Immunologic Outcomes of Allogeneic Stem Cell Transplantation: Graft-Versus-Host and Graft-Versus-Leukemia Responses and Implications for Future Therapy

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Abstract Allogeneic stem cell transplantation (allo-HCT) is a procedure with the potential to cure many malignant and nonmalignant diseases. The adoptive transfer of a donor immune system into a transplant recipient can result in allorecognition and reactivity of donor immune cells against host target tissues. This can lead to an immune attack against normal tissues in the recipient (graft-versus-host disease, GVHD) but also against the neoplastic cells themselves (graft-versus-tumor effect, GVT). While GVHD has long been recognized as a significant cause of morbidity and mortality after allo-HCT, there has been little progress in advancing the standards of care for GVHD prophylaxis and therapy, which have remain unchanged for more than two decades. Given the more recent recognition that much of the curative benefit of allo-HCT results from the GVT effect, rather than from the cytoreductive effects of conditioning chemotherapy, multiple strategies to take advantage of the GVT effect that aim to limit morbidity and mortality due to GVHD are under investigation, including cellular therapies employing the use of native or engineered graft populations enriched for antitumor responses, and employing donor lymphocyte infusions. Another critical question is how strategies to prevent and/or treat GVHD may be designed to limit the suppression of beneficial T cell responses against pathogens critical to limiting infections in the post-HCT setting. Research in murine models and human subjects has uncovered a great deal regarding the mechanisms of GVHD initiation and persistence, including clinical factors and graft constituents responsible for the acute and chronic forms of GVHD. A variety of cellular mediators, from antigen-presenting cells to effectors, including alloreactive T cells and B cells, have been characterized. Regulatory populations, including CD4+ regulatory T cells and invariant NKT cells, have also been shown to be capable of ameliorating GVHD intensity and survival in model systems. Given this clearer

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J.D. Rosenblatt et al. (eds.), *Advances in Tumor Immunology and Immunotherapy*, Current Cancer Research, DOI 10.1007/978-1-4614-8809-5_12,
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understanding of GVHD pathophysiology, a variety of novel clinical strategies are in development, from those utilizing classical inhibitors of T cell reactivity, to monoclonal antibody therapies to more novel approaches targeting specific signaling pathways in T cells and other mediators of inflammation. Recent meaningful progress has also been made in approaches using adoptive cellular therapies to decrease GVHD while maintaining or specifically augmenting GVT responses. These strategies bring promise for a future wherein more patients can receive allo-HCT for both malignant and nonmalignant diseases, with reduced rates of complications and improved overall survival.

1 Introduction

1.1 Hematopoietic Stem Cell Transplantation

The recognition that relatively small numbers of hematopoietic stem cells (HSCs) can regenerate the bone marrow function facilitated the use of high doses of chemotherapy and/or radiation [1] for the treatment of human malignancies and other diseases such as bone marrow failure syndromes, primary immune deficiencies, enzymopathies, and hemoglobinopathies [2]. Initially the procedure consisted of high-dose therapy (HDT, chemotherapy or radiation) followed by bone marrow transplantation (BMT). Later it was realized that HSCs are contained in umbilical cord blood and can also be mobilized and collected from the peripheral blood with apheresis. The donor of stem cells can be the patient (autologous hematopoietic stem cell transplantation, auto-HCT) or someone else (allogeneic hematopoietic stem cell transplantation, allo-HCT). This can be either an HLA-matched sibling donor (MSD allo-HCT), a haploidentical relative (haplo-HCT), or someone unrelated but HLA-matched with the patient (matched unrelated donor or MUD allo-HCT). In umbilical cord blood stem cell transplantation (UCB-HCT) the donor is usually unrelated.

1.2 Graft-Versus-Host Disease and Graft-Versus-Tumor Effect

Early in the HCT era, it was apparent that a subset of patients developed a declining course with evidence of inflammation in various organ systems, which in some cases could be lethal. These patients frequently did not have relapse of their malignancy or obvious infection and a term “secondary disease” and later “graft-versus-host disease” (GVHD) (reviewed in [3–5]) was coined to define this process. It became apparent from animal models and in the human clinical setting that GVHD was mediated mainly by alloreactive T cells, since the incidence of GVHD was low in the setting of syngeneic (i.e., identical twin), autologous or T cell-depleted HCT (TCD allo-HCT).

However, it was also recognized that the relapse rates of malignancies were lower after allo-HCT than after auto-HCT. Moreover, relapses were higher after TCD allo-HCT but lower in patients with GVHD (especially chronic GVHD), implying that not only the HDT but also the donor immune system was critical to keep recipients in remission. Subsequently, it was shown that for patients relapsing without GVHD following allo-HCT, the infusion of donor lymphocytes (DLI) could make the malignancy regress or enter another remission, in some cases without the development of GVHD following DLI. The terms graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) have been used to describe this donor immune reactivity against the recipient malignancy [6]. Since the recognition of GVL in allo-HCT, many transplanters have decreased the intensity of HDT, especially for slow-growing malignancies (e.g., follicular lymphomas). We call these attenuated therapies reduced-intensity conditioning (RIC) [7]. An extreme extension of this approach is to rely almost exclusively on GVL and to give only modest doses of immunosuppressive therapy (to avoid graft rejection, mediated by residual host immunity) and then to allow the donor immune system fight the neoplasm (non-myeloablative conditioning, NMA). The introduction of RIC and NMA conditioning regimens allowed the application of HCT to older patients or those with comorbid conditions, which is critical given that most diseases curable by HCT increase in incidence with age. Given the significant morbidity and mortality associated with GVHD and the curative potential of GVT, a critical problem faced by transplant immunologists has been the dissociation of these two phenomena.

1.3 Acute GVHD

Understanding the pathogenesis of GVHD (and of GVL) is essential to facilitate the selective manipulation of these clinical events after HCT. However, a critical problem is that GVHD is an extremely pleomorphic entity. There is an acute and a chronic form (aGVHD and cGVHD, respectively) and also an overlap syndrome that can combine features of both. Initially defined by the time of their onset (day +100 after HCT being the date that separated the two forms), aGVHD and cGVHD are now more appropriately distinguished by their clinical manifestations. aGVHD usually affects the skin, the gastrointestinal (GI) tract and the liver, whereas cGVHD typically involves mucosal surfaces, the eyes and the skin (but can involve nearly any organ system). More severe manifestations of aGVHD may include GI disease (e.g., diarrhea, nausea, emesis, abdominal pain, or failure to thrive, depending on the segment of the GI tract that is targeted), and signs of severe hepatic dysfunction (jaundice, encephalopathy, bleeding, hypoalbuminemia) and severe skin involvement (e.g., generalized maculopapular rash that can progress to erythroderma and exfoliation) [8]. If aGVHD happens before day +14 (usually before engraftment), this is called hyper-acute GVHD and is associated with an adverse prognosis [9]. We now also recognize that a late-onset form of aGVHD may occur even well beyond post-transplant day +100 (delayed-onset aGVHD), in some cases due to the tapering of immunosuppression.

1.4 Chronic GVHD

Chronic GVHD also manifests quite variably [10]. Patients may have sicca symptoms (xeropthalmia, xerostomia) with or without arthralgias/arthritis, oral lichenoid changes, skin rash, poikiloderma, skin lichenification, and/or systemic sclerosis (scleroderma), eosinophilic fasciitis or polymyositis. They can also develop liver dysfunction and cholestasis, anorexia, nausea, emesis, weight loss, malnutrition, bronchiolitis obliterans (BO), or cryptogenic organizing pneumonia (COP, formerly BOOP). Other less common manifestations include glomerulonephritis with or without nephrotic syndrome, hypogonadism, and other hormonal deficiencies. Serosal inflammation with pleural effusions or ascites and nervous system involvement are very rare.

2 Pathobiology of aGVHD (Fig. 1)

2.1 Effector Cells

Classically, the pathogenesis of aGVHD has been defined by three phases: Initiation, effector, and augmentation phase. The main effectors in aGVHD are the donor T cells, given the established preventive effects of TCD and T cell-directed immunosuppressive agents. Both αβ T cells and γδ donor T cells contribute to aGVHD. Either CD4+ or CD8+ T cells are *sufficient* to induce aGVHD. Of the CD4+ T cell subsets, naïve donor T cells seem to be the main effectors [11]. In contrast, central memory CD4+ cells have less ability to induce aGVHD [12], while effector memory CD4 cells seem to be incapable of GVHD induction [13]. It is interesting that both central memory and effector memory T cells have been shown to mediate GvL. Although helper T cell polarization is less clear in humans than in murine models, evidence suggests that both T_H1 and T_H17 subsets may contribute to aGVHD [14], while T_H2 cells have a more controversial, but probably detrimental, role. The main population of human regulatory T cells (T_{REG}) (delineated by the CD4+CD25^{hi}Foxp3+ phenotype) appears to be protective for GVHD and may relatively spare GVL responses [15].

