Separating graft-versus-leukemia from graft-versus-host disease in allogeneic hematopoietic stem cell transplantation

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Abstract

Routine methods to maximize the graft-versus-leukemia (GvL) activity of allogeneic hematopoietic stem cell transplantation (HSCT) without the detrimental effects of graft-versus-host disease (GvHD) are lacking. Depletion or inhibition of alloreactive T cells is partially effective in preventing GvHD, but usually leads to decreased GvL activity. The current model for the pathophysiology of acute GvHD describes a series of immune pathways that lead to activation of donor T cells and inflammatory cytokines responsible for tissue damage in acute GvHD. This model does not account for how allotransplant can lead to GvL effects without GvHD, or how the initial activation of donor immune cells may lead to counter-regulatory effects that limit GvHD. In this review, we will summarize new findings that support a more complex model for the initiation of GvHD and GvL activities in allogeneic HSCT, and discuss the potential of novel strategies to enhance GvL activity of the transplant.

Keywords

allogeneic hematopoietic stem cell transplantation; cytokine; dendritic cell; donor lymphocyte infusion; graft-versus-host disease; graft-versus-leukemia; immunosuppressant; natural killer cell

The first description of graft-versus-host disease (GvHD) was made by Billingham, who documented the cytopathic effect of injecting allogeneic leukocytes into developing chicken embryos and the ‘wasting disease’ seen in neonatal mice injected with allogeneic leukocytes from unrelated strains [1]. Billingham postulated that three conditions must exist for GvHD to occur in allogeneic cell transplantation: the recipient must be immunocompromised, the donor and recipient must be antigenically different, and the donor graft must contain immunologically active effector cells [2]. Soon after Billingham’s initial report, Mathe documented hematopoietic engraftment, GvHD and a durable antileukemic effect in a patient with relapsed antilymphoblastic leukemia who received bone marrow (BM) grafts from multiple related donors [3]. More recently, the pathophysiology of acute GvHD (aGvHD) has been described by Ferrara as a three-step process of donor T-cell activation [4] consisting of:

- Pretransplant radiation/chemotherapy conditioning regimen causing tissue damage in the recipient, including breakdown of the gut epithelial barrier and release of inflammatory cytokines;
- Activation of antigen-presenting cells (APCs) and amplification of inflammatory factors;

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Donor T-cell activation and expansion.

This paradigm for the initiation of GvHD in allogeneic hematopoietic stem cell transplantation (HSCT) is usually portrayed as a sequential series of events in which initial tissue damage leads to release of lipopolysaccharide (LPS), upregulation of inflammatory cytokines, activation of APCs, activation of donor cytotoxic T lymphocytes (CTLs), and finally epithelial damage by activated T cells and cytokines that leads to increased gut permeability and additional inflammatory cytokine release, maintaining a positive-feedback cycle for aGvHD. This model for GvHD represents the conceptual underpinning for studies designed to reduce the proinflammatory cytokines that initiate aGvHD [5], including the development of the ‘nonmyeloablative’ approach [6] and the use of tumor necrosis factor (TNF) blockade early post-transplant [7]. The release of LPS into the blood and the role of inflammatory cytokines following myeloablative conditioning in the initiation of GvHD have been confirmed in murine HSCT model systems and in observational clinical studies. Data from murine models have demonstrated that myeloablative conditioning with total-body irradiation leads to tissue injury and the release of inflammatory cytokines [8–10] that enhance early host APC activation [4, 11]. Clinical data have supported this model with evidence of increased levels of LPS and TNF-α in the transplant recipients and clinical utility of drugs that block TNF significantly [12]. However, the model has significant limitations in explaining the critical immunological events that occur in allogeneic HSCT, and lacks descriptions of counter-regulatory effects that may limit the extent or severity of aGvHD, and graft-versus-leukemia (GvL) reactions that are selectively targeted to tumor.

Thus, a fundamental question in the field is whether the mechanism or effectors of GvL are fundamentally different from those of GvHD or whether GvL simply represents a subset of GvH reactions. Biologically, it has been suggested that, in the setting of transplants between human leukocyte antigen (HLA)-matched donors and recipients, GvL represents a form of alloimmune response against minor histocompatibility antigen (MiHA). In certain malignancies with clonotypic gene rearrangements that generate tumor-specific antigens (such as chronic myeloid lymphoma [CML], with t(9;22) and the neo-oncogene fusion protein, BCR-ABL), the existence of tumor antigen-specific donor T cells has been demonstrated, and the frequencies of cytotoxic antitumor specific T cells has been shown to increase with clinical regression of malignancy post-transplant [13,14]. In many murine model systems [8,15] and in the clinical setting of allogeneic HSCT following reduced-intensity conditioning [16], GvL appears to be a result of donor cytotoxic effectors directed against host hematolymphoid cells. In most allo geneic transplants, GvL has been seen as part of the spectrum of GvH effects, usually characterized by a predominance of antigen-specific cellular immune responses directed against cells of the hematopoietic lineages. However, a striking exception to this generalization has been the clinical regression of metastatic renal cell carcinoma following withdrawal of immunosuppressive drugs after a reduced-intensity conditioning regimen and allogeneic HSCT [17]. GvL and graft-versus-tumor (GvT) immune responses can be associated with the development of either aGvHD or chronic GvHD (cGvHD) [18], although the strength of the statistical association of GvL with GvHD is greatest with cGvHD [19]. The basis for the greater association of GvL with cGvHD may be the clinical observation that aGvHD represents a more generalized state of donor-derived inflammation, with non specific tissue damage mediated by activated donor effector cells and the local release of inflammatory cytokines. By contrast, cGvHD represents more antigen-specific cellular and humoral immune responses directed against specific epithelial tissues and host-type hematopoietic cells. Thus, fundamental distinctions between GvL and aGvHD are the difference between antigen-specific and antigen-nonspecific immunity, and the difference between cellular (antigen specific) immunity directed toward hematopoietic targets versus broad (nonspecific) immune responses directed against epithelial targets (Figure 1).
This review will highlight new information from preclinical model systems and evolving clinical practices that describe the possibility of selectively activating GvH reactions that may result in GvL without increasing the risk of severe GvHD. We will also discuss the role of counter-regulatory immune effects that limit GvH reactions, including the role for donor dendritic cells (DCs) in modulating GvHD, graft rejection and GvL activities, and new strategies to enhance GvL through vaccination (Figure 1 & Table 1).

**Modifying/selecting T cells**

**Depleting T cells from the graft**

One initial strategy to separate GvL from GvHD was to deplete or impair the function of donor T cells in the graft using anti-T-cell antisera [20–22], but randomized clinical trials showed no overall therapeutic advantage of this method of nonspecific T-cell depletion (TCD) compared with control patients who received T-cell-replete grafts [23–25]. Newer technologies for HSC graft engineering have used monoclonal antibodies to reduce the number of T cells in allogeneic HSC grafts (TCD). Recipients of TCD allogeneic grafts experience a lower incidence of both aGvHD and cGvHD but a higher risk of opportunistic infection and post-transplant relapse [24,26]. Another strategy for T-cell reduction is positive selection of CD34+ hematopoietic stem cells from the graft. However, a randomized clinical trial of CD34+ cell selection in allogeneic HSC from HLA-identical related donors showed an increased incidence of relapse in recipients of CD34+ selected grafts compared with patients who received the nonselected grafts, with no survival benefit to recipients of CD34+ selected grafts [27]. Thus, neither TCD using antibodies with broad specificity against all T cells, nor CD34+ HSC selection methods, appear to be successful strategies to separate GvL from GvHD. Champlin has compared TCD with different methods (narrow-specificity and broad-specificity antibodies, CAMPATH antibodies, elutriation and lectins/sheep erythrocytes) and noted that different methods of TCD result in different outcomes in allo geneic HSCT. Recipients of grafts T-cell depleted using narrow-specificity antibodies have higher leukemia-free survival than recipients of grafts that were T-cell depleted using broad-specificity antibodies [28].

