HOSPITAL PHYSICIAN®

HEMATOLOGY BOARD REVIEW MANUAL

### STATEMENT OF EDITORIAL PURPOSE

The *Hospital Physician Hematology Board Review Manual* is a study guide for fellows and practicing physicians preparing for board examinations in hematology. Each manual reviews a topic essential to the current practice of hematology.



# Diagnosis and Management of Acute and Chronic Graft-versus-Host Disease

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## Table of Contents

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## INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment option for several hematologic malignancies and other congenital diseases including immunodeficiencies or hemoglobinopathies. When the first allografts were performed, most patients given bone marrow (BM) from donors other than homozygotic twins developed skin, gut, and/or liver injury. This disease was defined by Billingham in 1966 as graft-versus-host disease (GVHD). He also described 3 standard tenets for GVHD pathophysiology, which remain valid today even with rapid advances in this area: (1) donor graft must have immune-competent cells, (2) recipient must be incapable of rejecting the graft, and (3) recipient must have tissue antigens not present in the donor.

GVHD is the most common cause of non-relapse mortality (NRM) after allo-HSCT and is associated with significant morbidity, escalated and prolonged immunosuppression therapy (with increased risk of infectious complications), organ dysfunction, and impaired quality of life (data summarized at Center for International Bone Marrow Transplant Research [CIBMTR] Web site: www.cibtmr.org).[1] Clinically significant acute GVHD (aGVHD) continues to affect up to 70% of patients receiving allo-HSCT. The incidence of chronic GVHD (cGVHD) can be as high as 75% in HSCT recipients who survive 100 days. Although cGVHD is associated with lower relapse rates of the primary malignant disease,[2] severe cGVHD results in higher NRM, up to 12%.[3] Importantly cGVHD is associated with significant morbidity, organ dysfunction, and impaired quality of life and increased incidence of secondary malignancies.[3]

## DEFINITIONS OF ACUTE AND CHRONIC GVHD

GVHD has classically been distinguished as aGVHD, which arises before the 100 days post-HSCT mark (D100), or cGVHD, which occurs after D100. However, these 2 classical forms are separate pathophysiological entities, and thus the classical definitions of aGVHD and cGVHD were revised in 2005 in the National Institutes of Health's (NIH) new classification. The definitions are now based on distinct clinical characteristics rather than time post-transplantation. cGVHD can occur either as an extension of aGVHD (progressive), after a disease-free interval (quiescent), or with no previous aGVHD (de novo). However, characteristic symptoms of cGVHD can occur before D100, and aGVHD can present beyond 3 months post-transplant, particularly after a reduced-intensity conditioning regimen. With the new classification, 2 new GVHD categories were also recognized: late-onset aGVHD (after D100) and overlap syndrome, which has features of both aGVHD and cGVHD (**Figure 1**).[4] Studies aimed at validating the NIH consensus criteria have shown that the incidence of cGVHD with the new criteria was 20% to 45% lower than with the conventional D100 landmark.[5–7]

## PATHOGENESIS

### TRIGGERS FOR GVHD INDUCTION

#### Disparities Between Histocompatibility Antigens

Tissue compatibility is determined by genes of the human leukocyte antigen (HLA) system on the short arm of chromosome 6. The haplotype describes the group of HLA genes that are inherited together from each parent. The basic rule in HLA inheritance is that a patient has a 25% chance of sharing the same 2 parental haplotypes with any 1 of their siblings, and a 50% chance of sharing only 1 haplotype with a sibling. This rule may be faulted by the linkage disequilibrium, which means that certain alleles occur together with a greater frequency than would be expected by chance (nonrandom gametic association). High-resolution typing pre-transplant is mandatory to detect such linkage disequilibrium before sibling transplant and to avoid mismatches.

The homologous HLAs corresponding to major histocompatibility complex (MHC) class I (HLA-A, -B, -C) and

