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Approaches for the prevention of graft-versus-host disease following hematopoietic cell transplantation

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Summary

Allogeneic hematopoietic cell transplantation (HCT) is an important therapeutic option for malignant and non-malignant diseases, but the more widespread application of the therapy remains limited by the occurrence of graft versus host disease (GVHD). GVHD results from immune-mediated injury by donor immune cells against tissues in the HCT recipient, and can be characterized as acute or chronic depending on the time of onset and site of organ involvement. The majority of efforts have focused on GVHD prevention. Calcineurin inhibitors are the most widely used agents and are included in almost all regimens. Despite current prophylaxis strategies, 40–70% of patients remain at risk for developing GVHD. Herein, we review standard and emerging therapies used in GVHD management.

Keywords

GVHD; prophylaxis; treatment; hematopoietic stem cell transplantation; calcineurin inhibitors; acute; chronic

Clinical Features and Grading of GVHD

GVHD arises when donor T lymphocytes respond to mismatched protein antigens expressed on host T-cells. The most influential protein mismatches are human leukocyte antigens (HLAs) [1]. The incidence of acute GVHD is directly related to the degree of mismatch between HLA proteins expressed by the HCT donor and recipient [2]. Even in patients that receive HLA-matched (HLA-A/B/C/DRB1) grafts, however, GVHD arises in approximately 40% of patients due to differences in minor histocompatibility antigens, and requires systemic therapy [3].

GVHD presents in an acute or chronic form. Acute GVHD typically occurs within the first 100 days after transplant, but it can also present later as late-onset acute GVHD. The organs principally affected in acute GVHD include the skin, liver and gastrointestinal (GI) tract [4].

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Skin GVHD is characterized by a diffuse maculopapular rash with a predilection for the palms, soles, ears, neck, and dorsal surfaces of the extremities and malar regions. Signs and symptoms of GI involvement include profuse diarrhea, vomiting, anorexia, nausea and abdominal pain. Liver GVHD is characterized by cholestatic hyperbilirubinemia. Virtually all manifestations of GVHD require exclusion of other causes.

An overall grade of acute GVHD is assigned based on the individual stages of GVHD involvement of each of the three main target organs (skin, liver GI). The skin is usually the first to demonstrate clinical manifestation of acute GVHD, and is staged with a score of 0 to 4 based on the percent of body surface area involvement in the macropapular rash (stage 0, no macropapular rash; stage 1, rash <25% body surface area (BSA); stage 2, 25–50% BSA; stage 3, generalized erythoderma or rash >50% BSA; stage 4, generalized erythoderma plus bullous formation and desquamation >5% BSA). Liver GVHD is staged solely based on the serum bilirubin level (stage 0, <2mg/dL; stage 1, 2–3 mg/dL; stage 2, 3.1–6 mg/dL; stage 3, 6.1–15 mg/dL; stage 4, >15 mg/dL). The GI tract is staged based on the volume of stool output per day in adults (patients >50kg in weight), or stool output per kilogram bodyweight [5] in children (stage 0, <500 mL/day or <30 mL/kg; stage 1, >500 mL/day or >30 mL/kg; stage 2, >1000 mL/day or >60 mL/kg; stage 3, >1500 mL/day or >90 mL/kg; stage 4, severe abdominal pain with or without ileus, or grossly bloody stool, regardless of stool volume). Acute upper GI GVHD can manifest as persistent nausea, vomiting and anorexia. A positive upper GI biopsy in this clinical setting is confirmatory of GI GVHD (stage 1). These stages of individual organ involvement are combined to produce an overall grade, which has prognostic significance. The most widely used system for grading acute GVHD is a modified version of the Glucksberg Scale [6]. Mild, grade I acute GVHD, consists of stage 1 or 2 skin involvement without liver or GI involvement. Moderate, grade II GVHD, consists of stage 3 skin involvement or grade 1 liver or GI involvement. Grade III, severe, acute GVHD consists of stage 0–3 skin, with stage 2–3 liver or GI involvement. Finally, grade IV, very severe and life-threatening acute GVHD, consists of stage 4 skin, liver or GI involvement. Notwithstanding, although current scoring criteria are widely implemented, recent studies have demonstrated that they don't optimally predict outcomes. Thus, studies have been proposed or are underway to investigate refined acute GVHD scoring criteria that better predict response, survival, and TRM than current approaches [7–9].