**Delayed administration of allogeneic T cells**

Recipients of TCD grafts can achieve better immune reconstitution if they receive a subsequent donor lymphocyte infusion (DLI). The rationale for delayed DLI is to avoid the generalized inflammatory state present in the first weeks following conditioning that leads to activation of host DCs and upregulation of MHC-II expression on host epithelial cells and can increase the severity of aGvHD [10,29]. Recipients of a DLI scheduled at later times post-transplant have fewer remaining host DCs and lower levels of inflammatory cytokines [4,30], a condition that should lead to lower levels of donor T-cell activation. Data from MiHA-incompatible mouse HSCT model systems showed that delayed DLI preserved the GvL effect of allogeneic transplant with a reduced risk of GvHD [15,31], but equivalent protection from GvHD was not seen in murine HSCT across MHC disparities [32] or in clinical transplant protocols in humans utilizing prophylactic administration of small numbers of donor T cells 1–2 months post-transplant [33,34]. In murine transplant models, recipients had more GvL activity when DLI was administered 3 weeks post-transplant compared with recipients of DLI administered 12 weeks post-transplant [35], and DLI given to lymphopenic recipients caused more GvHD than in nonlymphopenic recipients [36], indicating that the balance between GvHD and GvL using DLI is complex. Prophylactic DLI has not resulted in consistent separation of GvL from GvHD, particularly in patients who might benefit most, those with advanced malignancies such as acute leukemia and myeloma who require higher doses of donor T cells for disease control and have a greater risk for post-DLI GvHD [37].
DLI to treat relapse

While prophylactic DLI has not gained widespread use in nonrelapsed HSCT recipients, post-transplant DLI is very effective in treatment of relapsed slow-growing hematopoietic malignancies such as CML or low-grade non-Hodgkin lymphoma [38,39]. One strategy to reduce the risk of GvHD, myelosuppression and DLI-related mortality in patients with relapsed CML has been to begin with a low dose of DLI and then to escalate the dose of T cells for subsequent DLI on a planned schedule. In a clinical study of DLI in patients with CML, recipients of escalating DLI (median total dose: 1.9 × 10^8/kg, range 0.01–3.3 × 10^8/kg) had a 10% incidence of GvHD compared with 44% incidence among recipients of a single administration of an equivalent number of donor lymphocytes (median dose: 1.5 × 10^8/kg, range doses: 0.6–5.3 × 10^8/kg) [40]. This study and others have suggested the use of initial doses of DLI not exceeding 0.2 × 10^8 mononuclear cells/kg [41], or T-cell doses of 0.1 × 10^8 cells/kg from a matched-related donor for patients with low-grade hematological malignancies relapsed after allogeneic transplantation. In the absence of GvHD or complete response of the relapsed malignancy, subsequent escalating doses of DLI are given until clinical response or the development of GvHD [42]. Clinical studies of DLI have demonstrated that intra-individual dose escalation of DLI in patients with relapsed CML is safer, and equally effective in terms of a GvL effect as bolus administration of an equivalent number of donor lymphocytes [42,43]. In relapsed CML, the alloreactivity and GvHD activity of DLI from matched-related donor and recipients of grafts from HLA-matched volunteer unrelated donors were similar [44]. For other hematological malignancies in which the fraction of proliferating malignant cells is greater than in CML, including acute leukemia or high-grade lymphomas, the efficacy of DLI to treat relapse is much less and cytoreductive chemotherapy is often required for initial disease control, releasing inflammatory cytokines and increasing the risk of GvHD [45]. Overall, the hazard risk for adverse events including GvHD is worse for recipients of volunteer unrelated donor transplants than for sibling transplants, possibly due to the effect of allele-level mismatches that were not recognized when the patients were transplanted [16,46]. In the setting of recipients of grafts from haploidentical, partially HLA-matched donors, a median T-cell dose of 3 × 10^4 cells/kg (range 1 × 10^4 to 11 × 10^4 cells/kg) has been used [47,48], although overall risks of GvHD following DLI for HLA-mismatched donor—recipient pairs are much higher at equivalent donor T-cell doses than in the setting of HLA-matched donors [49]. When DLI from HLA-mismatched donors is given prophylactically or in the setting of relapse, concomitant administration of immunosuppressive drugs may prevent severe aGvHD while retaining GvL activity [50]. A recent report showed that infusion of recipient-type T cells in recipients of nonmyeloablative conditioning with mixed chimerism induced GvL and no GvHD [51]. The mechanisms for this selective GvL effect have not been addressed, but may include release of Th1/Tc1 cytokines by the recipient-type T cells. Thus, DLI may be effective in the setting of relapse, but the balance between GvL and GvHD effects is unpredictable.

Elimination or reduction of alloreactive T cells

Several approaches to reduce GvHD have focused on reducing the alloreactive T-cell component of the graft, by physically removing activated T cells [52–54], eliminating activated T cells by incorporation of 4,5-dibromorhodamine 123 and exposure to light [55], tolerizing donor T cells against host-type alloantigen [8,56], passively eliminating alloreactive T cells in mixed lympho cyte cultures with ‘third-party’ stimulators [57], or culturing with IL-2 and anti-CD3 to generate activated but ‘nonalloreactive’ donor T cells [58]. None of these approaches has been adopted as part of routine clinical practice due to incomplete ability to separate GvL from GvHD. Recent data using photodynamic purging of alloreactive T cells look promising as a method to enhance cellular immune reconstitution in recipients of T-cell-depleted allografts from haploidentical donors, without increasing aGvHD [59]. However, recipients of photodynamic-treated T cells did develop cGvHD, and the long-term antileukemic activity of
treated T cells has yet to be established [55,60]. Transduction of donor T cells with a viral thymidine kinase ‘suicide gene’ allows some post-DLI control of GvHD by targeting the genetically modified donor T cells with gancyclovir [61–63]. This approach is limited though by lack of uniform transduction frequencies, immune responses to the thymidine kinase gene product, and reduced alloreactivity of T cells after culture [64,65].