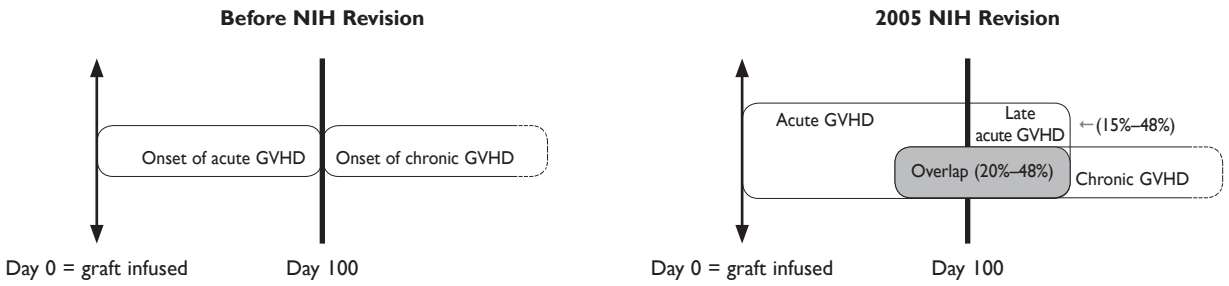


**Figure 1.** National Institutes of Health (NIH) classification of graft-versus-host-disease (GVHD).

class II (HLA-DR, -DQ, -DP) are co-dominantly expressed and differ in their structure, tissue distribution, and peptide presentation characteristics. MHC class I molecules are expressed on most nucleated cells, and peptides presented by these molecules are derived from degradation of cytoplasmic proteins by the proteasome. MHC class II molecules are expressed on antigen-presenting cells (APCs; eg, dendritic cells, monocytes, macrophages, B cells), which bind external antigens that have been internalized by endocytosis/phagocytosis and degraded to peptides that are recognized by CD4+ helper cells. Even in an HLA-matched setting, there is a substantial risk of GVHD mediated by incompatibilities of minor histocompatibility antigens (mHAs). The expression of mHAs is wide and variable. Thus, different mHAs may dictate variable phenotype, target organ involvement, development kinetics of GVHD, and antitumor responses after allogeneic HSCT.[8] Notably, not all mHAs induce equivalent immune responses.

### Molecular Damage Induced by Conditioning Regimen

Acute GVHD is an exaggerated but typical inflammatory mechanism mediated by activated donor immune cells infused into a genetically disparate recipient. This innate immune response is triggered by conditioning regimens that cause mucosal damage. Tissue injury induces release of tissue factor, pro-inflammatory cytokines (eg, interleukin [IL]-1, tumor necrosis factor [TNF]-α, IL-6, and other interferon [IFN] family members),[9,10] and surface antigens associated with malignancy, all of which can act as a molecular pattern triggering inflammation pathways that play a critical role in GVHD pathogenesis by enhancing presentation of self-peptides to donor immune cells.[11,12] This process is described as a *cytokine storm*. Related to conditioning toxicity, lipopolysaccharides from bacterial translocation in gut may induce similar inflammatory proteins.[13]

### Sensors of GVHD

Dendritic cells are described as "professional" APCs and are essential for initiation of the clonal expansion of alloreactive T cells. Every residual host APC primed by conditioning can directly present alloantigen.[14] As a result of conditioning and the GVHD response, in the days or weeks after transplantation donor APCs may assume this role and gain access to recipient mHAs in surrounding host tissues.[15,16] Professional APCs have the ability to present endogenous and exogenous antigens to donor CD8+ and CD4+ cells, respectively. Recent data support the involvement of recipient non-hematopoietic APCs, such as mesenchymal cells, in the initiation and maintenance of lethal GVHD.[17] A second signal through T-cell costimulatory molecules and their ligands on APCs is essential to achieve T-cell activation, proliferation, differentiation, and survival.[18] An in vivo blockade of these positive costimulatory molecules (eg, CD28, inducible costimulator, and CD40) reduces GVHD,[19–21] whereas blockade of inhibitory signals, such as programmed death-1 and cytotoxic T-lymphocyte antigen 4, exacerbates aGVHD in murine models.[22,23]

## MEDIATORS OF GVHD

The critical role of T cells in the pathophysiology of aGVHD is established. Evidence suggests that alloreactive donor T cells consist of several subsets with different stimuli responsiveness, activation thresholds, and effector functions.