Chronic GVHD is a complex, multisystem disorder with myriad manifestations that can involve essentially any organ [10]. Chronic GVHD is typically characterized by fibrosis, although some signs and symptoms are shared between acute and chronic disease, including erythematous rash, nausea, vomiting, diarrhea and liver dysfunction. The incidence of chronic GVHD ranges from 30% in recipients of fully HLA-matched HCT to 60–70% in recipients of mismatched or unrelated donor HCT [10]. Older recipient age and the occurrence of acute GVHD are the most important risk factors for chronic GVHD [11]. The median time of diagnosis is 4–4.5 months after HCT, depending on the source of donor cells. However, the classification of chronic GVHD is based on the specificity of signs and symptoms rather than the criterion of time of onset. Chronic GVHD can evolve from acute GVHD, develop after resolution of acute GVHD with immunosuppressive therapy or present *de novo*. In some patients, clinical features of acute and chronic GVHD may be present simultaneously (overlap syndrome) [12].

Diagnosis and scoring the severity of chronic GVHD is challenging given the limited understanding of its pathophysiology and often co-existing acute GVHD manifestations [13]. Historically, the classification criteria proposed by the Seattle Group was the most commonly adopted staging system [14]. However, because of difficulties classifying patients according to the restricted criteria, new consensus criteria for diagnosis and staging of chronic GVHD have been developed by the NIH Consensus Development Project and were recently reported in an updated, comprehensive, form [13]. Eight organs and sites are scored for chronic GVHD involvement, including the skin, mouth, eyes, GI tract, liver, lungs, joints and fascia, and the genital tract. Each site is scored from 0 to 3 (no involvement to severe involvement). Both the number of sites involved and the severity score at each site are used to calculate a global severity score. Although performance status is also assessed on a 0 to 3 scale, it is not used to derive the global score. Based on the global severity score, chronic GVHD is classified as mild, moderate or severe [13].

Pathophysiology of GVHD

The mechanism that belies GVHD is an exaggerated but prototypic immune response against foreign antigen(s). Murine models have been central to the understanding of the pathophysiology of GVHD. Largely on the basis of studies in these models, GVHD is commonly described as having 3 phases [4]: (1) activation of antigen presenting cells (APCs); (2) donor T-cell activation; and (3) target organ damage by effectors. In the first phase, APCs presenting mismatched antigens are activated by sensing innate inflammatory mediators (TNF- α , IL-1, IL-6) and pathogen-associated molecular patterns (PAMPs) released in response to recipient tissue damage during the conditioning regimen. In the second phase, donor T-cells proliferate and differentiate into effector cells in response to interaction with peptide antigen complexes and co-stimulatory molecules on the APCs. The alloantigen composition of the recipient determines which donor T-cell subsets differentiate and proliferate. In HLA-matched HCT, acute GVHD may be induced by either or both CD4⁺ and CD8⁺ responses to minor histocompatibility antigens [2]. Activation of donor T-cells against disparate antigens results in rapid production of a cascade of cellular mediators (such as T-cells and NK cells) and soluble inflammatory agents (cytokines, chemokines, reactive oxygen species). These molecules work together to amplify local tissue damage and to further promote inflammation and tissue damage in the third phase of GVHD pathophysiology. Interrupting this cycle at any point has the potential to limit, or prevent altogether, acute GVHD. However, the anti-tumor effects (graft-versus-leukemia; GVL) that underlie the curative efficacy of allogeneic HCT for malignant conditions also rely on functional cellular responses against tumor antigens.

Delineation of the pathophysiology of chronic GVHD has been hampered due to a lack of animal models representative of the spectrum of features in the human disease process [15]. Based on findings in human studies, alloreactive antibodies [16, 17], B-cells [18–21], conventional T-cells [22, 23] and T regulatory cells [24, 25] are dysregulated in chronic GVHD patients and appear to play a role in its complex immune pathogenesis. However, the precise contributions of, and communication between, other cells and subsets (antigen presenting cells), antigen (autologous, allogeneic), and inflammatory mediators (cytokines, chemokines) to the pathophysiology of chronic GVHD are poorly understood. Two recently

developed murine models appear to more closely recapitulate the clinical features of human chronic GVHD [26, 27]. It is hoped that these models will allow further elucidation of the pathophysiology of chronic GVHD.