Another population of "regulatory cells", the invariant NKT (iNKT) cells can act prophylactically early on by expanding CD4$^+$CD25^{hi}FoxP3 T_{REG} in a IL-4 dependent fashion [16], but the role of iNKT cells can vary depending on the way they have been activated and polarized.

B cells can also augment aGVHD by promoting alloantigen presentation. However, it appears that some B cell subsets can have a protective role in the effector phase by producing IL-10 [17]. B cells that produce high quantities of IL-10 and co-express Foxp3 (regulatory B cells, B_{REG}) have been described, although their importance in GVHD is uncertain [18].

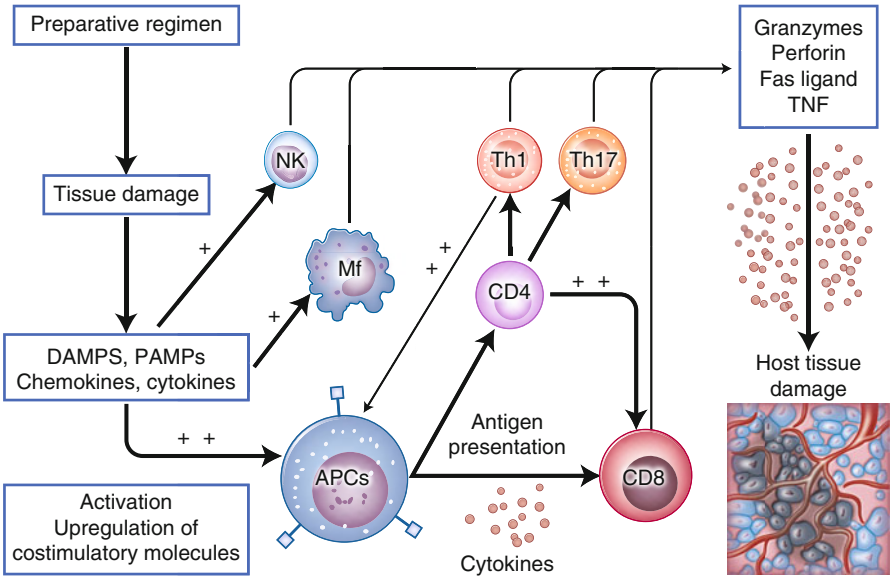

Fig. 1 PATHOBIOLOGY OF ACUTE GVHD: Professional antigen presenting cells (APCs), mainly recipient dendritic cells, become activated under the influence of danger signals that are produced by damaged tissues and microbes. Donor T cells recognize recipient antigens with their T cell receptor and are costimulated by the activated APCs. They are polarized to Th1 or Th17 under the influence of cytokines produced by APCs and monocytes. T cells become activated and produce cytokines, cytotoxic and pro-apoptotic molecules that mediate target-cell damage

2.2 Tissue Damage: Cytokines, Perforin, and FasL

The damage to target organs by T cells is mediated by both cell–cell contact and cytokines. Both FasL and perforin mediate apoptosis through cell–cell contact [19]. FasL-mediated apoptosis may be important in hepatic and cutaneous GVHD, while TNFα operates mainly in intestinal GVHD. Both IFNγ and IL-2 perpetuate aGVHD-induced inflammation and tissue damage, but in experimental systems, administration of these cytokines immediately after allo-HCT appears to *prevent* severe GVHD. The protective effect of IL-2 may be mediated by expansion of T_{REG} [20]. T_H17 cells play a significant role in GI aGVHD via IL-17 and IL-21 production [21]. T_H17 cells are induced by IL-6 and TGFβ and their survival and proliferation are supported by IL-23. High levels of IL-6 increase the T_H17/T_{REG} ratio, which may potentiate GVHD, especially in the GI tract.

2.3 T Cell Priming by Antigen-Presenting Cells

The classic teaching is that intrinsic antigens are degraded in proteasomes and presented through MHC class I to CD8+ cells, whereas exogenous phagocytosed antigens are presented after endosomal degradation to CD4+ cells through MHC class II. It has however been recognized that exogenous antigens can be presented directly to CD8 through MHC class I molecules, a phenomenon known as *cross-priming* or *cross-presentation*. This helps donor antigen-presenting cells (donor APCs) to prime donor CD8+ cells directly (without interference of CD4+ cells) against recipient antigens (for example minor histocompatibility antigens, miHA) [22]. After priming, T cells require costimulation (signal two) or otherwise become anergic. The classic second signal is provided by the B7 molecules (CD80 and CD86) of APCs via the CD28 receptor on T cells. Subsequently, cytokines produced by APCs (signal three, e.g., IL-12 and TNFα) further activate T cells and skew their polarization (T_H1 vs. T_H2). Ligation of CD40 on APCs by CD40L (on activated T cells) helps the APC produce the third signal and to upregulate their co-stimulatory molecules [23].

2.4 T Cell Priming: Are Professional APCs Really Required?

Classically, it was demonstrated that in CD8-mediated aGVHD in MHC-matched pairs, recipient (host) APCs of hematopoietic origin are *required* [24] to present antigens to donor (naïve) T cells to initiate GVHD. These APCs can be professional myeloid dendritic cells (mDCs) or "less professional" APCs of hematopoietic origin (macrophages or B cells). Subsequently, it was shown that in CD4-mediated GVHD in MHC-matched pairs, both *recipient and donor hematopoietic APCs* [25, 26] are *sufficient* to prime donor T cells. Lately, this dogma has been called into question following the observation that plasmacytoid dendritic cells can also induce aGVHD [27]. Subsequently, it was shown that recipient non-hematopoietic tissues can actually present the antigens to previously activated (in an antigen-independent fashion) donor CD4 cells, resulting in lethal aGVHD. These non-hematopoietic APCs express vimentin and α-smooth muscle actin and are probably *myofibroblasts*. They express MHC class II and co-stimulatory molecules as well. The *intestinal epithelial cells* themselves, under inflammatory conditions and in the presence of IFNγ, can also express MHC class II and present antigens [28]. It has even been shown that the collective depletion of B cells, mDCs, and pDCs was insufficient to inhibit aGVHD [29].

2.5 Co-stimulatory Molecules

Blockade of CD80 and CD86 either by antibodies or by CTLA4-Ig was shown to ameliorate aGVHD [30]. Similar attenuation of GVHD resulted from targeting of the OX40L–OX40 system, the CD137 (4-1BB), the ICOS, the CD153-CD30 axis,

and the LIGHT-HVEM pathway. Targeting of the CD40 pathway may induce tolerance [31]. CTLA-4 seems also to play a role in tolerance, while blocking the co-inhibitory molecule PD-1 appears to aggravate aGVHD. Co-blockade of CD28 and ICOS has an additive effect in GVHD prevention [32] as does blocking anti-CD40L in CD28-knockout animals. Both soluble and membrane-bound CD30 in CD8 central memory and in CD8 effector cells are increased in aGVHD patients, while CD30+ cells were increased in the gut of patients with GVHD [33]. Nevertheless, none of these co-stimulatory axes seem to be independently essential for aGVHD induction.

2.6 Innate Immunity Receptors

Irrespectively of which cell presents the alloantigens to donor T cell, that "antigen-presenting cell" has to be stimulated or activated (whether it is a host tissue cell or a professional APC). It is well known that immature DCs can actually be tolerogenic [34] and increase T_{REG}. APCs seem to be activated through a variety of mechanisms including toll-like receptors (TLRs), NOD-like receptors (NLRs), receptors for damage-associated molecular patterns (DAMPs), CD40, and cytokines. In GVHD mice models, the activation of TLR4 by bacterial lipopolysaccharide (LPS) potentiated GVHD [35]. Similarly deleterious in mice models was the activation of TLR9 [36] by CpG repeats and of TLR7 (the receptor for single-stranded RNA) by a strong agonist (R-848). Activation of TLR5 by flagellin decreases GVHD [37]. NOD2 (an intracellular receptor of muramyl dipeptide, a component of bacterial cell walls) seems to be protective [38]. P2X7 is a DAMP receptor for ATP and it also seems to potentiate GVHD [39], while P2X7 blockade has a protective effect. Strategies of gut decontamination with antibiotics (to reduce APC activation) are actively being investigated.

2.7 Target Antigens

In MHC-matched allo-HCT, donor T cells can recognize as foreign MHC-epitope complexes on the surface of recipient cells if the epitope source is a protein that differs between the donor and the recipient. *Gene polymorphisms* are responsible for the generation of different epitopes on the recipient that are not present on the donor. These antigens are frequently called minor histocompatibility antigens (miHA) [40]. Examples include the male-specific H-Y antigens and the antigens HA-1, HA-2, HPA-3, PECAM (CD31), and PANE-1. Some of these antigens have tissue-dependent expression patterns that could be responsible for *tissue-specific aGVHD*. Some miHA can induce very strong allo-immune reactions [41].