Selecting memory T cells

Recently, several lines of investigation in murine transplantation models have shown that donor T cells with a naive phenotype coexpressing CD62L and CD45RA or CD62L and CCR2 have higher GvHD activity compared with memory T cells. Unfractionated or naive CD4 T cells cause GvHD in a MiHA-incompatible murine transplant system, and fluorescence-activated cell sorting (FACS)-purified CD4+CD44+CD62L− memory CD4 T cells do not induce GvHD [66,67]. Similar results have been reported using a MHC-mismatched HSCT model, demonstrating that transfer of unseparated allogeneic CD3 T cells or immunomagnetic-activated cell sorting (MACS)-purified CD3+CD62L+ naive T cells resulted in rapid death from GvHD, while transfer of MACS-purified CD3+CD62L− memory T cells resulted in 100% survival without repetitive GvHD [68]. In our allogeneic murine HSCT models, ex vivo fludarabine treatment of donor T cells led to selective depletion of the CD4+CD44low naive T-cell subset and reduced the GvHD activity of DLI while retaining GvL effects [29,69]. Follow-up experiments using FACS-purified donor T-cell subsets showed that CD4+ CD44low naive T cells cause GvHD while CD4+CD44high memory T cells do not [70]. Based upon the simplicity of a short (24 h) ex vivo exposure to fludarabine as a novel method to deplete naive T cells from DLI, we are initiating a Phase I clinical trial of this approach in recipients of allogeneic HSC with post-transplant poor indices of impaired cellular immune reconstitution. Results of continuing studies will be of interest as novel methods are developed to separate GvHD from GvL based upon ex vivo ‘purging’ of allo reactive T cells or those with greater inflammatory potential.

Enhancing activated γδ T cells

γδ T cells are a unique and minor T-cell subset that are distinct from conventional αβ T cells. Besides producing IFN-γ and TNF cytokines [71], γδ T cells express killer cell immunoglobulin-like receptor (KIR) CD94/NKG2, similar to natural killer (NK) cells, and directly kill targets through cell—cell contact [72]. γδ T cells are enriched in epithelial tissues (gastrointestinal tract and skin, among others), and contribute to pathogen-specific immune responses [73]. Defects in γδ T cells lead to organ-specific immunopathology or increased risk of infections [73,74]. γδ T cells support the maturation of DCs through direct cell—cell contact and through TNF-α synthesis by γδ T cells [75]. Allografts from donors deficient in γδ T cells result in reduced rates of GvHD and decreased T-cell activation in murine MHC-mismatched models [76]. Patients who received higher numbers of donor γδ T cells had a higher incidence of grade II—IV aGvHD (66 vs 40%) [77]. In clinical transplantation, the incidence of aGVHD in recipients of allogeneic blood HSC grafts from unrelated donors was significantly correlated with the graft content of γδ T cells [77].

While ex vivo-activated γδ T cells facilitated donor stem cell engraftment without causing GvHD [78], nonstimulated donor-type γδ T cells were ineffective in protection from graft rejection. The graft facilitation conferred by activated donor γδ T cells was independent of Fas ligand (FasL) or perforin expression and could be achieved by multiple infusions of low doses of γδ T cells, suggesting that the continued presence of γδ T cells in vivo was important for promoting alloengraftment [79]. Clinical data from recipients of allogeneic BM grafts depleted of αβ+ T cells have shown that 5-year leukemia-free survival was significantly higher among patients with increased numbers of γδ T cells measured in the blood at day 60 post-transplant compared with patients with normal or decreased numbers of γδ T cells (54.4 vs 19.1% and
70.8 vs 19.6%, respectively) [80]. Of note, patients who received higher numbers of donor γδ T cells did not have a higher incidence of aGvHD [80]. These contradictory results on the GvHD activity of γδ T cells indicate that interaction of γδ T cells with conventional T cells in the graft and the activation status of γδ T cells may be critical for their ability to augment GvL and facilitate engraftment without causing GvHD [78].

Selecting regulatory T cells

In human and animal models, a specific sub-population of regulatory T cells (Tregs) has been identified, which is characterized by coexpression of CD4, CD25 and the transcription factor Foxp3 [81]. Tregs constitute 5–10% of peripheral CD4+ T cells in the blood of mice and 1–2% in humans, and control the development of autoimmunity and transplant rejection [82]. Since intracellular staining for Foxp3 expression must be performed on fixed/permeabilized cells, any experiments with viable purified Tregs must use the broader CD4+CD25+ phenotype. Human CD4+CD25+ Tregs in the blood can suppress mixed leukocyte reactions [83], and murine donor CD4+CD25+ Tregs prevent aGvHD. Donor CD4+CD25+ Tregs can suppress alloreactive donor CD4+CD25+ T cells and the donor Treg content of the graft is correlated with the development of GvHD [84–86]. While donor CD4+CD25+ Tregs inhibit alloreactive donor T cells that cause GvHD, the GvL effect of the allograft was not inhibited in murine models [87–89]. In addition, there is evidence that the Treg-specific transcription factor Foxp3 is reduced in patients with both aGvHD and cGvHD [90]. However, the relationship between the number of CD4+CD25+ Tregs in the blood and the onset of cGvHD is controversial, with some reports showing that reduced numbers of Foxp3+ or CD4+CD25+ Tregs are associated with cGvHD [91], while other reports indicate that higher absolute numbers of circulating CD4+CD25+ Tregs are found in patients with cGvHD following allogeneic HSCT [92]. Technical issues in accurately phenotyping CD4+CD25+ Tregs may contribute to this uncertainty [93]. A recent report shows that the FOXP3 promoter is demethylated in suppressive Tregs [93]. This may be useful in monitoring Tregs in clinical settings, and suggests an intriguing approach of enhancing Treg function through hypomethylating drugs. Efforts to expand antigen-specific Tregs ex vivo have used exogenously added IL-2 and stimulation through CD3 and CD28 in order to generate large numbers of cells for use as adoptive cell therapy. Infusion of ex vivo-activated and -expanded donor CD4+CD25+ Tregs can inhibit GvHD, while ex vivo depletion of CD4+CD25+ Tregs from the graft, or in vivo CD25 depletion of recipient mice before HSCT, enhances GvHD in the recipients [94]. Thus, adoptive transfer of functional Tregs in patients with cGvHD is an attractive approach that is currently being tested in the clinic at a few academic centers [95]. The widespread application of this technology will be limited by the need to both select and expand the relatively rare Tregs ex vivo. Of note, most immunosuppressive medications inhibit all T-cell subpopulations including Treg, except sirolimus, which does not cause significant suppression of Tregs [96]. In addition, treatment of isolated leukocytes with extracorporeal photopheresis (cells incubated with 8-methoxypsoralen and exposed with UVA radiation) lead to increased levels of Tregs and reduced the incidence and severity of GvHD in murine models [97].

Modifying/selecting other cells in graft

Donor sources

A number of different sources of hematopoietic stem cells have been successfully used in a llogeneic HSCT, with grafts obtained from donor BM, G-CSF-mobilized blood, and umbilical cord blood (UCB) from both related and unrelated donors [98]. Overall, the GvL activities of these three different graft sources appear to be similar in adults [99–103]. In pediatric transplants, BM-HSCT and peripheral blood-HSCT are similar with regard to risk of aGVHD, but BM-HSCT is associated with lower risks of cGVHD, mortality and cancer relapse [98]. In a meta-analysis of pediatric studies, there were no differences in grade III—IV aGvHD or 2-
year overall survival when UCB-HSCT was compared with matched unrelated donor BM-HSCT, but cGVHD was decreased in children receiving UCB-HSCT [104]. Selection of a specific source for a hematopoietic allograft is unlikely to have a major effect on the balance between GvL and GvHD in adult patients. An ongoing randomized clinical trial (BMT CTN 0201) is comparing donor and patient outcomes among donor—recipient pairs randomized to either BM or G-CSF-mobilized peripheral blood transplants. Initial review of outcome data from the study will be available in 2011.