### CD4+ and CD8+ T Cells

The CD4+ T cells interact with MHC class II molecules of APCs, whereas CD8+ T cells interact with MHC class I antigens. It is currently believed that these subsets are the terminal effector of GVHD. As mentioned previously, aGVHD as well as an antitumor effect may be induced by CD4+ T cells, CD8+ T cells, or both subsets responding to mHAs. Several studies investigated the T-cell repertoire that emerges during GVHD to determine if a few clones could be involved in the disease, and thus if a specific depletion of donor inoculum could prevent GVHD. If data suggest that a restricted repertoire is involved, the

GVHD-associated peptide molecules have yet to be precisely defined.[24,25]

### Regulatory T Cells

FOXP3 is a member of the forkhead/winged-helix family of transcription factors and is identified as the lineage-defining transcriptional master regulator in regulatory T cells (Tregs), which represent a subset of CD4+ cells. Tregs have the regulatory function of effectively down-regulating the immune response in order to prevent autoimmunity, suppress uncontrolled immune response, and induce immunologic tolerance.[26] In murine models of GVHD, depletion of Tregs from splenocytes is sufficient to increase GVHD severity and lethality after MHC-mismatched transplantation. These results provided the first evidence that Tregs transplanted at physiological ratios (ie, as constituents of the peripheral T-cell pool) ameliorate the detrimental effects of alloreactive donor CD4+ and CD8+ effector T cells in the host.[27]

### EFFECTORS AND AMPLIFIERS

The effector phase that leads to GVHD target organ damage is a complex cascade involving cytolytic cellular effectors such as CD8+ lymphocytotoxic cells (CTLs), CD4+ T cells, natural killer cells, and the inflammatory molecules detailed below. The cytotoxic activity of cellular effectors requires achievement of immunologic synapse to target tissues through activation of perforin/granzyme (cytotoxic granule stored in CTLs is released upon recognition of target cells to induce lysis by perforation of target cell membrane) or Fas/FasL or TNFR/TRAIL pathways, which trigger a death-inducing signal complex.[28] Various danger signals are a major stimuli for inducing cytokine generation and initiating the innate and then adaptive immune response leading to GVHD. Therefore, a pro-inflammatory cytokine environment is still critical for amplifying cellular damage in GVHD, and cytokines are important drivers of pathology and "protection" after HSCT. The major cytokines promoting GVHD pathology are IFN-γ, TNF-α, and IL-1. The regulatory cytokines countering these pathogenic cytokines are IL-10 (produced by Tregs) and, early after transplant, transforming growth factor β.[29]

### CHRONIC GVHD: DISTINCTIVE PATHOPHYSIOLOGIC FEATURES

Although animal models have been useful in understanding aGVHD pathophysiology, such models do not reflect the complexity of cGVHD. Chronic GVHD is considered an allo- and autoimmune disorder characterized by dysregulation of the immune system caused by donor-derived reactive CD4+ and CD8+ T-cell populations, leading to organ injury. Some studies support a role for alloreactive CD4+ T cells that arise in impaired thymus via negative selection in the pathophysiology of cGVHD. The involvement of B lymphocytes in cGVHD was highlighted by cGVHD patients who responded to B-cell depletion therapy based on rituximab.[30] There have been many examples of autoantibody formation in patients with cGVHD, particularly antibody responses to H-Y mHA,[31] which is consistent with findings showing that BAFF levels (cytokines acting as a B-cell activator) are higher in patients with cGVHD than in those without.[32,33] Another hallmark of cGVHD pathophysiology is the association between the immune response and development of fibrosis, especially in skin.[34]

## DONOR AND GRAFT CHARACTERISTICS

### DONOR SOURCE

When a sibling donor shares with a recipient the same 2 haplotypes inherited from their parents, he/she is considered HLA genotypically identical and defined as a matched related donor (MRD) compatible for HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 at the allele level at all loci on both chromosomes; this is considered a full *12/12 match donor.* A matched unrelated donor (MUD) is matching for HLA-A, -B, -C, -DRB1, and -DQB1, and defined as a *10/10 match donor.* A haploidentical donor shares only 1 haplotype inherited from a first-degree parent of the recipient and is considered a *6/12* HLA match donor. First-degree relatives imply biological parents, siblings, half siblings, or children.

There is a direct association between the number of donor–recipient HLA mismatches and the risk for NRM. However, transplant outcome studies have shown that not all HLA mismatches result in a deleterious clinical outcome. For instance, in a large cohort of 3860 transplantations with myeloablative conditioning, Lee and colleagues demonstrated that each mismatch is associated with a 9% to 10% decrease in survival, and single mismatches for HLA-A or -DRB1 may be more poorly tolerated than those for HLA-B and -C.[35]