Standard agents for acute GVHD

Calcineurin inhibitors

Cyclosporine and tacrolimus are calcineurin inhibitors that are structurally distinct but have similar mechanisms of action. Cyclosporine complexes with cyclophilin, and tacrolimus complexes with FKBP12, to inhibit calcineurin and block the dephosphorylation, nuclear translocation and transcriptional function of nuclear factor of activated T-cells (NFAT), thereby reducing T-cell function (Table 1; Figure 1) [28]. In one study, GVHD prophylaxis with tacrolimus reduced the risk of acute GVHD, and TRM without increasing relapse after unrelated donor HCT compared to cyclosporine prophylaxis [29]. However, the relapse rate was significantly higher using tacrolimus prophylaxis after HCT from HLA-matched sibling donors [30]. Combination prophylaxis, such as cyclosporine or tacrolimus plus methotrexate, led to a notable reduction in GVHD and improved survival compared to either agent alone [31]. Since that time, CNI-based therapies have been the standard-of-care for GVHD prevention.

Methotrexate

Methotrexate attenuates T-cell activation at low, non-cytotoxic, doses [32]. Preclinical studies demonstrated its efficacy in GVHD prevention [33]. Although first used as monotherapy; prophylaxis with a combination of cyclosporine-methotrexate proved superior over single agent use [31]. Two multicenter, randomized, prospective trials conducted in the mid-1990s demonstrated that tacrolimus-methotrexate decreased the incidence of acute GVHD compared with cyclosporine-methotrexate, but did not significantly impact overall survival [34, 35]. Some centers thus favor the tacrolimus-methotrexate combination over the cyclosporine-methotrexate combination [36].

Mycophenolate mofetil

Mycophenolate mofetil (MMF) selectively inhibits inosine monophosphate dehydrogenase in T-cells (Figure 1). MMF has shown synergistic activity when combined with any CNI for GVHD prophylaxis. This regimen is used widely after non-myeloablative transplants and cord blood transplants [37], but is just now being formally tested in randomized trials. A multi-center phase III study is underway to determine the most promising GVHD prevention approach (cyclosporine-MMF vs cyclosporine-MMF-sirolimus) after non-myeloablative conditioning and unrelated donor HCT ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01231412): NCT01231412). The efficacy of MMF after myeloablative transplants is not well-established. Phase I and II clinical trials have reported less mucositis and faster neutrophil engraftment with the combination of cyclosporine and MMF compared to MMF alone after myeloablative transplant, but no improvement in incidence of grade 2–4 acute GVHD [38].

Investigational approaches for acute GVHD prevention

T-cell-targeted strategies

The mainstay of GVHD prophylaxis and treatment is T-cell manipulation. While broadly depleting T-cells is a very effective means of prevention of GVHD, there are concerns of increased risks of delayed immune reconstitution, infection, graft failure, and relapse [39].

Sirolimus—Sirolimus (rapamycin) binds to the intracellular protein FKBP12 and inhibits the mammalian target of the rapamycin (mTOR) pathway to block IL-2 mediated signal transduction leading to cell-cycle arrest in naïve T-cells [40] (Figure 1). Sirolimus effectively prevented lethal GVHD in experimental models, which led to its clinical use in GVHD prophylaxis [41].

Patients undergoing myeloablative mismatched unrelated HCT that received sirolimus combined with tacrolimus-methotrexate had lower incidence of grade 2–4 acute GVHD compared with historical controls in an initial study [42]. The combination of tacrolimus-sirolimus (without methotrexate) was tested in the related donor setting and demonstrated low incidences of grade 2–4 acute GVHD, neutrophil recovery, and encouraging overall survival at 1-year [43], but other single-institution studies followed with mixed reports [44], [45]. An open-label, multicenter, phase III randomized controlled trial (RCT), conducted in 304 patients undergoing HCTs from a related donor, demonstrated equivalent grade 2–4 acute GVHD incidence and 2-year overall survival for tacrolimus-sirolimus or tacrolimus-methotrexate prophylaxis regimens [46]. However, neutrophil engraftment was more rapid and mucositis was less severe in patients that received tacrolimus-sirolimus.

Anti-thymocyte globulin—Anti-thymocyte globulins (ATG) are polyclonal immunoglobulins directed against antigens expressed on human T lymphocytes (Figure 1), resulting in T-cell cytolysis [47]. ATG is sometimes included as routine GVHD prophylaxis for patients undergoing unrelated or mismatched donor HCT. ATG has also been studied in a RCT, combined with cyclosporin and methotrexate prophylaxis, for patients undergoing myeloablative conditioning HCT from matched unrelated donors [48]. The addition of ATG decreased grade 2–4 acute GVHD and chronic GVHD but did not significantly impact survival, was associated with delayed neutrophil and platelet engraftment, and increased incidence of EBV post-transplant lymphoproliferative disease [48]. A definitive study of ATG in adult patients undergoing bone marrow or peripheral blood stem cell transplantation from unrelated donors is currently underway (NCT01295710).

