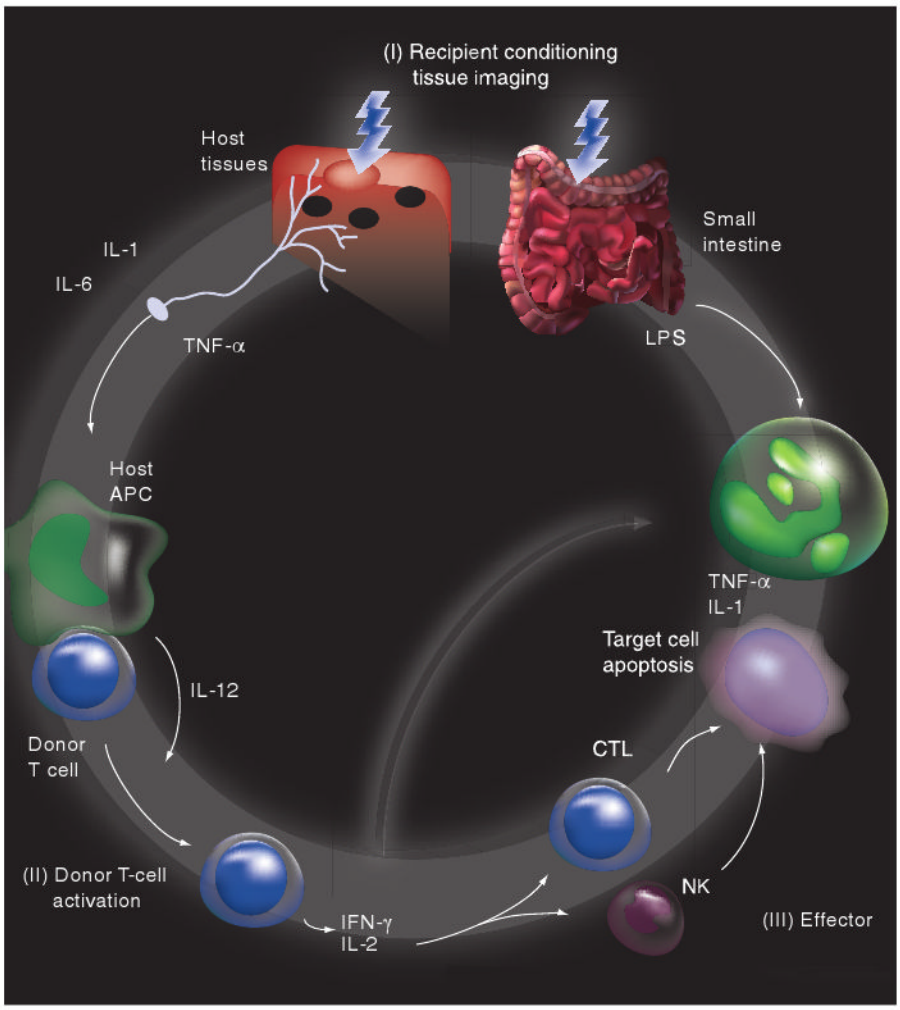

Figure 1. Etiopathogenesis of acute graft-versus-host disease
Modified with permission from [245].

Table 1
Modified Glucksberg criteria for acute graft-versus-host disease grading

Organ stage	Skin*	Liver	Gut
1	Rash < 25%	Bilirubin 2-2.9 mg/dl	Diarrhea 500-1000cc/d or biopsy-proven upper GI involvement
2	Rash 25-50%	Bilirubin 3-6 mg/dl	Diarrhea 1000-1500cc/d
3	Rash > 50%	Bilirubin 6.1-15 mg/dl	Diarrhea 1500-2000cc/d
4	Generalized erythroderma with bullae	Bilirubin > 15 mg/dl	Diarrhea > 2000 cc/d or severe abdominal pain with or without ileus
Overall grade			
I	Stage 1 or 2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2 or 3 or	Stage 2–4
IV	Stage 4 or	Stage 4	

* Use ‘rule of nines’ to determine body surface area.

Data from [1].

Table 2
Definite and probable manifestations of chronic graft-versus-host disease

Organ system	Definite manifestations of chronic GVHD	Possible manifestations of chronic GVHD
Skin	Scleroderma (superficial or fasciitis), lichen planus, vitiligo, scarring alopecia, hyperkeratosis pilaris, contractures from skin immobility, nail bed dysplasia	Eczematoid rash, dry skin, maculopapular rash, hair loss, hyperpigmentation
Mucous membranes	Lichen planus, noninfectious ulcers, corneal erosions/noninfectious conjunctivitis	Xerostomia, keratoconjunctivitis sicca
GI tract	Esophageal strictures, steatorrhea	Anorexia, malabsorption, weight loss, diarrhea, abdominal pain
Liver	None	Elevation of alkaline phosphatase, transaminitis, cholangitis, hyperbilirubinemia
GU tract	Vaginal stricture, lichen planus	Noninfectious vaginitis, vaginal atrophy
Musculoskeletal/serosa	Nonseptic arthritis, myositis, myasthenia, polyserositis, contractures from joint immobilization	Arthralgia
Hematologic	None	Thrombocytopenia, eosinophilia, autoimmune cytopenias
Lung	Bronchiolitis obliterans	Bronchiolitis obliterans with organizing pneumonia, interstitial pneumonitis

GI: Gastrointestinal; GU: Genitourinary; GVHD: Graft-versus-host disease.