2.8 Trafficking

L-selectin (CD62L) is important for homing of naïve T cells to lymphoid structures. P-selectin is expressed on the endothelium and is important for T cell–endothelium interaction, while its ligand (PSGL1) is upregulated during GVHD. The integrin α4β7 is essential for recruitment of T cells back to the intestines [42]. Multiple chemokine receptors affect T cell trafficking in GVHD including CXCR3, CCR4, and CCR5. The sphingosine-1 receptor FTY720 in experimental GVHD seems to maintain alloreactive T cells in the lymph nodes preventing them from migrating to the inflammatory site [43].

3 Strategies to Prevent aGVHD

3.1 Find the Best Compatible Donor

Matched sibling donors are preferred. A high-resolution match between donor and recipient at HLA-A, B, C, and DR is usually required for MUD allo-HCT in Western world countries. National Marrow Donor Program (NMDP) data initially showed that HLA-DQ and HLA-DP mismatches did not have a significant impact on GVHD [44]. Later, an analysis of 8500 transplant pairs demonstrated permissive and nonpermissive HLA-DP mismatches and that nonpermissive mismatches were associated with higher rates of severe aGVHD, non-relapse and overall mortality, but lower incidence of relapse, relative to HLA-DP-matched pairs [45]. Similarly, a mismatch at the minor histocompatibility locus coding for HA-1 increases the risk for GVHD. KIR/KIR ligand mismatch in the haploidentical setting seems to prevent relapses of myeloid malignancies through NK cell-mediated lysis of leukemia after conditioning with TBI and T cell depletion (and without post-transplant immunosuppression) [46]. Furthermore, NMDP data showed that KIR genotyping could identify that donors with high content of the so called “B-motifs” conferred lower relapse risk to recipients with AML but not ALL [47].

MHC haplotype match implies identical genetic material in the entire MHC coding region that seems to encode multiple other genes that affect transplant outcome. MHC haplotype match decreases significantly the GVHD risk. The high-resolution HLA typing that is performed routinely does not guarantee MHC haplotype match. Since 22 % of the high-resolution HLA-identical (allelic match) unrelated donor-recipient pairs do not share identical MHC haplotypes, novel array methodology has been developed to improve matching [48].

The source of stem cells (bone marrow or peripheral blood) also affects the risk of cGVHD with PBSCs conferring higher risk than bone marrow. In the setting of nonmalignant disease (e.g., aplastic anemia) the lack of a GVL-associated benefit has been shown to favor marrow transplantation, due to the excess morbidity and mortality associated with cGVHD.

3.2 Other Factors Affecting GVHD Incidence

Other donor factors that have been suggested to affect the incidence of GVHD are age, gender, and parity and, more recently, the possible use of statins [49]. Older donor age has been associated with an increased incidence of both severe aGVHD and cGVHD and decreased overall survival after allo-HCT. Male recipients who have female multiparous donors (especially mothers of multiple sons) have higher incidence of cGVHD.

3.3 Standard Prevention

So far, the standard of care for GVHD prevention has been the use of low-dose methotrexate (given repeatedly in low doses) plus long-term therapy using a calcineurin inhibitor (CNI, cyclosporine or tacrolimus). The addition of corticosteroids as a third medication, even in a delayed fashion (e.g., starting 2 weeks after transplant) is not beneficial. In UCBT, methotrexate is often replaced by mycophenolate mofetil (MMF) to limit the duration of post-transplant cytopenias.

In most centers some degree of in vivo T cell depletion (ATG or alemtuzumab, which depletes both B and T cells) is applied in the MUD setting. Some centers routinely utilize ex vivo lymphocyte depletion for MUD or even MSD allo-HCT. In allo-HCT for benign diseases the use of ATG is more common even in the MSD setting to reduce GVHD. A recent retrospective analysis compared the outcomes with ATG, alemtuzumab or no TCD after reduced-intensity allo-HCT in patients with hematologic malignancies. The use of ATG compared to no TCD was associated with less GVHD, higher relapse rate and lower 3-year overall survival [50]. The use of alemtuzumab decreased GVHD further but disease relapse and infections were more common.

For decades, some degree of bacterial decontamination of the bouel has been applied at most centers, and it is possible that reducing bacterial load at the time of intestinal mucosal injury may limit danger signals that result in APC activation. Most centers use an oral fluoroquinolone, although in the MSD setting, lower incidence of aGVHD was observed in patients who received ciprofloxacin and metronidazole compared to ciprofloxacin alone.

3.4 Novel Pharmacological Approaches

To improve historical results with CNI-based therapy, recent studies have combined tacrolimus with the mTOR inhibitor sirolimus [51], which allows earlier engraftment and decreases mucositis at the expense of higher risk for thrombotic microangiopathy and sinusoidal obstruction syndrome (SOS), especially when myeloablative doses of

busulfan are used. A phase III trial is prospectively comparing the combination tacrolimus-methotrexate with tacrolimus-sirolimus across BMT Clinical Trials Network (CTN) institutions. CNI use is associated with nephrotoxicity, may inhibit T_{REG}, and may harm thymic stroma, potentially impairing immune reconstitution. Sirolimus relatively spares T_{REG}, so attempts to combine sirolimus to improve the ratios of T_{REG}/T_{CON} are under exploration. In one small study, the combination of sirolimus with MMF after busulfan-based conditioning increased the incidence of SOS. In another pilot study, pre-transplant alemtuzumab was given in combination with post-transplant sirolimus in adults with hemoglobinopathies with promising results [52].

In a mouse model, the post-transplant use of IL-2 with sirolimus [20] expanded natural T_{REG} and increased the induction of T_{REG} from CD4+CD25-T_{CON}, increasing the T_{REG}/T_{CON} ratio, and decreasing GVHD incidence. In humans low-dose IL-2 (1 MU/m^2/day × 8 weeks) has been tried in a population of patients with steroid-refractory chronic GVHD (cGVHD) with a remarkable 52 % response rate, facilitating steroid tapering [53].

3.5 Total Lymphoid Irradiation/ATG

In an attempt to increase T_{REG} in the setting of NMA conditioning, low-dose total lymphoid irradiation (TLI) (total of 8 Gy in ten fractions) was combined with ATG (thymoglobulin, total dose = 7.5 mg/kg). The risk of both acute and chronic GVHD were very low, an effect postulated to be caused by radioresistance of iNKT and IL-4 dependent expansion of T_{REG} [54].

3.6 Post-transplant Cyclophosphamide

Another intriguing recent approach to decrease GVHD incidence is the use of post-transplant cyclophosphamide (50 mg/kg × 2 doses on days +3 and +4). At the expense of delayed engraftment, the rates of aGVHD, and especially cGVHD, are remarkably low despite only limited use of other post-transplant immunosuppressive medications [55]. It is believed that cyclophosphamide kills proliferative alloreactive donor T cells that have been primed at this early interval.

3.7 Cytokine-Directed Antibody Therapies

The addition of the 2A3 monoclonal antibody against IL-2Rα (CD25) or the addition of human recombinant IL-1R antagonist (Anakinra) to cyclosporine and methotrexate did not further prevent GVHD. TNFα seems to play a role in the pathogenesis

of GVHD and the Michigan group has shown that day +7 TNFR1 (compared to the pre-transplant level) is predictive of occurrence of severe GVHD [56]. Etanercept (soluble TNF-receptor) was tested in a phase II trial in high risk for GVHD patients and decreased the d +7 TNFR1 levels but only in the non-TBI setting (compared to historic controls). That group of patients sustained only a 14 % grade III–IV aGVHD and the 1-year overall survival was 69 % [57]. Infliximab an antibody against TNFα failed to decrease the expected incidence of a GVHD when it was added to cyclosporine-methotrexate.

3.8 Pentostatin

A phase I/II study in MUD or mismatched related allo-HCT sought to evaluate the effect of omission of day +11 methotrexate and addition of pentostatin (1.5 mg/m^2 on days +8, +15, +22, and +30) to tacrolimus and ATG. This strategy decreased severe aGVHD but also increased the graft failure rate [58]. The study met its statistical endpoint for success.

3.9 Maraviroc

Maraviroc (Selzentry) is a CCR5 oral antagonist which inhibits the RANTES-CCR5 interaction and is FDA-approved for AIDS. In a recent phase I/II trial, maraviroc at a dose of 300 mg twice a day on days −2 to +30 decreased the incidence of severe aGVHD (d +180 cumulative grade III–IV aGVHD of 6 %) without increasing NRM or reducing GVL [59].