Alemtuzumab—Alemtuzumab is a humanized monoclonal antibody that binds to the CD52 receptor to deplete lymphocytes by complement fixation and antibody-dependent T-cell-mediated cytotoxicity [49] (Figure 1). Treatment with alemtuzumab before allogeneic HCT reduced the incidence and severity of GVHD, and mortality [49]. However, the drug was also associated with delayed immune reconstitution, increased graft failure and disease recurrence [39]. In HLA-mismatched unrelated HCT, alemtuzumab was also associated with increased graft failure, but incidence of GVHD and overall survival were equivalent to matched related donor HCT, suggesting a potential role for alemtuzumab in the mismatched setting [50]. Alemtuzumab has also been incorporated in the conditioning regimen for non-

malignant diseases, where it was associated with favorable outcomes [51]. However, the optimal dose has not been determined and its efficacy has not been formally tested in a RCT [52].

Pentostatin—Pentostatin inhibits adenosine deaminase and blocks the metabolism of 2'-deoxyadenosine to induce depletion of lymphocytes through apoptosis (Figure 1) [53]. In a recent controlled dosing study, patients undergoing matched (unrelated or related) or mismatched related donor HCT that received pentostatin-tacrolimus-methotrexate for GVHD prophylaxis, demonstrated an encouraging incidence of grade 2–4 acute GVHD compared with control patients that received tacrolimus-methotrexate [54]. A multicenter trial is also assessing the safety and efficacy of pentostatin for both prevention of graft rejection by host cells, and the induction of GVHD by donor cells after donor lymphocyte infusion, in patients with low or falling T-cell chimerism after transplantation from HLA-matched donors (NCT00096161).

CTLA4-Ig—CTLA4-Ig (abatacept) blocks co-stimulation signals to inhibit T-cells (Figure 1). Randomized clinical trials have indicated its safety [55], although chronic use increases risk of infection [56]. In preclinical studies, CTLA4-Ig ameliorated GVHD [57]. Only 2 of 10 patients developed grade 2–4 acute GVHD in a recent feasibility study of adding abatacept to cyclosporine-methotrexate for GVHD prevention following unrelated donor HCT. However, seven patients showed cytomegalovirus (CMV) or Epstein-Barr virus (EBV) reactivation [58]. A phase II multicenter, randomized, double-blind RCT of abatacept combined with CNJ-methotrexate following unrelated donor HCT is currently being conducted (NCT01743131).

Regulatory T-cells— T_{regs} are important regulators of tolerance to self- and allo-antigen (Figure 1) [59]. T_{regs} suppress the early expansion of alloreactive donor T-cells and limit GVHD while maintaining the graft-versus-leukemia (GVL) effect in preclinical models [60]; thus, infusions of human T_{reg} are being tested in clinical trials for GVHD prevention. Infusion of T_{regs} isolated from partially HLA-matched umbilical cord units, expanded in *ex vivo* culture, decreased the incidence of grade 2–4 acute GVHD to 43% in patients that received T_{reg} infusion compared with 61% in historical controls, despite the fact that 25% of patients received less than the targeted T_{reg} dose [61]. When donor T_{regs} were co-infused with conventional T-cells in haploidentical HCT, 26 of the 28 enrolled patients achieved sustained donor engraftment, lethal GVHD was minimized, and no cases of chronic GVHD were reported. However, four patients developed lethal infections [62]. Despite challenges with T_{reg} purity and number, these trials established feasibility. Several phase I and phase I-II studies are underway to further assess this approach (NCT# 01660607, 00602693, 01818479).

B-cell targeted strategy: Rituximab

Rituximab is a chimeric monoclonal antibody targeted against CD20⁺ B lymphocytes, which have also been implicated in the pathogenesis of GVHD [18] (Figure 1). Retrospective, single-institution analyses and registry data have evaluated the potential role of rituximab for GVHD prevention. Of patients with CD20⁺ non-Hodgkin lymphoma (NHL) who received

rituximab pre-transplant as part of the conditioning regimen or post-transplant for disease control, none developed GVHD [63]. Patients with CD20⁺ malignancies who received rituximab within 3 months of HCT also experienced reduced incidence of grade 2–4 acute GVHD compared with patients who did not receive rituximab [42]. Furthermore, 435 patients with B-cell lymphomas registered in the CIBMTR database and had exposure to rituximab within 6 months before HCT had decreased acute GVHD and a survival benefit [64]. A phase II study of rituximab on prevention of acute GVHD after unrelated allogeneic HCT is underway (NCT01044745).