**Selecting natural killer cells**

The non MHC-restricted ability of donor NK cells to kill allogeneic tumor and host APCs in HLA-mismatched [11,105,106] and HLA-matched [107] transplant recipients has been demonstrated to decrease the incidence of tumor relapse as well as aGvHD in preclinical models and clinical HSCT. The immunological activities of NK cells are regulated by cell surface polymorphic proteins known as KIRs that consist of both inhibitory receptors (e.g., inhibitory KIR and CD94/NKG2A) and activating receptors (e.g., activating KIR, CD94/NKG2C and NKG2D). Inhibitory KIRs recognize and engage their ligands — MHC-I molecules on the surface of target cells — and initiate inhibitory signals that block NK activation and cytotoxicity. By contrast, activating receptors bind ligands on the surface of the target cells and trigger NK cell activation, which causes lysis of target cells [108,109]. Transplants with related donors that share only a single haplotype (haploidentical donors) may have potent NK alloreactivity based upon MHC-I and KIR gene mismatches [110].

Clinically measurable NK alloreactivity is inversely proportional to the presence of donor T cells, and the lack of extensive T-cell depletion in haploidentical transplantation is associated with high GvHD rates that diminish the potential benefits from graft-derived NK-cell alloreactivity [11]. Therefore, use of donor NK cells to prevent tumor relapse without GvHD in the context of TCD allografts may provide a novel mechanism to enhance GvL without GvHD [111].

**Manipulating DCs & DC subsets**

Much current research using preclinical model systems has focused on the role of host-type APCs in the initiation of GvHD [15,112,113]. Host APCs have been shown to be required for CD8+ T-cell-dependent GvHD in a MiHA-mismatched murine model of HSCT [4,112] and the initiation of CD4+ T-cell-dependent GvHD [114]. The role of donor DCs is controversial, as some reports, utilizing a rapidly growing and p210-transfected tumor, have not shown a role for donor DCs in GvL, while other studies, utilizing the same transplant model system but different tumor cell lines, have shown a modest ability of donor DCs to augment GvL activities [4].

**Selecting donor DC subsets to augment GvL activity**—Donor APCs may increase the severity of CD8+ T-cell-dependent GvHD, supporting the idea that both donor and host APCs may modulate alloreactivity of donor T cells in allogeneic HSCT [115]. Our own recent data have shown that selective depletion of CD11b+ donor DC subsets from BM or the addition of FACS-purified CD11b− donor DCs to purified donor HSCs and T cells results in enhanced GvL effects without increased GvHD [116].

**Manipulating donor DCs in graft rejection**—Recently, the Blazar group described the effect of CpG oligonucleotide ligands for Toll-like-receptor (TLR)9 on host and donor APCs in a murine model of allogeneic HSCT [117]. Administration of CpG-activated donor and host DCs resulted in enhanced rates of graft rejection and accelerated GvHD [117]. In a murine transplant model, using nonmyeloablative doses of busulfan, we have observed that mixed chimerism and tolerance were induced with costimulatory blockade (anti-CD40 ligand
monoclonal antibody) [118]. Pretransplant administration of either viable donor splenocytes or purified T cells during costimulatory blockade enhanced bidirectional host-versus-graft and GvH tolerance [118]. The addition of apoptotic (irradiated) donor cells activated host APCs and triggered graft rejection in this model. By contrast, pre-transplant administration of FACS-purified donor CD11b+ BM APCs led to increased anti-inflammatory cytokines and enhanced donor engraftment, while the administration of donor CD11b− BM APCs led to activation of host T cells and decreased donor engraftment. These data are consistent with an effect of the Th1 cytokines, particularly IFN-γ, in augmenting alloreactivity following non myeloablative conditioning and allogeneic HSCT [119]. In murine myeloablative allogeneic HSCT, donor BM plasmacytoid (CD11b−) DCs polarized donor T cells toward Th1/Tc1 immunity and enhanced GvL activity without increasing GvHD, while donor BM myeloid (CD11b+) DCs skewed donor T cells toward Th2/Tc2 immunity and decreased GvHD and GvL activity [Waller EK et al., Unpublished Data]. These data support the role of donor DCs in GvH and GvL activity in allogeneic HSCT and are in contrast to the dominant viewpoint that stresses the importance of host DCs rather than donor DCs in allogeneic transplantation (Figures 1 & 2) [120].

Selecting inhibitory DC populations in GvH—Another recently described DC population is the tolerogenic DCs or regulatory DCs that suppress Th1/Tc1 immune responses through higher levels of IL-10 and TGF-β secretion coupled with reduced secretion of proinflammatory cytokines (IL-1β, IL-6, TNF-α and IL-12p70) [121–124]. Distinct subsets of mature splenic DCs from mice have been shown to have intrinsic tolerizing abilities via IL-10 production and have a critical role in peripheral immune tolerance by limiting the activity of autoreactive T cells [125–127]. CD8α+ CD4+ myeloid tolerogenic splenic DCs suppressed IFN-γ production of pathogenic Th1 cells and mitigated experimental autoimmune encephalomyelitis [128], consistent with our results using CD11b+ myeloid DCs in which decreased Th1 polarization was seen in a logogeneic transplant recipients [129].

The development of tolerogenic DCs from immature DCs requires IL-10 and TGF-β [130] or vasoactive intestinal peptide (VIP) [131] in vivo. DCs conditioned ex vivo with VIP expressed low levels of costimulatory molecules and induced anergic Tr1-like cells. We have shown that allogeneic HSCT using VIP-knockout (VIP-KO) donor mice resulted in increased GvHD severity and higher numbers of antigen-specific antiviral T cells following allogeneic HSCT and post-transplant challenge with murine cytomegalovirus [132 & Waller EK et al., Unpublished Data]. These data indicate that VIP probably promotes the generation of tolerogenic DCs in vivo, but the anti-tumor effect of exogenous VIP or the use of BM or T cells from VIP-KO donor mice is unknown.