4 Treatment of aGVHD

4.1 Initial Therapy

Low-grade skin aGVHD can be treated with topical corticosteroids (e.g., triamcinolone), but higher grade aGVHD is treated with systemic glucocorticoids. Most centers start methylprednisolone (MP) at 2 mg/kg/day in divided doses. The calcineurin inhibitor that was used for prophylaxis is typically continued when steroids are added. Strong antimicrobial prophylaxis is started against bacteria, herpesviruses, fungi, and against *Pneumocystis jiroveci*. Close monitoring for reactivation of chronic viral infections (e.g., CMV and EBV) is essential, as high rates of reactivation are common during steroid therapy.

Several studies have assessed outcomes with combination therapies, relative to initial therapy with steroids alone. Indeed, the upfront use of daclizumab (anti-CD25) in combination with steroids increased early mortality. The combination of infliximab + MP was not superior to MP alone [60]. Similarly a combination of ATG + prednisone was not better than prednisone alone. However, a phase II trial of initial etanercept + MP compared favorably to contemporary patients with GVHD treated with MP alone [61]. A randomized phase II study for initial treatment of aGVHD started patients on MP and maintained CNI and randomized them to either MMF, pentostatin, denileukin diflitox, or etanercept [62]. The combination of MMF and MP was the winner, yielding a CR rate of 60 % and improved overall survival at 9 months. A confirmatory phase III trial is needed.

If aGVHD is controlled, steroids are tapered relatively rapidly (e.g., 0.2 mg/kg every 5 days until a dose of 1 mg/kg is reached). Then the dose is tapered more gradually (e.g., a 10–15 % reduction weekly). Nevertheless, a lot of these patients will develop extensive chronic GVHD. For patients who do not respond well to steroids (steroid-refractory) or in whom steroids cannot be tapered (steroid-dependent) the prognosis is very poor.

4.2 Studies of Immunosuppressive Therapies for Steroid-Refractory GVHD

Multiple studies have been conducted in the setting of steroid-refractory aGVHD (SR-GVHD) [63]. Although multiple therapies have demonstrated efficacy, all effective therapies increase the rates of opportunistic infections and/or disease relapse. Most of these patients experience a significant decrease in performance status and experience direct and indirect (e.g., infection-related) complications.

Daclizumab is a monoclonal antibody against CD25. It is usually given at a dose of 1.0 mg/kg iv on days 1, 4, 8, 15, 22 and is associated with very good control of aGVHD in at least half of patients with SR-GVHD. Unfortunately the results are temporary in most cases and many patients develop extensive cGVHD.

Infliximab is a monoclonal anti-TNFα antibody which is usually given iv at a dose of 10 mg/kg on days 1, 8, 15, 22. Although very good responses at the range of 60 % have been described especially for GI SR-GVHD, such responses are usually short-lived. The medication is associated with high rates of opportunistic infections including mycobacterial infections.

Etanercept which is soluble TNFα receptor is usually given subcutaneously at a dose of 25 mg twice a week for 4 weeks and then once a week for 4 additional weeks (12 total doses). It has demonstrated encouraging activity in about half of patients with SR-GVHD (mainly GI aGVHD).

The combination of etanercept and daclizumab has demonstrated responses in more than 50 % of patients but the long-term effects of the combination were disappointing with most patients succumbing to infection or GVHD. Similarly the

combination of infliximab–daclizumab resulted in a 47 % response rate, although all patients eventually died. However, the same combination was more efficacious in a pediatric population with SR-GVHD, with 68 % of children alive 31 months later [64].

Horse ATG (ATGAM), once a standard treatment for SR-GVHD, was more recently shown to have limited efficacy, with only 5 % of 69 patients surviving long-term. Similarly another study of rabbit ATG (Thymoglobulin) resulted in only 2/36 (6 %) evaluable patients with SR-GVHD being long-term survivors. ATG, however, has been used in combination with etanercept with or without MMF while maintaining MP and CNI. The response rate was high (80 %) with a median survival of 224 days. From the 16 patients on the study, five died from infection, two from GVHD and one from relapse of the underlying malignancy [65].

Alemtuzumab is a very immunosuppressive medication and should be used carefully. Early administration (second line) and low doses (10 mg iv weekly) was more effective than late administration (third line after salvage with ATG/etanercept) and higher doses (10 mg iv daily for 5 days). The former mode of administration gave a 70 % response rate in 20 evaluable patients. Half of the responses were complete and the median survival was 280 days.

The Dana-Farber group reported on the use of denileukin diflitox, which is a conjugate of IL-2 with diphtheria toxin. Hepatotoxicity was the dose-limiting toxicity but the response rate was high with 50 % CR, 21 % PR, and 1/3 of patients surviving at least 6 months [66].

MMF (1,000 mg orally twice daily) yielded a response rate of 42 % but with very few long-term survivors (16 %). Pentostatin at a dose of 1.5 mg/m^2 iv daily for 3 days gave an impressive 64 % CR rate with 26 % of patients with SR-GVHD surviving at 1 year [67]. Sirolimus at relatively high doses benefited 57 % of patients, although only one patient survived more than a year. Other studies have shown better results with sirolimus, either as salvage or as a frontline treatment of aGVHD, in patients in whom steroids could not be used [68, 69].

Another approach to treat acute SR-GVHD is extracorporeal photopheresis (ECP) in which peripheral blood lymphocytes are separated and incubated with 8-methoxypsoralen and then irradiated with ultraviolet A (UVA) before they are returned back to the patient. ECP requires a central catheter and frequent treatments. ECP may result in apoptosis of cells taken up by APCs that become tolerogenic and increase the number of T_{REG}. In the first pilot Austrian study, 21 patients with SR-GVHD grades II–IV were treated with ECP. 57 % of patients were alive after a median follow-up of 25 months. The response rates were very high for grade II–III GVHD, but only 12 % of patients with grade IV aGVHD responded to the treatment. Most other studies have consistently showed encouraging activity for grade II–III with lower efficacy in more severe GVHD [70].

The use of mesenchymal stem cells (MSCs) initially yielded great enthusiasm. Early reports and a phase II study were promising, though a phase III trial failed to demonstrate a statistically significant improvement over placebo in initial aGVHD and SR-GVHD [71].

5 Selected Novel Approaches/Proposals for Prevention and Treatment of Acute GVHD (Table 1)

- Tocilizumab, an anti-IL-6 monoclonal antibody. Besides its inflammatory properties, IL-6 participates in the T_H17 cell differentiation. Absence of IL-6 can skew T cell differentiation to induced regulatory cells (iT_{REG}). In a pilot study [72] four of six patients with acute SR-GVHD responded to this agent.
- Low-dose IL-2 followed by sirolimus. IL-2 has to be given right away after stem cell infusion (before priming of potentially alloreactive T cells). The intent is to expand donor natural T_{REG} and to utilize sirolimus to selectively limit the function/proliferation of alloreactive T_{CON}. This strategy is being investigated in the setting of both initial prophylaxis and steroid-refractory GVHD therapy.

Table 1 Selected novel immune manipulations for prevention or treatment of GVHD

Target	Method	Aim
↑Treg	Infusion of CD4+CD25+CD127(−) cells (before HCT)	Prevent aGVHD
	Low-dose IL-2 post-HCT	Prevent aGVHD or treat cGVHD
	Photopheresis (preferential expansion of Treg)	Treatment of cGVHD
↑iNKT	Total lymphoid irradiation (0.8 Gy × 10) before HCT	Prevention of aGVHD
	Liposomal a-galactosylceramide (REG-2001) after HCT	
↑Th17	Ustekinumab (anti-IL-12 and -IL-23)	Prevent or treat aGVHD by preventing expansion of Th17
	Tocilizumab (anti-IL-6)	
↑Tnaive	Anti-CD45RA before HCT	Prevention of aGVHD through depletion of naïve CD4 cells
Gut flora	Rifaximin, metronidazole peritransplant	Prevention of a GVHD through decreasing TLR stimulation
CD80 CD86	CTLA-4 Ig (Abatacept, Belatacept)	Prevent aGVHD by blocking co-stimulation and inducing anergy
α4β7	MLN-002 (monoclonal antibody)	Prevent homing of T cells to gut
CCR5	Maravicor (oral drug inhibiting RANTES-CCR5 interaction)	Prevent homing of T cells
CD30	Brentuximab vedotin	Kills alloreactive T cells
Proteasome	Bortezomib, Carfilzomib	Prevent cGVHD Prevent aGVHD
BAFF	Belimumab	Prevent, treat cGVHD
HDAC	Vorinostat, romidepsin, panobinostat	Prevent aGVHD. HDAC inhibitors decrease the efficiency of antigen presentation