In certain circumstances where care should be taken not to prolong the donor search unnecessarily (especially for advanced stage leukemia), a single-locus mismatched donor can be used with acceptable risks of transplant-related mortality while improving graft-versus-leukemia effect. This is termed a "permissible" mismatch. Another recent study from the CIBMTR showed that mismatches in alleles C*03:03/C*03:04 were most frequent (68.7%) among the transplants with a single allele level mismatch in HLA-C, and that the 7/8 C*03:03/C*03:04 mismatch group was not significantly different from the 8/8 HLA matched transplants in any transplant outcome.[36]

In the United States, 75% of Caucasian patients have an HLA-A, -C, -B, or -DRB1–matched donor, and 94% have a donor with a single HLA mismatch. Among African Americans, 31% of patients have an HLA-matched donor, and 69% have a donor with a single HLA-mismatch.[37] However, MRDs or MUDs cannot be identified or mobilized in time for as many as 40% to 50% of patients, and the availability of MUDs can be quite low for ethnic minorities and patients with mixed-race backgrounds. In contrast, almost all patients have an available related donor with whom they share a single HLA haplotype. One study reported that at least 1 HLA-haploidentical first-degree relative could be identified for more than 95% of patients.[38]

Another potential source of transplantable stems cells is umbilical cord blood (UCB). HLA matching for unrelated UCB transplantation generally focuses on 3 loci (HLA-A, -B, and -DRB1) and has less strict matching criteria: a UCB unit must match at least 4 to 6 markers at HLA-A, -B, and -DRB1.[39] A collaborative work by the CIBMTR and Eurocord established that additional matching at HLA-C may improve outcomes.[40] Together, UCB and haploidentical transplantation are efficient ways to address the issue of availability of well HLA-matched donors.

The cell dose is a critical determinant of hematopoietic recovery and early mortality after UCB transplantation because the graft cell dose is directly correlated to the time to engraftment. Classically, UCB transplants have had a longer time to engraftment as compared to conventional transplant and thus lead to higher risk for infections during the first month. In addition, due to a higher number of naïve T cells in the UCB graft, the immune reconstitution is delayed as well. One strategy to increase the number of UCB cells for recipients weighing more than 50 kg is to infuse 2 UCB units.[41] Ponce and colleagues reported similar survival among double-UCB, MRD, and MUD patients.[42] Several other alternatives to augment the number of cells in UCB such as UCB expansion strategies, enhancing the homing of UCB stem cells, and combining a haplo-transplant to support the engraftment are being investigated. These strategies have recently been reviewed.[43]

### STEM CELL AND T-CELL SOURCES

The graft contains both stem cells and donor T cells. Currently, 3 stem cell sources are available for allo-HSCT: BM, granulocyte-colony stimulating factor–mobilized peripheral blood stem cells (G-PBSCs), and UCB. The T-cell–replete haploidentical HSCT can be processed either with BM or PBSCs, depending on the conditioning and prophylaxis of GVHD.

Several large randomized trials of transplantation between HLA-identical siblings showed that PBSCs resulted in better engraftment but increased the risks of cGVHD. Prospective studies have shown rapid engraftment using G-PBSCs. Concerning unrelated donors, the recent Blood and Marrow Transplant Clinical Trials Network (BMT CTN) randomized trial did not show significant differences in survival or aGVHD between the 2 major cell sources, but BM transplants tended to be associated with less extensive cGVHD and an average 5 and 7 days longer time to neutrophil and platelet engraftment, respectively.[44] The main differences between the cell sources are summarized in **Table 1**.

A BMT CTN randomized study is currently open to accrual (BMT CTN 1101) to compare progression-free-survival at 2 years in patients with hematologic malignancies receiving double unrelated UCB (dUCB) versus HLA-haploidentical related bone marrow (haplo-BM) in a reduced-intensity setting.

Beyond using genetics and disease status of the malignancy to select a donor, finances should be considered. The cost of collecting cells from the donor, his/her medical tests, and possible travel expenses can be high. The average cost is $3500 to $5000 for a related donor, $15,000 to $50,000 for an unrelated donor, and up to $50,000 for only 1 UCB unit. When available, MRDs are always preferred over other donor sources. The algorithm for donor choice used in most BMT centers when matched sibling donors are not available is summarized in **Figure 2**.

## ACUTE GVHD

### CLINICAL FEATURES AND DIAGNOSIS

Historically, aGVHD and cGVHD have been distinguished by the time of onset after transplantation. As discussed, NIH consensus emphasizes use of clinical features for this purpose given the separate pathophysiology of these 2 disease entities.[4] The NIH consensus also defines 2 new entities, namely late-onset aGVHD and overlap syndrome, as outlined above.