Expression of indoleamine 2,3-dioxygenase (IDO) by DCs is another tolerogenic mechanism that may regulate the GvHD activity of allogeneic T cells. IDO is an immunomodulatory enzyme secreted by immune cells that catalyzes the conversion of L-tryptophan to N-formylkynurenine, and could lead to the suppression of T-cell proliferation [133]. DCs expressing IDO suppress T-cell immune responses, indicating that these cells may represent a regulatory subset of DCs [134]. Human CD123+ regulatory DCs constitutively express IDO, which is also upregulated following stimulation with IFN-γ [135] or LPS [136,137]. Furthermore, mouse DCs induced by CD4+CD25+ Treg cells express IDO and suppress immune responses [138,139]. Tolerogenic DCs express signaling lymphocyte activation molmolecule (SLAM) and programmed death-1 ligand (PD-L1). The interaction of PD-L1 with PD-1 expressed on activated T- and B-lymphocytes leads to their apoptosis and inhibition of T- and B-cell responses. In addition, tolerogenic DCs express CD205, an antigen-uptake/processing receptor, and immunoglobulin-like transcript (ILT)3/ILT4, an inhibitory receptor of the ILT family that leads to Treg development and suppresses T-cell immune responses [121,123,139–141]. In mice, B220+ plasma DCs, CD8α+ splenic DCs and CD19+ plasma DCs
contained within tumor-draining lymph nodes express IDO and inhibit anti tumor immune responses [140,141]. In humans, CD123^+CCR6^+ DCs acquired IDO functional activity after activation by IFN-γ or prostaglandin E_2, or following CD80/CD86 ligation by cytotoxic T-lymphocyte antigen (CTLA)4 expressed on Tregs [142,143]. PDL-1 expressed on DCs acts as a strong inhibitor of CD4 T-cell activation and helps maintain peripheral tolerance [144–146]. Thus, the use of IDO^+ DCs may be very significant as a novel strategy to limit GvHD, but the effect of IDO production by donor DCs on the GvL activity of the allograft is unknown.

**Donor mesenchymal stem cells**—Bone marrow-derived stromal progenitors, termed ‘mesenchymal stem cells’ (MSCs) are easily expanded *in vitro* from BM samples by culturing undifferentiated adherent progenitor cells that retain the ability to selectively differentiate along multiple mesenchymal lineages in response to specific signals [147]. MSCs have been used in preclinical murine [148] and primate [149] transplant models and in early-phase clinical trials [148,149] as a method of delivering cells with immunomodulatory and immunosuppressive activity to sites of tissue injury, including sites of aGvHD. Administration of MSCs at the time of allogeneic HSCT or following the development of aGvHD has been reported to reduce the incidence or severity of GvHD, respectively, potentially through local release of IL-10 [150], IFN-γ [151], or the secretion of metalloprotease-cleaved antagonistic forms of chemokines [152], although other reports indicate an absence of protection from GvHD using murine models [153]. MSCs from normal volunteer donors can be expanded *in vitro*, cryopreserved and administered to transplant recipients who develop aGvHD [154] and patients with steroid-refractory grades III—IV GVHD [155]; their clinical efficacy in this setting is currently being studied through ongoing randomized, placebo-controlled clinical trials. Prophylactic administration of MSCs has been associated with reduced risk of aGvHD in allogeneic HSCT, but also with an increased risk of disease relapse [156], suggesting that the immunosuppressive effects of this novel form of adoptive cell therapy may not selectively target GvHD over GvL activities. Broad clinical adoption of MSC into clinical practice will depend upon the results of current clinical trials.

**Pharmacological regulation of alloreactivity**

**Immunosuppressants**

Pharmacological prophylaxis against aGvHD is applied in nearly all patients undergoing allogeneic HSCT with T-cell-replete grafts. Prophylaxis is effective in preventing the development of aGvHD in 50–70% of patients treated with allogeneic grafts from HLA-matched donors, but still allows for clinically significant GvL effects [157]. Polyclonal anti-thymocyte globulin (ATG) as a part of the conditioning regimen with total body irradiation or busulfan in allogeneic HSCT accompanied by ciclosporin A and a short-course of methotrexate, significantly reduced aGvHD [23], but a beneficial effect of ATG treatment on overall survival has not been seen in clinical trials [158]. To control aGvHD, or to suppress host alloantigen recognition by the donor T cells, four classes of drugs are commonly used: glucocorticoids, calcineurin inhibitors, mTOR inhibitors and antimetabolites. Although they are effective as single agents, combinations of drugs are usually administered in clinical transplantation. For example, ciclosporin and tacrolimus, calcineurin inhibitors that inhibit the gene transcription of IL-2, IL-3, IFN-γ and other factors produced by antigen-stimulated T cells [159,160], are used along with methotrexate (MTX) as a standard prophylactic regimen to prevent aGvHD after allogeneic HSCT [161]. While combining multiple drugs as prophylaxis (e.g., the combination of ciclosporin, MTX and prednisolone (CSA/MTX/Pred) in allogeneic HSCT transplants delays the onset of early aGvHD in allogeneic transplant recipients, these more intensive prophylactic regimens have not led to an overall decrease in the incidence of GvHD or improved overall survival [162]. Sirolimus (rapamycin), a mTOR inhibitor, has also been used effectively alone and in combination with other immunosuppressants (corticosteroids,
ciclosporin, tacrolimus and mycophenolate mofetil) as a prophylactic medication and to treat steroid-refractory acute and cGvHD in HSCT recipients [163,164]. Effects of such drug combinations on DCs include inhibition of DC differentiation and maturation, reduction of costimulatory molecules and IL-12, reduced allostimulatory capacity, and inhibition of nuclear factor (NF)-κB activity [165].

Reducing the dosage of prophylaxis for GvHD should theoretically reduce the risk of relapse and toxicity after allogeneic HSCT, but prospective clinical trails have not demonstrated an overall survival advantage associated with this strategy due to increased GvHD-related mortality [166]. Therefore, adjusting the doses of drug combinations of calcineurin inhibitors, anti-metabolites, and steroids as GvHD prophylaxis is unlikely to have a major effect on enhancing the GvL activity of an allogeneic HSCT graft without producing concomitant effects on GvHD. Of interest, sirolimus has direct anti-tumor activity against lymphoid malignancies [167–169] and has been also shown to stimulate the generation of Tregs [170]. The clinical advantage of sirolimus as a prophylactic drug compared with a calcineurin inhibitor is being tested in prospective clinical trials in pediatric patients with acute lymphoblastic leukemia and in adult patients with lymphoma.

Histone deacytelase inhibitor

Another novel agent, the histone deacetylase (HDAC) inhibitor suberoylanilide hydroxamic acid (SAHA), has potential for separating GvL effects from GvHD. SAHA binds to the catalytic site of HDAC6 and leads to reversible enzyme inhibition [171]. SAHA in nanomolar concentrations decreases the production of TNF-α, IFN-β, IL-1β and IL-12 by non-malignant cells [172]. Administration of SAHA reduced secretion of inflammatory cytokines and reduced GvHD without suppressing the GvL activity of donor CTLs [173,174]. In addition, SAHA in micromolar concentrations has direct antitumor activity, inducing cell-cycle arrest and apoptosis in tumor cells by selectively suppressing (e.g., cyclin A) and increasing (e.g., caspase 9) expression of several genes in tumor cells [175,176].

Cytokines

Th1 cytokines, including IL-1, IL-6 and TNF-α produced by monocytes/macrophages, and IFN-γ and IL-2 produced by CD4 Th1/CD8 Tc1 cells, are involved in the development of aGvHD, while Th2 cytokines including IL-4, IL-5 and IL-10 produced by CD4 Th2/CD8 Tc2 cells appear to be more important in the process of cGvHD [177,178]. Regulating or controlling these cytokines could potentially inhibit the development or reduce the severity of aGvHD or cGvHD. The effect of inhibiting specific cytokines on GvL is, however, unknown (Figure 2).