Abbreviations: *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease, *HDAC* histone deacetylase, *BAFF* B-cell activating factor, *Treg* regulatory T cells, *iNKT* invariant natural killer T cells, *TLR* toll-like receptor, *IL* interleukin, *HCT* hematopoietic stem cell transplantation

- Ustekinumab: This represents an antibody against p40, which is shared by the cytokines IL-12 and IL-23. IL-12 has been proposed as a third signal for polarization of T cells to T_H1, while IL-23 facilitates T_H17 differentiation. This has to be given for prevention before T cell polarization happens. A patient with SR-GVHD having failed multiple treatments received this agent and responded completely, but later died of bacterial sepsis [73].
- CD45RA-depleted grafts: CD45RA is expressed on naïve T cells primarily responsible for GVHD induction, and therefore might selectively deplete alloreactivity without compromising GVL and immune reconstitution.
- Bortezomib: By giving bortezomib during conditioning, there is a possibility of sensitization of tumor stem cells to chemotherapy. Also, bortezomib, when is given before and just after allo-HCT, may be able to decrease antigen presentation by MHC class I, impair the maturation of dendritic cells and reduce donor GVHD-mediating T cells [74, 75]. Bortezomib may also be used later in an attempt to decrease cGVHD, through its effect on post-germinal center B cells and plasma cells. Multiple studies are ongoing [76].
- Statins: Use of statins by the donor before stem cell collection and by the recipients before and after transplant may decrease GVHD through direct effects on T cells as well as inhibition of activation of APCs. Retrospective studies of outcomes in donors taking statins and prospective studies of recipients are underway.
- Liposomal a-galactosylceramide (RGI-2001, Regimmune, Inc.) is a molecule that if presented through CD1d to invariant NKT cells, increases the T_{REG}/T_{CON} ratio via iNKT-T_{REG} crosstalk and decreases GVHD. Animal models have shown promising results [77] and the molecule is being tested in a multicenter phase I clinical trial, given immediately post-HCT.
- Inhibition of α4 or α4β7 integrins: If this is done early, it can inhibit migration of potentially alloreactive T cells to the gut. Natalizumab is a α4-specific antibody approved for multiple sclerosis and Crohn disease as a monthly infusion. It is associated with opportunistic infections, including JC-associated progressive multifocal leukoencephalopathy (PML). Novel therapies (e.g., vedolizumab) have demonstrated encouraging results in inflammatory diseases of the gut [78] with the potential to be less immunosuppressive than natalizumab.
- Histone diacetylase inhibitors (HDACi): Vorinostat/panobinostat/romidepsin: In animal models, HDACi reduce GVHD by inhibiting upregulation of costimulatory molecules and secretion of inflammatory cytokines by APC. Their effect seems to be mediated by upregulation of IDO [79]. HDACi also promote the generation and function of T_{REG} [80]. HDACi are being investigated in the setting of GVHD prophylaxis.
- Infusion of T_{REG}. These cells have been reported to decrease GVHD while preserving GVL. Their immunophenotype is CD4+ CD25+ CD127− CD62L+ FoxP3+. Ex vivo expansion and infusion has allowed subsequent low-dose donor T_{CON} infusion in haploidentical allo-HCT in an Italian study [81]. Multiple studies are underway in the setting of conventional and UCB-HCT.

- Brentuximab vedotin: It has been shown that CD30+ T cells number is high in situ in patients with gut aGVHD. Treatment with the anti-CD30 immunotoxin brentuximab vedotin maybe helpful in patients with steroid-refractory or steroid-dependent gut aGVHD, although initial data have shown significant myelosuppression.
- PKCθ inhibitors: In an animal model PKCθ inhibition attenuated T_H1 responses and accentuated T_{REG} function, thereby selectively inhibiting GVHD while preserves GvL and antiviral responses [82]. Sotrastaurin (AEB071), although inferior than tacrolimus in the human renal transplant setting, increased the survival of primates with a renal allograft in combination with a CNI. Its efficacy is currently studied in the solid organ transplant setting in combination with tacrolimus (vs. tacrolimus + MMF).

6 Chronic Graft Versus Disease

6.1 Pathogenesis and Translational Implications

cGVHD is a very frequent complication after allo-HCT with an incidence rate up to 70 %. It is a major determinant of disability and most patients with extensive disease require long-term immunosuppression to control the disease. Its incidence increases with mismatch donor–recipient pairs and with MUD allo-HCT compared to matched MRD allo-HCT. The use of a female donor (especially multiparous) in a male recipient also increases GVHD incidence. cGVHD incidence also increases in the setting of PBSCT vs. BMT and with increasing donor and recipient age. Conversely, ex vivo TCD, ATG [83], or alemtuzumab given before allo-HCT are protective, implying that T cells play a significant role at least in the initiation phase.

6.2 B Cells and cGVHD

Recently it has been appreciated that B cells contribute to cGVHD pathogenesis since many of its manifestations resemble auto-immune diseases (e.g., systemic sclerosis). Anti-host antibodies (e.g., the anti-H-Y anti-male antibodies in cases of gender disparity) [84] and also the agonistic antibodies against the PDGFR have been implicated in the pathogenesis of sclerodermatous cGVHD, which may respond to PDGFR inhibitors like imatinib [85]. Rituximab (an anti-CD20 Ab) may have efficacy in established cGVHD [86] and may prevent cGVHD development [87].

6.3 BAFF and B Cell Homeostasis in cGVHD

Patients with cGVHD have elevated levels of B-cell activating factor (BAFF) and decreased numbers of naïve B cells (B cell dysregulation) [87]. Experiments in mouse models of arthritis have shown that BAFF promotes T_H17 differentiation [88]. BAFF is also important for the survival of plasma cells and its level is high in patients with myeloma. Belimumab [89] is a monoclonal Ab against BAFF, which has been approved for advanced SLE. Its use could possibly affect B cell dysregulation, plasma cell proliferation, and T_H17 polarization in cGVHD. If plasma cells play a role in cGVHD then targeting them with bortezomib may be beneficial. At least one study is evaluating bortezomib in chronic pulmonary cGVHD, with the intent of decreasing the signaling of pro-fibrotic TGF-β1 signaling.

6.4 Direct and Indirect Targeting of Regulatory T Cells

T_{REG} are thought to be beneficial in cGVHD and since they are dependent on IL-2, a Dana-Farber study of low-dose IL-2 in steroid-refractory cGVHD gave very good results [53]. For the same reason, sirolimus is increasingly used in cGVHD [90] instead of calcineurin inhibitors, since it is thought that mTOR inhibitors respect T_{REG}. Extracorporeal photophoresis (ECP) is commonly used successfully in cGVHD and one of its mechanisms of action is thought to be related to T_{REG} upregulation [91].

6.5 First-Line Treatment of cGVHD

Initial therapy of cGVHD is becoming increasingly standardized. If the patient has limited skin involvement or mild involvement of two organs (e.g., sicca symptoms and limited skin involvement) without lung involvement and without thrombocytopenia (PLT < 100,000) or hyperbilirubinemia (total bilirubin > 2 mg/dL), then topical (skin, mouth, eyes) steroids or topical calcineurin inhibitors or oral ursodiol (for isolated elevation of alkaline phosphatase) can be tried with close follow-up. Otherwise the patient should be started on prednisone at 1 mg/kg/day. The combination of oral prednisone and a CNI was not superior to prednisone alone in recipients of bone marrow with moderate cGVHD and without thrombocytopenia [92]. Many physicians, however, prefer such a combination in severe forms of extensive cGVHD or for cGVHD and concurrent thrombocytopenia or when fast tapering of steroids is needed [93–96]. CNI addition is also favored if cGVHD onset concurred with withdrawal of previous prophylactic CNI.

6.6 mTOR Inhibition

Many theorize that mTOR inhibitors are better steroid partners because they are more favorable to T_{REG}. For that reason, the current BMT-CTN trial 0801 randomizes patients to either prednisone-sirolimus or to prednisone-sirolimus plus a calcineurin inhibitor. Irrespectively of the initial treatment, responders stay on an initial high steroid dose initially, with only gradual taper thereafter. Flares of cGVHD can happen with faster tapering. The partner drug of prednisone should be maintained at therapeutic plasma levels during the entire period. Ursodiol for liver disease, topical steroids and minimally absorbable steroids like oral budesonide and oral beclomethasone can be used in combination during this period. The role of extracorporeal photopheresis as an addition to a steroid-based initial treatment of cGVHD is an objective of an ongoing clinical trial. When cGVHD develops during treatment of aGVHD (progressive onset cGVHD) the prognosis is more likely to be adverse.