Chemokine and cytokine inhibition strategies

Maraviroc

CCR5 has been shown to mediate GVHD in murine models through its role in lymphocyte migration to target tissues (Figure 1) [65, 66]. Maraviroc is a CCR5-receptor antagonist and has been investigated, in conjunction with tacrolimus-methotrexate, for GVHD prophylaxis [67]. In patients with high-risk hematological malignancies undergoing reduced intensity conditioning HCT, cumulative incidences of grade 2–4 acute GVHD at day 100 and day 180 were favorable, but 1-year relapse rates were high [67]. The role of this drug in the unrelated donor HCT setting is currently being explored (NCT01785810).

TNF- α inhibition

Murine and human studies demonstrate a role for TNF- α in the induction of GVHD [68, 69]. Higher plasma TNF- α levels during a patients' conditioning regimen correlated with higher incidence of acute GVHD and greater likelihood of mortality [70]. Delivery of etanercept (two recombinant human TNF receptor p75 monomers fused to the Fc portion of human immunoglobulin G₁) during the pre- and peri-transplant period significantly decreased TNF- α release after conditioning and delayed the onset of acute GVHD [71]. Etanercept, combined with standard tacrolimus-methotrexate prophylaxis, reduced TNFR1 ratios and provided encouraging 1-year survival in patients undergoing myeloablative, unrelated donor HCT [72]. However, a randomized 4-arm phase II trial demonstrated that the combination of etanercept and corticosteroids as initial therapy, at the time of acute GVHD diagnosis, was comparable or inferior to combination therapy with corticosteroids and other agents (MMF, denileukin or pentostatin) [73].

Interleukin-2 receptor antagonists

Daclizumab is a humanized IgG1 monoclonal antibody and basiliximab is a chimeric monoclonal antibody. Both bind the α -subunit of IL-2 receptor (IL-2R α , or CD25) to selectively inhibit T-cell activation (Figure 1). A randomized trial of daclizumab combined with steroids for initial treatment of acute GVHD was halted after a planned interim analysis that showed equivalent GVHD response rates but inferior 100-day survival compared with steroid-placebo controls [74]. However, a recent retrospective analysis in patients who underwent unrelated donor HCT and received basiliximab or daclizumab combined with standard GVHD prophylaxis reported favorable acute GVHD incidence and 2-year survival. Basiliximab-treated patients demonstrated lower incidence of chronic GVHD compared with daclizumab [75]. The addition of basiliximab to standard cyclosporine prophylaxis after

matched (related or unrelated) non-myeloablative HCT is undergoing current prospective evaluation (NCT00975975).

Interleukin-6 inhibition

Interleukin (IL)-6 plays an essential role in inflammation and immune regulation [76]. IL-6 and IL-6R levels are increased during GVHD in murine models, and IL-6 blockade reduces GVHD severity [77]. Early blockade of IL-6 was recently tested in a clinical trial of GVHD prevention following myeloablative or reduced-intensity conditioning and allogeneic HCT. In addition to standard GVHD prophylaxis with cyclosporine- methotrexate, patients received tocilizumab, the human neutralizing monoclonal antibody against IL-6R, on day 1 following HCT. Immune reconstitution was preserved in recipients and favorable incidence of day 100 grade 2–4 and 3–4 acute GVHD were demonstrated [78]. These early findings are encouraging and further evaluation in a multicenter study is currently being conducted.

Other novel strategies

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) interact with, and modulate effector functions of, innate and adaptive immune cells [79]. Early feasibility studies demonstrated the safety of infusing autologous human MSCs [80]. Co-infusion of *ex vivo* expanded, third party, MSC at the time of high-risk HCT, may reduce GVHD. However, the benefit of reduced incidence of GVHD with co-infusion of MSCs was offset by increased relapse in patients who received HLA-identical sibling matched HCT [81]. In another study, MSC co-infused with haploidentical HCT did not change the incidence of GVHD nor impact overall survival, relapse-free survival, non-relapse mortality (NRM), relapse or infection incidence [82]. As treatment for refractory acute GVHD, bone marrow-derived MSCs from third-party donors resulted in a 75% overall response rate compared to 42% in patients not infused with MSC, and did not impact the incidence of infection or tumor relapse [83]. A phase I/II trial is accruing patients to evaluate the safety and feasibility of bone marrow-derived MSC infusions to systemic corticosteroids for newly-diagnosed acute GVHD (NCT02379442).