Blocking pathways of inflammatory cytokines & Th1/Tc1 cytokines—The release of inflammatory cytokines following tissue damage from the conditioning regimen is central to the Ferrara model of aGvHD of GvHD, but experimental model systems have not consistently shown that blocking or inhibiting these cytokines reduces the incidence or severity of GvHD. Inhibition of IL-1 activity with IL-1R antagonist (IL-1Ra) decreased steroid-resistant GvHD [179], but IL-1Ra during conditioning and 10 days immediately after transplantation was not sufficient to reduce GvHD [180]. The role of TNF in the initiation of GvHD is supported by clinical data showing that patients with higher levels of TNF-α release in their blood during conditioning had a higher incidence (90%) of grade II—IV aGvHD and higher mortality (70%) [181,182]. Blocking TNF-α and IL-1 by administering the combination of dimeric human TNF-α receptor Fc fusion protein and hamster antimouse IL-1 receptor after allo genetic SCT significantly decreased or prevented the progression of liver GvHD in murine models [113,183], and protected against skin and gastrointestinal injury [184]. Pharmacological suppression of soluble TNF reduced GvHD morbidity and mortality without affecting the activation and expansion of donor T cells and GvL activity in a MiHA-mismatched
Regulating Th1/Tc1 & Th2/Tc2 cytokines in the initiation & augmentation of GvHD & GvL—Donor Th1/Tc1 responses are generally required for antitumor immune responses after allogeneic HSCT [192–196], although some tumors may be sensitive to both Th1/Tc1 and Th2/Tc2 immune responses [197]. The interest in clinical administration of cytokines to augment GvL effects has been tempered by concerns that exogenous inflammatory cytokines will induce or aggravate aGvHD. While IFN-γ is a prototypic Th1 cytokine [198], and is central in the Ferrara model of GvHD in the pathway of donor T-cell activation, blockade of IFN-γ has not been successful to prevent or treat GvHD. In nonmyeloablative mouse transplant models, the administration of IFN-γ accelerated GvHD, and use of donor T cells from IFN-γ KO mice resulted in less GvHD [119,199]. However, a similar role for IFN-γ has not been observed in the setting of myeloablative conditioning. In model systems that utilize myeloablative doses of irradiation (leading to tissue injury and release of inflammatory mediators), administering exogenous IFN-γ led to donor T-cell tolerance towards host alloantigens, and reduced aGvHD [119,200,201]. Moreover, IFN-γ produced by allogeneic specific Treg cells maintains the regulatory function [202]. Furthermore, IFN-γ may decrease severity of GvHD through suppression of Th17 [203] and induce allreactive T-cell apoptosis [204]. Post-transplant administration of IL-12 or IL-18, cytokines that induce IFN-γ, protected lethally irradiated recipients against GvHD in a Fas-dependent fashion [205–207]. Significantly, administering high doses of IL-2 immediately after HSCT also protected animals from GvHD mortality [208,209]. Paradoxically, the protective effect of IL-2 may be mediated by suppression of IFN-γ [200]. In addition, recent murine allogeneic transplant experiments showed that IFN-γ suppresses Th17 immune/inflammatory responses and decreases the severity of GvHD [203,210].

Role of Th2/Tc2 cytokines in GvL & GvHD—In contrast to the GvL effect associated with Th1/Tc1 cytokines, Th2/Tc2 cytokines have been implicated as part of the pathophysiology of cGvHD and may inhibit the development of aGvHD. Th2 cytokines (IL-4, IL-5, IL-10 and TGF-β) have been shown to be upregulated in an experimental model system and in patients with cGvHD [211–213], but Th2/Tc2-biased immune responses have been associated with a lack of protective immunity against malignancy [213,214]. Administration of IL-4 to donor mice skewed CD4+ T cells towards a Th2 phenotype and resulted in a decreased incidence of lethal GvHD compared with control mice that received unmanipulated donor T cells [215,216]. Human studies showed that an elevated precursor frequency of IL-4-secreting alloreactive donor T cells is associated with decreased GvHD [217]. On the other hand, transplantation of IL-4 KO T cells reduced GvHD in murine models [218–220]. IL-10, another Th2 cytokine, may modulate GvHD activity through suppression of IL-12 production and expression of costimulatory molecules by DCs [221,222]. IL-10 induced apoptosis of freshly isolated or cultured plasmacytoid DCs [223] and inhibited inflammatory cytokine (IL-1, IL-6 and TNF) production by activated monocytes/macrophages.
and proinflammatory chemokine (Mip-1α, -1β, -3α, -3β, IL-8, IP-10 and Mip-2) production by activated monocytes [228–230]. However, IL-10 administration in murine model systems did not cause decreased GvHD [231]. In humans, the level of IL-10 in blood correlated with the severity of GvHD [232]. The correlation of serum IL-10 levels and GvHD notwithstanding, systemic administration of IL-4 or IL-10 as experimental prophylaxis of GvHD was either inefficient or toxic [233–235]. Thus, administration of Th2 cytokines has not been an effective strategy to reserve GvL activity while reducing GvHD.

Enhancing IL-11 & keratinocyte growth factor—IL-11 and keratinocyte growth factor (KGF) may protect against conditioning-related tissue damage and enhance wound repair, and have been used in allogeneic HSCT to reduce conditioning-related toxicity and increase thrombopoiesis [236,237]. IL-11 administration decreased GvHD severity in allogeneic HSCT by diminishing conditioning-induced gut injury and prohibiting the release of LPS into the systemic circulation, while preserving perforin-dependent GvL activity [238–240]. IL-11 after HSCT also polarized donor T cells toward Th2/Tc2 development in vivo, subsequently decreasing Th1/Tc1 cytokine production by T cells and IL-12 secretion by DCs, as well as decreasing systemic TNF-α levels and GvHD severity [241]. Clinical administration of IL-11 in allogeneic transplant recipients has been limited by multiorgan toxicity of the commercial agent [242].

Keratinocyte growth factor reduced GvHD and reduced weight loss and dermatitis in mice following nonmyeloablative transplantation utilizing low-dose total-body irradiation and cyclophosphamide-based chemotherapy as the conditioning regimen [243]. In a Phase I clinical trial in allogeneic HSCT recipients, KGF reduced the incidence and severity of mucositis in patients, but had no significant effect on engraftment, aGvHD, cGvHD or survival [5,244].

Mitigating the effect of IL-17 on GvHD—IL-17 is a proinflammatory cytokine that enhances the synthesis of chemokines, matrix metalloproteases and other inflammatory cytokines (IL-6) [245]. Although a number of cell types synthesize IL-17, including CD8 [246], γδ T cells [247] and NK cells [247], Th17 CD4+ T cells represent a unique regulatory subset that is generated from Th0 cells. Th17 CD4 T cells are polarized to synthesize IL-17, IL-21, IL-22 and TNF-α under conditions of low levels of IL-12 and high levels of IL-23 and other inflammatory signals [248–251], and require IL-23 for stabilized development [252]. Data from murine studies show that Th17 cells are involved in various autoimmune diseases [248]. The role of Th17 cells in the development of GvHD has not been fully elucidated. Using IFN-γ KO mice as T-cell donors, we have observed Th1 and Th17 polarization of donor T cells associated with increased inflammation and GvHD [Waller EK et al., Unpublished Data]. IL-17 contributes to the early development of CD4-mediated GvHD by promoting production of proinflammatory cytokines [253]. By contrast, a recent report that showed deficiency of donor Th17 T cells led to increased Th1 immunity and enhanced GvHD [254]. These contrasting results may be due to the proinflammatory condition induced by Th17, leading to subsequent activation of host DCs and enhanced donor Th1/Tc1 polarization of donor T-cell immune responses [254].