6.7 Second-Line Treatment of cGVHD

Patients with cGVHD who do not respond to steroid-based treatment (steroid-refractory) or in whom the dose of prednisone can't be tapered below 1 mg/kg/day after 3 months (or fail tapering below 0.5 mg/kg/day) require additional systemic treatment. Agents that have shown efficacy and used frequently include ECP, rituximab, sirolimus, imatinib (for sclerodermatous and pulmonary GVHD), pentostatin, and mycophenolate. Other approaches less commonly employed include switching to the alternative calcineurin inhibitor, pulses of methylprednisolone, methotrexate, infliximab, thalidomide [97], clofazimine, hydroxychloroquine, cyclophosphamide, etanercept [98], oral retinoids, PUVA, alemtuzumab, low dose of thoracoabdominal irradiation [99], and infusion of mesenchymal stem cells. There is a paucity of randomized trials and durable complete responses are only occasionally seen. The use of immunosuppression is associated with many side effects including opportunistic infections and secondary malignancies.

6.8 cGVHD: Organ-Specific Interventions

Organ-specific management of cGVHD can sometimes decrease the needs for potent systemic immunosuppression and improve results [93, 100, 101]. For cutaneous cGVHD topical medium to high potency steroids like triamcinolone or clobetasol are used except from the face and the flexural areas where only mild potency steroids are allowed. Use of topical calcineurin inhibitors like tacrolimus or pimecrolimus can help and is associated with less skin atrophy. Emollients help

pruritus and xerosis. Oral anti-histamines, gabapentin, or doxepin are used for intense pruritus. The risk for skin infections (viral, fungal, and bacterial) and malignancies with both steroids and CNI is increased. Sunscreen use is very important. For sclerodermatous cGVHD, physiotherapy should be employed to avoid contractures. ECP can be used as second-line steroid-sparing treatment. UVB and PUVA may be helpful, especially when there is no access to ECP.

In ocular GVHD, artificial tears, and cyclosporine drops help. For severe xerophthalmia, plugging the lacrimal ducts has been tried successfully. Patients with acute/subacute onset of impaired vision and ocular pain should be referred to an ophthalmologist to diagnose and treat disorders like uveitis, retinal problems, herpetic infections, and cataracts. Oral cGVHD is very common and oral solutions of dexamethasone, budesonide, or betamethasone have been used successfully. For significant xerostomia, pilocarpine is used in a similar fashion to patients with Sjogren's syndrome.

All patients with cGVHD are at increased risk of infection, and prophylaxis is required against pneumococcus, viruses, PCP, and fungi (posaconazole preferred for patients on high-dose immunosuppression). Immunoglobulin deficiency should be corrected, and pneumococcal, influenza, and Hemophilus influenza vaccines should be given. Screening for CMV is required.

Pulmonary cGVHD should be confirmed by biopsy and infections must be ruled out. BOOP is usually responsive to steroids, but bronchiolitis obliterans (BO) is problematic. Inhaled corticosteroids in addition to systemic immunosuppression may help. Monthly pulses of steroids have been used. Imatinib and ECP can be beneficial. Oral azithromycin and oral montelukast are often prescribed. Infections are frequent and vaccines, antimicrobial prophylaxis and Ig replenishment are all employed [102]. Long-term prognosis of BO is dismal. All patients with cGVHD on steroids should be monitored and treated for osteoporosis and hormonal (thyroid, gonadal, adrenals') deficiencies.

7 Antitumor Post-transplant Immune Manipulation (Table 2)

Relapse following allo-HCT carries a relatively ominous prognosis. There are three approaches against post-transplant neoplastic relapse: (a) Prevention, (b) Preemptive therapy of minimal residual disease, and (c) Treatment of clinical relapse. The following sections review selected strategies that may be employed against post-HCT relapse.

7.1 Immunomodulatory Molecules

One of the best examples of preventive immunotherapy post-transplant is the use of the immunomodulatory molecules, thalidomide [103], and lenalidomide, for

Table 2 Selected approaches to decrease relapse after allogeneic HCT

Approach	Rationale	Potential problems
Lenalidomide	Augment NK and T cell attack against myeloma MRD	Myelosuppression, GVHD
Abl-TKIs (imatinib, dasatinib, nilotinib, bosutinib, ponatinib)	Target MRD in CML and Ph + ALL	Myelosuppression, immunosuppression
Ibrutinib	Minimize MRD in CLL and B-NHL by targeting Btk	GI symptoms, fatigue, hypogammaglobulinemia
5-Azacytidine	Decrease relapse of myeloid malignancies	Myelosuppression
Rituximab	Decrease relapse of CD20+ malignancies may reduce cGVHD	Hypogammaglobulinemia, myelosuppression
Ipilimumab	Inhibit immunologic tolerance by inhibiting CTLA-4	Aggravation of GVHD, immune endocrinopathies
CT-011	Inhibit anergy by blocking PD1	GVHD?
IL-2, IL-7, IL-21	Boost T cell function	Capillary leak syndrome, fever, arthralgia, GVHD?
Peptide vaccines (WT-1, PR1)	Educate the immune system to attack antigens over-expressed in malignant cells	Low immunogenicity
Dendritic cell vaccines ± TLR7/TLR9 agonists	Enhance cancer cell antigen presentation	Complicated production of the vaccine
CARs	Join an immunoglobulin recognizing a cancer antigen to the TCR signaling cascade	Difficult production, decreased survival of engineered T cells, requires costimulatory receptors and a virus as a vehicle of the genes
NK cell infusion	Augment innate immunity	May need cytokine treatment for enhanced efficacy
Preemptive DLI	Augment GvL	GVHD
Donor with KIR ligand mismatch and/or donor with activating KIR receptors (e.g., KIR2DS1)	Increase NK activity against mainly myeloid malignancies	Difficult to find such donors

Abbreviations: *NK* natural killer cells, *MRD* minimal residual disease, *GVHD* graft-versus-host disease, *TKI* tyrosine kinase inhibitor, *CML* chronic myeloid leukemia, *ALL* acute lymphoblastic leukemia, *NHL* non-hodgkin lymphoma, *CLL* chronic lymphocytic leukemia, *Btk* bruton kinase, *Ph* Philadelphia, *GI* gastrointestinal, *PD1* programmed death-1, *TLR* toll-like receptor, *TCR* T cell receptor, *CAR* chimeric antigen receptor, *GvL* graft versus leukemia, *KIR* killer-immunoglobulin-like receptor, *WT-1* Wilms tumor antigen 1, *Abl* Abelson kinase

prevention of myeloma relapse after auto-HCT. Both have been associated with improved progression-free survival (PFS) and lenalidomide use has been correlated with improved OS as well [104]. It is interesting that the doses used are lower than the conventional anti-myeloma doses and it has been theorized that is not only the

anti-myeloma effect but the immune-stimulatory effect of lenalidomide which is responsible for the improved outcome. Lenalidomide increases NK cell cytotoxic function mainly through NKG2D upregulation. It also increases ADCC function of NK cells. In fact lenalidomide has been successfully used with DLI post-allo-HCT in myeloma and trials are being conducted using lenalidomide after allo-HCT for high-risk MDS and AML, especially those with 5q- known to respond to lenalidomide.

7.2 Tyrosine Kinase Inhibitors

Although imatinib, dasatinib, and nilotinib have been used both prophylactically and therapeutically for CML relapse post-HCT [105], this is not considered an immune manipulation by itself, although these agents may also influence immune function. Similar post-transplant maintenance may be seen in the future in CLL using agents like PI3Kδ inhibitors or Btk inhibitors. Interestingly, Btk inhibition reduces GVHD in murine models [106] suggesting a role for B cells in pathogenesis.

7.3 Hypomethylating Agents

Hypomethylating agents like 5-azacytidine are being used post-allo-HCT to decrease the relapse rate of AML. It may be possible that 5-azacytidine enhances GvL (upregulates the expression of leukemia antigens) without exacerbating GVHD (increases Tregs). Recent encouraging data have emerged from studies treating high-risk patients with myeloid malignancies with post-HCT therapy [107].

7.4 Anti-Lymphoma Antibodies

Rituximab was tested successfully as a strategy to prevent relapses of aggressive B cell lymphomas after auto-HCT [108]. After allo-HCT the use of antibody therapy can buy time for an effective GVL to develop and may also facilitate phagocytosis of the targeted cells and tumor antigen cross-priming. Recently it has been shown clearly in preclinical models that both the agonistic anti-CD137 [109] and the antagonistic anti-CD47 can potentiate the effect of monoclonal antibodies including rituximab in preclinical models [110]. Both of these strategies may prove beneficial to prevent and treat post-transplant relapse; however, the use of agonistic CD137 post-allo-HCT must be viewed with caution, given GVHD exacerbation in murine models.