Gene-modified T-cells

Feasibility and potential efficacy of introducing suicide genes into allogeneic T-cells to allow induction of cell death in the event of acute GVHD was established in phase I-II studies using virus-derived genes [84]. Subsequently, to avoid potential immunogenicity, an alternative suicide gene was developed by fusing an inducible human caspase 9 gene (iCasp9) to human FKBP12 (Figure 1) [85]. In five pediatric patients who had haploidentical HCT and received infusions of iCasp9-expressing T-cells, skin GVHD developed in four patients and concomitant liver GVHD occurred in one patient [86]. Each of these four cases of GVHD resolved within 24 hours of infusion of the dimerizer drug, AP1903, to induce death of iCasp9 T-cells [86]. On follow-up of 10 patients, long-term persistence of iCasp9 T-cells was noted. T-cell reconstitution was accelerated, and provided sustained protection from major pathogens without inducing acute or chronic GVHD [87]. A safety/efficacy study is underway to determine the optimal dose of haploidentical iCasp9-modified T-cells

to deliver and assess immune reconstitution and relative contributions of endogenous and infused gene-modified T-cells (NCT01494103).

Cyclophosphamide

Delivered post-transplantation, cyclophosphamide induced stable mixed chimerism and reduced GVHD following non-myeloablative conditioning in experimental models of MHC-mismatched bone marrow transplantation [88]. Clinically, post-transplantation cyclophosphamide has been studied in combination with tacrolimus-MMF after non-myeloablative haploidentical HCT [89], alone after myeloablative HLA-matched donor (related or unrelated) HCT [90], and with cyclosporine-MMF after myeloablative haploidentical HCT [91]. However, in a recent comparison with matched historical controls, patients who received post-transplantation cyclophosphamide alone after reduced-intensity conditioning and HLA-matched (related or unrelated) HCT, the incidence of acute GVHD, NRM, and overall survival were worse with post-transplantation cyclophosphamide than with tacrolimus-methotrexate prophylaxis [92]. Further studies aim to more clearly define the optimal patient population, conditioning intensity, and graft source of post-transplant cyclophosphamide for GVHD prevention, including investigating its use in combination with cyclosporine after myeloablative conditioning (NCT01427881) or with tacrolimus-MMF after myeloablative or reduced-intensity conditioning (NCT01010217). One recent trial also demonstrated that post-transplantation cyclophosphamide and bortezomib were feasible and may be effective GVHD prophylaxis after reduced-intensity transplantation from matched donors [93].

Bortezomib

Bortezomib is a dipeptide boronic acid that blocks NF- κ B activation (Figure 1) to reduce activation, proliferation, and survival of T-cells and abrogate GVHD [94]. Clinically, bortezomib can control GVHD, but timing may be important [95]. Bortezomib combined with tacrolimus-methotrexate in high-risk patients undergoing reduced-intensity conditioning HLA-mismatched unrelated donor HCT resulted in encouraging incidences of grade 2–4 acute GVHD, chronic GVHD, NRM, relapse, and overall survival [96]. A randomized phase II trial of bortezomib combined with either tacrolimus-methotrexate or tacrolimus-sirolimus compared with tacrolimus-methotrexate in this patient population is underway (NCT01754389). A recent phase I study also reported that bortezomib in combination with high-dose cyclophosphamide after HCT from matched siblings or unrelated donors after reduced-intensity conditioning was safe and resulted in encouraging levels of acute GVHD that merit further evaluation [93].

Histone deacetylase inhibition

Histone deacetylase (HDAC) inhibition leads to accumulation of hyper-acetylated histones and alterations in gene transcription and expression (Figure 1). In pre-clinical HCT models, HDAC inhibitors suppressed pro-inflammatory cytokine production, reduced GVHD, and preserved GVL by modulating indoleamine-2,3-dioxygenase-dependent APC function in a STAT-3-dependent manner [97]. HDAC inhibitors also enhanced natural T_{reg} functions [98]. Clinically, use of the HDAC inhibitor vorinostat, delivered as GVHD prophylaxis in combination with tacrolimus-MMF after reduced-intensity conditioning HCT demonstrated

favorable day 100 incidence of grade 2–4 acute GVHD and 2-year relapse and overall survival rates [99]. Consistent with pre-clinical findings, vorinostat reduced circulating proinflammatory cytokines, increased the number and function of T_{reg}, increased STAT-3 acetylation, and induced indoleamine-2,3-dioxygenase [100]. Safety and efficacy of vorinostat, combined with tacrolimus-methotrexate, after myeloablative, unrelated donor HCT is currently underway (NCT01790568).