Inhibiting or blocking effector mechanisms of donor T-cell cytotoxicity

FasL and perforin are two effector mechanisms expressed in cytotoxic T cells that are potentially the targets for novel drugs that could reduce the effector phase of GvHD. Preclinical experiments using knockout strains lacking one or both of these effector pathways support their role in the development of GvHD. Perforin-KO effecto memory donor T cells retained GvL activity in murine models. However, GvL activity of effecto memory T cells was decreased when both FasL and perforin cytolytic pathways were defective [67]. The Fas—FasL pathway is involved in the development of hepatic GvHD in a nonirradiated allogeneic HSCT murine

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model [255]. In sublethally irradiated murine MHC-mismatched HSCT models, both perforin/granzyme and FasL-KO donor T cells did not prime lethal GvHD [256]. By contrast, donor CD4 T cells ‘knocked out’ for both perforin/granzyme and FasL pathways caused similar mortality as wild-type CD4 T cells in a murine allogeneic HSCT model using lethal irradiation as conditioning [257], and perforin/FasL double-KO CD8 T cells caused significant GvHD [258]. Ex vivo treatment of lymphocytes with L-leucyl-L-leucine methyl ester (LLME) depleted perforin-containing alloreactive T cells and protected recipients from GvHD [259]. The T-cell repertoire and proliferative capacity of LLME-treated DLI product in response to allo-stimulation in a one-way MLR was comparable with that of untreated donor T cells [260]. These data suggest that the roles of perforin and FasL in GvHD vary according to the intensity of conditioning and/or the inflammatory cytokines induced by conditioning, and that small molecule drugs intended to selectively inhibit these pathways are unlikely to be broadly effective in preventing aGvHD in the clinical setting.

Targeting tumor-associated antigens & vaccination strategies

Minor histocompatibility antigens

Minor histocompatibility antigens encode peptides that function as strong HLA-restricted alloantigens with differential tissue distribution [261]. Selective tissue distribution of many MiHA on hematopoietic cells make them attractive targets for antigen-specific donor T cells that have selective GvL activity and lack the capacity to cause generalized GvHD. MiHA HA-1 and HA-2 are expressed on hematopoietic cells, and aberrantly on some solid tumors, including kidney cancer, but not generally on epithelial tissues. These MiHAs have been explored as targets to prime the antitumor effect of allogeneic HSCT without inducing severe GvHD in preclinical models and pilot clinical studies [262]. T cells from HA-1- and HA-2-negative donors will target HA-1- or HA-2-positive tumor cells. HA-1- or HA-2-specific CTLs are capable of eradicating human solid tumors in a highly MiHA-specific manner in vitro, accompanied by IFN-γ release. In vivo, HA-1-specific CTLs distribute systemically and prevent human breast cancer metastases in immunodeficient mice [262]. HA-1-specific CTLs infiltrate and inhibit the progression of fully established metastases [263]. While donor T cells reactive against MiHA could result in GvL without GvHD when these antigens are restricted to hematopoietic tissues, GvHD has been observed in murine model systems when only recipient hematopoietic cell express MHC alloantigen [113], but it is possible that donor T cells reactive against MiHA could result in GvL without GvHD in recipients with MiHA restricted to hematopoietic tissues. Clinical application of anti-MiHA CTLs has been limited by the use of HLA-restricted peptide as vaccines and patients with specific HLA haplotypes as recipients, and the relatively small number of MiHA that have been well characterized [264].

Tumor vaccines

To enhance tumor-specific immune cytolytic activity without inducing GvHD, vaccines based upon tumor-associated antigens have been widely studied. Several tumor-specific antigens (proteinase 3, Wilms’ tumor 1 and BCR-ABL) can induce tumor-specific alloreactivity, and tissue-specific MiHAs (HA-1, HA-2 and H-Y) have been explored in preclinical models and tested in Phase I and II clinical trials [262,265–268]. Vaccines against MiHA (HA-1, HA-2 and H-Y) and leukemia-specific antigens (proteinase 3, Wilms’ tumor 1 and BCR-ABL) [264] could be ideal for enhancing GvL without augmenting GvHD. As an alternative to vaccination with defined tumor associated antigens, direct vaccination with irradiated, autologous tumor cells engineered to secrete GM-CSF has resulted in documented antitumor immunity in a few patients with metastatic tumor [269,270]. In addition, vaccines comprised of DCs fused with tumor cells may induce T-cell responses targeted to tumor associated antigen leading to GvL without GvHD [271].

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Predicting which patients are at increased risk for GvHD

Biomarkers to predict GvHD

Biomarkers for GvHD have been widely studied [272–275]. GvHD biomarkers could provide a means for prognosis of GvHD and serve as early indicators for the development of GvHD, and to monitor GvHD progression. Paczesny et al. described a panel of four biomarkers (IL-2-receptor-α, TNF-receptor-1, IL-8 and hepatocyte growth factor) that are associated with the subsequent diagnosis of aGvHD among allogeneic HSCT recipients [275]. The levels of these biomarkers in blood are significantly higher in patients who develop GvHD than those in non-GvHD controls by a median of 29 days and provide a sensitive and specific tool for diagnosis of aGvHD and for the initiation of early treatment such as TNF antagonists. Another recent study used donor T-cell gene expression arrays to identify expression patterns associated with GvHD outcomes in recipients. The researchers determined that expression of several genes involved in TGF-β signaling were highly correlated with patient GvHD outcomes [276], a finding that may lead to the development of additional screening tools to improve the safety of allogeneic HSCT.

Gene polymorphisms that influence GvHD outcome

Recent studies have identified polymorphisms in the control regions of proinflammatory cytokines that are associated with higher production of these cytokines (IL-1, IL-6 and TNF) leading to more severe GvHD [277], while a polymorphism in the gene for the IL-1 receptor antagonist (IL-1Ra) in donors protects against aGvHD by downregulating IL-1 production [278]. There are two types of polymorphisms in the IFN-γ gene: one enhances and the other diminishes IFN-γ production. Curiously, patients with either gene polymorphism have an increased incidence of GvHD [279,280]. Different gene polymorphisms in the Th2 cytokine IL-10 can lead to high production of IL-10 and decrease the risk of aGvHD [281], or decrease the production of IL-10 and increase the incidence of aGvHD [282] and cGvHD [283]. L-selectin (CD62L) is a cell-adhesion molecule on the surface of naive and central memory T cells involved in homing of these T cells to secondary lymphoid tissues during immune responses. Polymorphisms of CD62L in donors or recipients lead to increased incidence of GvHD [284]. Gene polymorphisms of CD31, another cell-adhesion molecule, show association with development of GvHD [285,286]. These genetic studies have the potential to help identify patients at increased risk for severe GvHD and post-transplant death. Clinical use of these newly defined gene polymorphisms in allogeneic HSCT will require routine screening of recipients and donors for non-HLA gene polymorphisms as well as HLA matching.