7.5 Fighting Tolerance

CTLA-4 and PD-1 are two very important mediators of post-transplant immune tolerance. Positive clinical trials with antibodies against CTLA-4 and PD-1 have been reported in melanoma and other solid tumors and the anti-CTLA-4 antibody, and ipilimumab has already been granted FDA approval for the treatment of melanoma. Ipilimumab has generated responses in relapsed lymphoma after allo-HCT without inducing GVHD [111]. CT-011 is an anti-PD1 monoclonal being studied in myeloma patients in the post-auto-HCT setting, alone and in combination with a dendritic-myeloma fussion cell vaccine. Another approach to release the brakes of immune response is to inhibit MDSCs. 1-methyl-D-tryptophan is an oral IDO inhibitor and is being tried in solid tumors.

7.6 Cytokines as a Boost

Enhancement of antitumor T cell responses can be tried with cytokines. The selection and dose of cytokine(s) are critical since for example high doses of IL-2 or IFNγ can lead to activation-induced cell death (AICD) and T cell exhaustion. IL-21 is not associated with CD8 exhaustion or AICD and in viral illnesses decreases the percentage of exhausted CD43++/PD-1++ CD8 cells [112]. IL-21 has been tried in metastatic renal cell carcinoma and melanoma and induced responses [113]. IL-15 has been shown to be critical for memory T cells and for optimal NK function and is not associated with T cell exhaustion or AICD. It has been studied in immunotherapy trials of NK cell infusion in AML (University of Minnesota) and in melanoma after lymphodepleting chemotherapy and adoptive transfer of tumor infiltrating lymphocytes (TILs) at NCI. IL-7 plays a role in T cell homeostasis and broadens TCR repertoire and may decrease the frequency of natural T_{REG} which usually do not express CD127. IL-7 facilitates immune reconstitution and may increase GVHD but may also potentiate GVL. It is possible that a combination of such cytokines might be most beneficial. However optimal dosing combinations and schedules have not been determined.

Despite the concerns of T cell exhaustion and AICD, IL-2 has been given in solid tumors post-transplant. In melanoma, clinical trials demonstrated the utility of a non-myeloablative regimen of fludarabine and cyclophosphamide for T cell depletion followed by infusion of autologous stem cells and ex vivo expanded anti-melanoma T cells. These cells were either tumor infiltrating T cells or cells with an engineered anti-melanoma TCR. In the post-transplant environment of lymphopenia, T cells expanded rapidly via lymphopenia-induced proliferation and were activated by exogenously given IL-2. Durable responses were seen [114, 115].

7.7 Antitumor Vaccines

Another approach to prevent and treat post-transplant relapses is the administration of cancer peptide vaccines. Proteins that can be used for that purpose are minor histocompatibility antigens expressed in hematopoietic tissues like HA-1, HA-2, and HB-1. Other antigens include the WT-1 and the PR1 peptides alone or in combination. Both WT1 and PR1 peptide vaccines have induced immunologic and clinical responses [116, 117] with responses appearing improved with minimal disease burden. Other antigens that have been used as peptide vaccines include CD168 and a modified b3a2 fusion peptide in patients with CML. There are more questions than answers regarding the use of leukemia vaccines. What is the optimal antigen? What combination of antigens should be used? When and how often leukemia vaccines should be given? What is the optimal route of administration (intramedullary, intradermally, subcutaneously), and what is the optimal adjuvant? Should they be combined with molecules for breaking tolerance (e.g., anti-CTLA-4 antibodies) or with immune-stimulatory molecules (e.g., IFN, IL-2, IL-7, or IL-21)? Should chemotherapy be given first to debulk the tumor and create a lymphopenic environment to facilitate homeostatic expansion? What is the optimal combination of vaccines with cellular therapy (e.g., DLI)?

Besides peptide vaccines, investigators have tried to create immune responses with DC vaccines or with genetically modified leukemic cells (e.g., leukemic cells modified to secrete GM-CSF) [118]. DCs are often manipulated (e.g., by loading of mRNA via electroporation). Others have used fusion of dendritic cells with tumor cells (with myeloma or leukemia cells) [119] [120, 121]. Other studies are combining DC vaccines with TLR7 or TLR9 stimulation [122].

7.8 T Cell Engineering

A very promising strategy to treat post-transplant relapse is to use engineered T cells. These cells have been transduced with either a TCR specific for a tumor antigen of interest or a chimeric antigen receptor (CAR), which is a fusion immunoglobulin-like molecule able to recognize the target antigen. Recent attempts have combined transduction of CARs with other co-stimulatory molecules (e.g., a fusion of an immunoglobulin-like receptor with CD3ζ, CD28, and CD137). CARs used thus far in clinical trials include a CD19-specific CAR for recognition of precursor B-ALL and mature B cell neoplasms, a κ-light chain-specific CAR to target myeloma cells, and a CD30-specific CAR to recognize Hodgkin and anaplastic large cell lymphoma [123–127] One unresolved question is what cell should be the target for transfection. Options include naïve T cells, central memory cells, and EBV-specific effector

T cells. After infusion, expansion may be optimal in the setting of a lymphopenic environment, potentially yielding a benefit for chemotherapy administration. Recently, a partial response in a patient with indolent lymphoma who received a "third generation" CD20-specific CAR expressing CD28 and CD137 domains was reported [123]. Another study reported that six of eight patients with B-cell malignancies responded to an anti-CD19-CAR-transduced T cell infusion after lymphodepleting chemotherapy [127]. The T cell infusion was followed by IL-2. Patients with advanced neuroblastoma responded to anti-GD2-CAR-transfected T cells, with long-term persistence of transfected T cells [125, 128]. Complete remissions have been reported in two patients with advanced CLL by June and coworkers using CAR-T cells with investigators reporting significant CAR-T expansion and persistence, as well as profound B-cell depletion. A tumor-lysis syndrome was reported in a patient with CLL who received an anti-CD19/CD137 CAR-transduced T cell infusion. The patient stayed in remission, and there was a long-term persistence of transduced T cells which was attributed to the co-transfected CD137 [128]. More encouraging results are expected in the near future with CARs used either before or after HCT. Use of CAR may allow for increasing selective GVL responses, relative to currently employed nonspecific transfer of T cells such as DLI.

7.9 Natural Killer Cell and Cytotoxic Lymphocyte Infusions

Haploidentical NK cell infusion after high-dose fludarabine and cyclophosphamide lymphodepletion-induced complete remissions in five of 19 patients with poor-prognosis leukemia when NK cell infusions were followed by administration of IL-2 [129]. These results were not reproduced in patients with ovarian or breast cancer [130]. Disease-specific cytotoxic lymphocytes (CTLs) have been generated ex vivo and transfused. Clear responses following EBV-CTL infusions have been seen in EBV-related nasopharyngeal carcinomas [131, 132]. Similarly, responses have been obtained in melanoma patients treated with Melan-A-specific CTLs [133]. Transient responses of leukemias that relapsed post-transplant were elicited with miHA-specific CTLs at the expense of pulmonary toxicity [134].

7.10 Donor Lymphocyte Infusions

Despite encouraging results infusing antigen-specific T cells, the most common method of adoptive immunotherapy for post-transplant relapse is the infusion of non-specific donor lymphocytes (DLI) following withdrawal of immunosuppression.

In the last two decades since the original description of anti-leukemic effects of "buffy coat infusions" [135] we have enriched our knowledge about the sensitivity of different diseases to DLI and we have a better idea about the dose and the

frequency of DLI in different settings [6]. Diseases like CML and indolent lymphomas respond very well to DLI. Myeloma, Hodgkin, and CLL are also sensitive but not as much as CML. Aggressive lymphomas are less sensitive and AML typically responds best when chemotherapy has decreased the tumor burden. ALL is much less responsive to DLI [136–139].

The dose of DLI used is typically one log higher when the donor is a sibling, relative to that in unrelated transplants, because of the higher incidence of GVHD in the MUD setting. Escalating doses are typically given after 4–8 weeks, if no GVHD is seen and if responses are not optimal. The onset of a DLI response can be delayed and may take 2 months or more. The pace of disease growth and degree of donor–recipient mismatch usually determine the dose and timing of initial and subsequent DLI. Even with initial doses of 20 million CD3+ cells/kg in matched siblings, the treatment-related mortality is typically less than 5 % [140].

7.11 DLI in Chronic Myeloid Leukemia

The response rate of CML to DLI depends on the disease status. It is 90 % for cytogenetic relapses and even higher for molecular relapses. The response of chronic phase CML is 70 % but is lower than 35 % in accelerated phase and even lower in blast phase. Responses in chronic phase are usually durable. Adjuvant cytokines (IFNα, GM-CSF, etc.) may be helpful in conjunction with DLI for CML [141]. A TKI inhibitor can be tried before or concurrently with DLI depending on the previous patient exposure. One recent report examined CML patients who relapsed after allo-HCT who were treated with imatinib, DLI, or the combination [142]. Patients who received the combination did much better with the majority of them achieving durable CRs.