Atorvastatin

Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (Figure 1) and have effects on both T-cell and APC functions [101]. There have been inconsistent findings of the importance of statin use on GVHD outcomes [102],[103]. Interestingly, treatment of the HCT donor alone, or both donor and recipient, with any statin was recently shown to significantly reduce the incidence of grade 3–4 acute GVHD [104]. Atorvastatin is currently being investigated in phase II studies (NCT01665677, NCT01491958, NCT01527045).

Therapies for chronic GVHD

The choice of agents already used for prophylaxis against and/or the treatment of acute GVHD has bearing on the treatment of chronic GVHD, as do patient characteristics and institutional practice. A combination of systemic corticosteroids and a calcineurin inhibitor are most commonly employed as first-line therapy in patients with chronic GVHD. This treatment is recommended if 3 or more organs are involved or any single organ has a severity score of more than 2 [13]. If the response to this initial treatment is inadequate, additional immunosuppressants may be required [13].

Mycophenolate and phototherapy are two agents that are commonly employed in the treatment of chronic GVHD. Despite its common use, a recent double-blind, randomized, multicenter trial determined that the addition of mycophenolate to initial systemic treatment of chronic GVHD did not improve the efficacy of treatment [105]. Whether there is a beneficial role for mycophenolate in cases of refractory chronic GVHD requires additional investigation. Phototherapy can be effective treatment for chronic GVHD, and is administered either to the skin as PUVA or as extracorporeal phototherapy (in which leukocytes are obtained from the peripheral blood by apheresis, incubated with 8-methoxypsoralen, irradiated, and then re-infused to the patient). Significant responses have been observed in high-risk patients, with the best responses observed in skin, liver, oral mucosa, eye, and lung [106]. Experimental models suggest that these effects may result from increased production of IL-10 and the induction of T_{reg} cells [107, 108]. However, a small analysis of patient samples suggested that the positive effect of extracorporeal photopheresis may depend on a more generic re-adjustment of immune homeostasis [109].

Many emerging therapies for the treatment of chronic GVHD are agents that have been tested previously for the prophylaxis of acute GVHD, including bortezomib, rituximab, sirolimus, pentostatin, and low-dose IL-2. One recent study of bortezomib plus prednisone for initial therapy of chronic GVHD demonstrated an 80% response rate at week 15, and a decrease in median prednisone dose from 50 mg/day to 20 mg/day. The highest response

rates were observed in the skin (73%) and GI tract (75%), followed by liver (53%) [110]. Further studies will be required to further define the impact of bortezomib as add-on therapy for chronic GVHD.

The efficacy of rituximab in chronic GVHD suggests a potential role for B cells in its pathogenesis [111]. The GITMO (Gruppo Italiano Trapianti di Midollo Osseo) study reported an overall response rate of 65% in patients who received rituximab for refractory chronic GVHD [82, 83]. Overall, rituximab was well-tolerated and toxicity was limited primarily to infectious events [83].

Therapy with low-dose IL-2 has been investigated as a means to increase T_{reg} cells in patients with chronic GVHD [112]. Daily administration of IL-2 for a period of 8 weeks expanded T_{reg} in the periphery and augmented thymic T_{reg}. These findings were associated with clinical improvement in chronic GVHD manifestations and glucocorticosteroid dose reductions [112, 113].

The BMT CTN is also planning a phase II-III multi-center, randomized trial to compare various treatment combinations. It is hoped that this trial will inform more uniform and efficacious treatment of chronic GVHD.

Supportive care

Finally, supportive care and monitoring are vital components of GVHD management with emphasis on infection prophylaxis, physical therapy, nutritional status, pain control, and monitoring of drug-drug interactions and drug-related adverse effects [4]. Early recognition of high-risk features, such as thrombocytopenia, progressive onset chronic GVHD, extensive skin involvement with sclerodermatous features, and multiorgan involvement are also important considerations in the overall management [11]. Suggested monitoring interval and tests in adults have been published, and are also available for children and adolescents [114, 115]. NIH-sponsored consensus Working Groups have provided a comprehensive guideline for ancillary and supportive therapies in patients with chronic GVHD [116]. The National Marrow Donor Program has also issued long-term follow-up guidelines for survivors of allogeneic hematopoietic cell transplantation (HCT), available on their website (<https://bethematch.org/For-Patients-and-Families/Life-after-transplant/Guidelines-for-long-term-care/>, last accessed 14 July 2015).