Conclusion

Allogeneic HSCT has the potential to cure many patients with hematological and non-hematological cancers. However, allogeneic HSCT is associated with conditioning-related toxicities, GvHD and immune deficiency post-transplantation, all of which result in significant mortality and morbidity. A variety of approaches to separate GvL from GvHD in allogeneic HSCT in translational studies and clinical trials have been reviewed in this article. There is growing evidence that standard approaches using TCD or pharmacological immunosuppression are ineffective in consistently separating GvHD from the beneficial GvL effects. Progress in the field will require the development of novel techniques to promote activation of tumor-specific T cells and their durable reconstitution, and to potentiate their GvL activity without inducing GvHD. Promising approaches for clinical translation include depletion or suppression of subpopulations of donor T cells associated with GvHD including alloreactive and naive T cells; the use of donor NK cells that target MHC-mismatched hematopoietic cells; and the administration of specific donor DC subsets that can augment the GvL activity of donor T cells without increasing GvHD. Finally, vaccination against MiHA
(HA-1 and HA-2) and therapy using MiHA-specific T cells may be one of the most promising (but technically challenging) approaches. A more complete understanding of the earliest immunological events that occur following the infusion of donor T cells in transplant recipients is critical to the design and implementation of translational clinical studies to evaluate the most promising of these approaches.

**Future perspective**

Due to differences between animal models and human biology, the beneficial outcomes seen with many approaches to reduce GvHD and retain GvL that have been developed in animals have not been confirmed in clinical trials with patients. In addition, the kinetics and dynamics of cytokine secretion and their effects on GvHD have not yet been fully explored. Progress in the field will require the development of novel techniques to further explore the pathophysiology and immunobiology involved in GvHD and GvL. In the next 5–10 years, new information on specific cell—cell interactions involving donor NK cells, T cells and DC populations in allogeneic HSCT can be expected. Novel blocking antibodies, immunosuppressants, and small-molecule inhibitors (such as antisense RNA) of effector pathways (FasL and perforin) will be tested in the clinic in the setting of GvHD. More tumor-specific and tumor-associated antigens will be discovered, and vaccines based upon these antigens will be tested in the clinic to potentiate GvL activity without inducing GvHD.

### Executive summary

**Modifying/Selecting T Cells**
- Depletion or pharmacological immunosuppression is ineffective in separating graft-versus-host disease (GvHD) from the beneficial graft-versus-leukemia (GvL) effects.
- Selective depletion of αβ T cells and naive T cells decreases GvHD.
- Selective enhancement of γδ T cells, memory T cells, and regulatory T cells (Tregs) are promising approaches to enhance GvL and/or suppress GvHD.

**Graft Engineering**
- Donor natural killer cells have GvL activity without GvHD.
- Donor DC subsets may enhance donor T-cell GvL activity.
- Third party or donor mesenchymal stem cells can be used to prevent or treat GvHD.

**Pharmacological Regulation of Alloreactivity**
- Sirolimus increases Tregs with direct antitumor activity.
• IFN-γ is associated with GvL and inhibits GvHD.
• Th2/Tc2 cytokines (e.g., IL-10), receptor blockade, and blocking the FAS—FASL pathway in effector cells may have some effects in separating GvL from GvHD, but are not likely to be successful in clinical applications.

Vaccination to minor histocompatibility antigen & tumor-associated antigens
• HA-1 and HA-2 are expressed exclusively on hematopoietic cells.
• The tumor-associated antigens proteinase 3, BCR-ABL and Wt-1 can induce tumor-specific alloreactivity.

Biomarkers & genetic polymorphisms to predict GvHD
• Serum TNF-αR1, IL-2R, IL-8, HGF and donor T-cell gene expression predict development of acute GvHD.
• Cytokine and selectin gene (e.g., IL-6, TNF and CD62L) polymorphisms are associated with increased acute GvHD and GvL outcomes.

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■ of interest
■■ of considerable interest


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Figure 1. Immune cells and cytokines of host and donor in GvHD pathophysiology and GvL activity

Donor naive T cells, activated by direct alloantigen presentation by host antigen-presenting cells (APCs) and by indirect antigen presentation by donor APCs, are polarized into Th1/Tc1, Th2/Tc2, Treg or Th17 cells. Activated Th1/Tc1 cells directly attack allogeneic host organ/tissue (skin, gastrointestinal tract and liver) and initiate specific inflammatory immune responses that lead to aGvHD and GvL in hematopoietic targets. Activated donor Th2/Tc2 cells lead to cGvHD and GvL effects due to antigen-specific cellular and humoral immune responses. Th17 cells potentiate inflammation and lead to aGvHD. Donor Treg cells suppress GvHD mediated by activated T cells through cell—cell contact and cytokine secretion (IL-10 and TGF-β). The effect of Tregs on GvL activity remains to be fully determined.

Figure 2. T-cell immune polarization and cytokines involved in GvHD pathophysiology and GvL activity
The donor T cells include specific subsets with different cytokine profiles and varying GvL and GvHD activities. The T-cell subsets most likely associated with a clinical advantage in the balance between GvL and GvHD are shown in the colored area.

aGvHD: Acute GvHD; cGvHD: Chronic GvHD; GvHD: Graft-versus-host disease; GvL: Graft-versus-leukemia; ROR: Retinoic acid-related orphan receptor; STAT: Signal transducer and activator of transcription; Treg: Regulatory T cell.
### Table 1

Summary of clinical approaches to separate GvL from GvHD.

<table>
<thead>
<tr>
<th>Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Modifying/selecting donor T cells</strong></td>
</tr>
<tr>
<td>T-cell depletion</td>
</tr>
<tr>
<td>Delayed administration of allogeneic T cells</td>
</tr>
<tr>
<td>Elimination or reduction of alloreactive T cells</td>
</tr>
<tr>
<td>Selecting memory T cells</td>
</tr>
<tr>
<td>Escalating doses of donor T cells</td>
</tr>
<tr>
<td>Activated γδ T cells</td>
</tr>
<tr>
<td>Selecting regulatory T cells</td>
</tr>
<tr>
<td><strong>Modifying/selecting other cells in the grafts</strong></td>
</tr>
<tr>
<td>Mesenchymal cells</td>
</tr>
<tr>
<td>Selecting natural killer cells</td>
</tr>
<tr>
<td>Manipulating dendritic cells and dendritic cell subsets</td>
</tr>
<tr>
<td><strong>Pharmacological prophylaxis and modulators of alloreactivity</strong></td>
</tr>
<tr>
<td>Immunosuppressants</td>
</tr>
<tr>
<td>Histone deacetylase inhibitors</td>
</tr>
<tr>
<td>Cytokines</td>
</tr>
<tr>
<td>Inhibiting/blocking effector mechanisms of donor T cells</td>
</tr>
<tr>
<td><strong>Targeting tumor-associated antigens and vaccines</strong></td>
</tr>
<tr>
<td>Minor histocompatibility antigen</td>
</tr>
<tr>
<td>Tumor vaccines</td>
</tr>
<tr>
<td><strong>Predicting the severity and the incidence of graft-versus-host disease</strong></td>
</tr>
<tr>
<td>Biomarkers</td>
</tr>
<tr>
<td>Cytokine gene polymorphisms</td>
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</tbody>
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