7.12 DLI in Multiple Myeloma

Patients with myeloma frequently respond to DLI but higher doses are usually needed (100 million CD3+ cells/kg). The same recommendations for dose escalation as for CML patients apply because of the high chance of severe GVHD with higher CD3 doses. About 45 % respond and 25 % get a CR, but responses frequently are temporary, so that consolidation DLI should be considered in most cases [143–145]. In one study of 18 relapsed myeloma patients who received DLI in combination with thalidomide, the rate of CR was 22 % and ORR was 67 % with acceptable toxicity [146, 147]. In another study, DLI administered after following two cycles of lenalidomide in relapsed myeloma yielded a 2-year PFS of 50 % [148].

7.13 DLI in Acute Myeloid Leukemia

In AML, while complete responses to DLI are relatively low, DLI has shown to confer a survival benefit in relapsed AML patients, compared to chemotherapy alone (21 % vs. 9 %) [149]. Patients who received DLI with minimal disease burden fared better compared than AML patients who received DLI with active disease. Frequently, relapses happen in sanctuary sites like CNS and the gonads and consideration should be given to screen and treat these areas. Results of DLI for relapsed AML are better if the relapse happens later than 6 months after allo-HCT [150]. In a study of low-dose cytarabine followed by infusion of G-CSF mobilized donor PBSCs and subsequent treatment with GM-CSF in relapsed AML, ten of 36 patients survived for more than 5 years and fared better compared to those treated with DLI alone [151]. Some encouraging results have been obtained with lymphodepleting chemotherapy or low-dose 5-azacytidine before DLI, but it is uncertain if these approaches are better than traditional AML chemotherapy followed by DLI. Preliminary results of DLI after each second cycle of azacytidine showed sustained remission in five of 30 patients [152]. A second allo-HCT may be considered for young patients with relatively long disease-free interval since a CIBMTR report showed a 28 % survival at 5-years for patients with acute or chronic leukemias who underwent a second allo-HCT [153]. Schmid et al. reported on prophylactic DLI in AML patients. In this trial, high-risk AML patients received fludarabine–cytarabine–amsacrine, followed few days later by high-dose cyclophosphamide, low-dose TBI, and ATG. Patients without GVHD who were off immunosuppression started receiving prophylactic DLI on day +120. This yielded a remarkable 2-year leukemia-free survival of 40 %. A similar approach yielded 4-year survival of 61 % with upfront allo-HCT in complex cytogenetics AML [154–156]. In pediatric patients with incomplete donor chimerism, patients receiving prophylactic DLI achieved a much better event-free survival compared to others [157].

7.14 DLI in Lymphomas

Following DLI in relapsed follicular lymphoma after RIC allo-HCT, nine of 13 patients attained a sustained complete remission [158]. In 15 patients with mantle cell lymphoma who relapsed after RIC allo-HCT and received either DLI ($N=14$) or second allo-HCT ($N=1$) [159], 11 of 15 patients achieved a sustained remission. In 15 patients with DLBCL who had active disease after allo-HCT and were treated with different modalities including withdrawal of immunosuppression and/or DLI, six of 15 patients attained a sustained remission [160]. In patients with Hodgkin's lymphoma who had failed auto-HCT and then underwent a RIC allo-HCT, five of 15 patients who relapsed after allo-HCT and received DLI were in CR after a median of 45 months [161]. In a study of DLI outcomes in 17 patients with B-cell lymphoproliferative diseases, CRs were attained in all four patients with mantle cell lymphoma, three of four patients with follicular lymphoma, three of four patients with CLL but none of five patients with DLBCL or Richter transformation [162].

7.15 DLI in Acute Lymphoblastic Leukemia

Results in ALL are disappointing despite the fact that the first patient who survived long after DLI was a male with B-ALL who had a florid relapse after allo-HCT from a female donor [163]. In a study of ten patients with relapsed ALL post-allo-HCT who received chemotherapy (idarubicin + cytarabine + etoposide) followed by DLI, only one patient remained alive in CR, 900 days after DLI. The fact that patients received chemotherapy for disease control before DLI implies that probably not only the disease pace but an inherent ALL resistance to DLI may underlie these failures [164]. The poor outcomes of relapsed ALL after allo-HCT are confirmed by another report, wherein 44 patients with relapsed ALL received DLI with or without preceding chemotherapy and where 3-year survival was only 13 % [165].

7.16 Evolving Strategies for DLI

Potential strategies to enhance the efficacy of DLI in lymphoproliferative disorders include the use of disease-specific antibodies (e.g., rituximab, ofatumumab, or blinatumomab) before DLI and the use of engineered T cells as part of DLI. Another approach is to use preemptive DLI when MRD is detected or in cases of incomplete donor chimerism, especially after RIC allo-HCT for diseases that have a known poor prognosis following florid post-transplant relapse.

DLI infusions are associated with an approximately 35 % risk of GVHD [6]. While higher CD3+ cell dose is associated with increased GVHD risk, the incidence of GVHD remains lower after DLI than after ablative conditioning followed by T-replete grafts, perhaps since some host APC have been replaced by donor APCs or are suppressed by donor T_{REG} [166]. Prior host lymphodepletion (e.g., with fludarabine) [139] or concurrent use of IFNα and DLI increases the risk of GVHD. DLI after previous T cell-depleted transplant is also associated with higher rates of GVHD, perhaps due to a lack of donor T_{REG} [167]. If GVHD occurs, it is often responsive to treatment and many investigators give suboptimal immunosuppression or even tolerate lower degrees of GVHD until they see an improvement of the underlying malignancy [168, 169]. However, DLI may result in overt cytopenias and in extreme cases with marrow aplasia [170], especially if the recipient has completely lost donor chimerism. In such cases administration of a T cell replete stem cell product, rather than DLI alone, may prevent aplasia and restore donor chimerism.

8 Conclusions

Dramatic improvements in HCT, especially the widespread adoption of reduced-intensity conditioning regimens (given our understanding of the importance of GVL responses) have substantially expanded transplant utilization with reduced treatment-related morbidity and mortality. However, five decades into the HCT

era, GVHD, and relapse continue to remain vexing problems, resulting in symptom burden and mortality even when the transplant outcome is otherwise successful. The dissection of GVHD from effective GVL and pathogen-specific T cell responses remains a central intellectual challenge and may provide genuine hope for improved transplant approaches. Until then, the focus of clinical trials should be the prevention of GVHD, both the acute and the chronic forms, and on improved studies of initial therapy in both the acute and chronic settings. Adoptive cellular therapies (e.g., using $T_{REG,}$ transduced T cells, or innate immune cells including NK and iNKT cells) are also promising, although pharmacologic interventions that selectively inhibit alloreactivity, while sparing GVL-inducing cells and T_{REG} are also highly desirable strategies. Promotion of tolerance is another mechanism that may reduce GVHD, especially cGVHD. All of these strategies will benefit from an improved understanding of GVHD biomarkers (e.g., promising candidates including TNFR1, HGF, soluble CD25, BAFF, and others) that may facilitate preemptive treatment and early dose escalation or de-escalation of immunosuppression [56, 171–174]. Development of improved animal models that better replicate the human condition, and facilitate a better understanding of cGVHD, will also provide great benefit.

While this review has focused on immunotherapeutic strategies, optimization of conditioning regimens continues to be a priority. Incorporation of agents including gemcitabine [175–177], bendamustine [178] for lymphomas or novel agents like proteasome inhibitors; HDAC inhibitors in myelomas; or Btk or PI3Kδ inhibitors in lymphoid malignancies may also improve outcomes. Antibodies or immunotoxins (brentuximab, anti-CD22 immunotoxins, etc.) are also likely to be increasingly utilized in pre- and post-transplant conditioning and maintenance therapies. Additionally, targeting of putative cancer stem cell pathways (Notch, Hedgehog, β-catenin, etc.) during conditioning may also improve outcomes. Post-transplant maintenance/consolidation treatments have already given good results (e.g., lenalidomide, imatinib) and other promising strategies (e.g., hypomethylating agents in leukemias) are also being developed. Many of these strategies may have intended or unintended immunologic consequences, which should be assessed systematically when clinical trials of these agents are conducted.

In many settings, detection of early relapse before overt clinical signs are evident (e.g., by chimerism or by MRD evaluation) may allow us to more successfully modify the immune environment or apply novel agents. Overall, it is expected that a combination of these diverse new approaches in the next decade will substantially improve post-HCT disease control while decreasing early mortality and late effects of HCT, which may impair immune function and quality of life. To accomplish these aims, thoughtful and systematic basic, translational and clinical studies will be needed. These studies will require the careful cooperation of academic institutions, industry partners and regulatory agencies, but will yield a promising future for the rapidly growing field of HCT.

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