Conclusion and Future Perspective

As our population ages and increasingly carries higher co-morbidities, and as the number of patients receiving transplants from unrelated donors increases, the need for strategies to prevent – and treat – GVHD effectively is of paramount importance. CNI-based GVHD prophylaxis remains the standard of care. Newer therapies are showing promise and several are currently being tested in multi-center, randomized trials through the BMT CTN. In addition to identifying and testing new therapeutic approaches, recent work has also focused on early identification of patients at high risk for NRM due to GVHD, using plasma biomarkers to define prognostic risk strata for newly diagnosed acute GVHD [117]. Similar efforts have also been proposed in the context of chronic GVHD [118]. Implementing

biomarker-based GVHD scores, as opposed to, or in addition to, clinical GVHD scores, may have future utility in guiding risk-adapted therapeutic approaches at GVHD onset.

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Practice Points

- GVHD arises when donor T lymphocytes respond to mismatched protein antigens expressed on host T-cells.
- Acute GVHD typically occurs within the first 100 days after transplant, but it can also present later as late-onset acute GVHD. The organs principally affected in acute GVHD include the skin, liver and gastrointestinal tract.
- Chronic GVHD is a complex, multisystem disorder with myriad manifestations that can involve essentially any organ. Older recipient age and the occurrence of acute GVHD are the most important risk factors for chronic GVHD.
- Calcineurin inhibitor-based therapies have been the standard-of-care for GVHD prevention since the late 1980s. However, despite prophylaxis strategies, 40–70% of patients remain at risk for developing GVHD.
- Novel approaches for the prevention of acute GVHD, that target T-cells, B-cells, and inflammatory mediators known to be important in the pathogenesis of GVHD, or increase regulation of inflammation by T regulatory cells, are being investigated and may allow broader clinical application of allogeneic HCT.
- The agents used for prevention or treatment of acute GVHD influence the treatment of chronic GVHD, as do patient characteristics and institutional practice. The most common first-line therapy for chronic GVHD is a combination of systemic corticosteroids and a calcineurin inhibitor.
- Supportive care, monitoring and early recognition of high-risk features are also critical components of GVHD management.

Figure 1.

Mechanisms of actions of agents to prevent and/or treat GVHD. The medications and their cellular targets are illustrated. Infusions of T regulatory cells (T_{reg}) and mesenchymal stem cells (MSC) are depicted extracellularly. Abbreviations: ADA, adenosine deaminase; ATG, anti-thymocyte globulin; CCR5, C-C chemokine receptor 5; CTLA4, cytotoxic T lymphocyte antigen 4; Cy, cyclophosphamide; DHFR, dihydrofolate reductase; FKBP12, FK506 binding protein 12; GVHD, graft-versus-host disease; HAT, histone acetyltransferase; HDAC, histone deacetylase inhibitor; HMG CoA reductase, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase; iCasp9, inducible caspase 9; I κ B, nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor; IL, interleukin; IMPDH, inosine monophosphate dehydrogenase; MHC II, major histocompatibility class II; mTORC, mammalian target of rapamycin complex; NFATc, nuclear factor of activated T-cell cytoplasmic; TNFR, tumor necrosis factor receptor.

Table 1

Mechanisms of actions of agents for the prevention of GVHD

<i>Standard Agents</i>	<i>Molecular Target</i>
<i>T-cell targeted agents</i>	
Cyclosporin	Cyclophilin-calcineurin
Tacrolimus	FKBP12-calcineurin
Methotrexate	Dihydrofolate reductase
Mycophenolate mofetil	Inosine monophosphate dehydrogenase
<i>Investigational Approaches</i>	
<i>T-cell targeted agents</i>	
Sirolimus	FKBP12-mTOR
Anti-thymocyte globulin	Surface antigens on T-cells
Alemtuzumab	CD52 receptor
Pentostatin	Adenosine deaminase
CTLA4-Ig	CD80, CD86
Regulatory T-cell infusion	Multiple interactions, innate and adaptive immunity
<i>B-cell targeted agents</i>	
Rituximab	CD20
<i>Chemokine/cytokine targeted agents</i>	
Maraviroc	CCR5 receptor
Etanercept	TNF- α
Daclizumab	IL-2R α
Basiliximab	IL-2R α
Tocilizumab	IL-6R
<i>Other novel agents</i>	
Mesenchymal stem cells	Multiple interactions, innate and adaptive immunity
Suicide gene-modified T-cells	Inducible caspase 9
Cyclophosphamide	Guanine base of DNA
Bortezomib	26S proteasome
Vorinostat	Histone deacetylases
Atorvastatin	HMG Co-A reductase