Acute GVHD most commonly involves the skin, liver, and gastrointestinal (GI) tract. Rash is usually the first and most common manifestation of aGVHD and can occur early with engraftment of donor cells. It is usually characterized by maculopapular lesions, which can be intensely itchy or painful. Rash usually starts at the shoulders and neck, palms of hands, and soles of feet, and it may spread to all parts of the body in a morbilliform fashion. In severe cases, rash can develop into bullous lesions with toxic epidermal necrolysis and extensive desquamation. Skin biopsy is frequently obtained to correlate histological and clinical findings supporting the diagnosis or to rule out an alternative etiology like drug-related skin eruption. The classic his-

**Table 1.** Stem Cell Sources

| |
|---|
| **Bone Marrow** |
| Harvested under anesthesia |
| Engraftment delayed compared to PBSCs[44] |
| Average cost = $10,000 |
| **PBSCs** |
| Easily harvested, no requirement for anesthesia but unknown late effect of G-CSF for donor |
| Average cost for unrelated donor = $40,000 |
| **Umbilical cord blood** |
| Easily harvested, cryopreserved units are readily available |
| Acceptable partial mismatches |
| No possibility for donor lymphocyte injection |
| Delayed engraftment compared to bone marrow and PBSCs, leading to higher risk of infection if the cell dose is not increased by other means |
| Delayed immune reconstitution and loss of T-cell repertoire diversity leading to higher risk for viral infections[45] |
| Average cost = $50,000 per unit |

G-CSF = granulocyte-colony stimulating factor; PBSC = granulocyte-colony stimulating factor–mobilized peripheral blood hematopoietic stem cells.

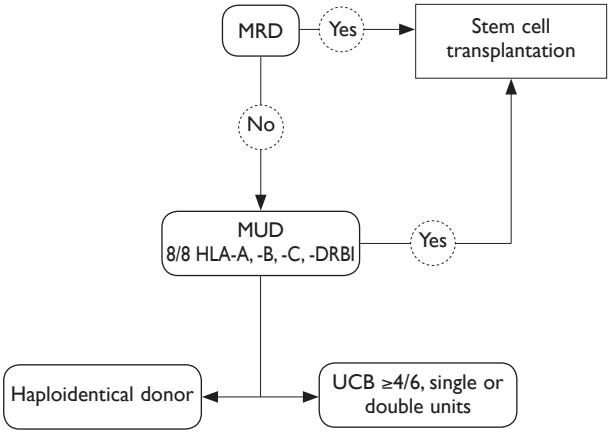


**Figure 2.** Algorithm for donor choice. CMV = cytomegalovirus; MRD = matched related donor; MUD = matched unrelated donor; UCB = umbilical cord blood.

tological features of aGVHD include apoptosis at the base of epidermis, dyskeratosis, and infiltration of lymphocytes.

Acute GVHD of the GI tract can involve both the small and large intestines. Upper GI involvement is more frequent; manifests as nausea, vomiting, anorexia, and satiety; and has a better prognosis. Acute GVHD of the lower GI tract manifests as secretory and profuse diarrhea and abdominal cramps; hematochezia indicates mucosal ulceration and poor prognosis.[46] Imaging studies show small bowel wall thickening and luminal dilatation (reported as "ribbon sign" on CT scan) and air-fluid levels suggestive of ileus. Histopathology, through endoscopy, colonoscopy, or rectal biopsy, is required to establish the diagnosis and to rule out infectious etiologies like infection with cytomegalovirus or *Clostridium difficile* or mucositis related to conditioning regimens. Histologic findings include patchy ulceration, apoptotic bodies and microabscesses in crypt bases, and loss of epithelial surface.

Acute GVHD of liver mostly presents with jaundice and cholestatic findings on hepatic function tests and sometimes tender hepatomegaly. Differential diagnosis usually includes veno-occlusive disease, toxicity from conditioning or other treatments, and infectious hepatitis. Histological features include bile duct and endothelial damage and lymphocytic infiltration of portal areas. Obtaining histopathologic confirmation of diagnosis, however, can be challenging as most patients still have thrombocytopenia following HSCT. Isolated aGVHD of liver is rare; other diagnoses should be strongly pursued in the absence of skin or GI involvement, and hepatic GVHD should be a diagnosis of exclusion.

Although skin, GI tract, and liver are the most common sites, alloreactivity has been reported to target other organs. Approximately 5% of HSCT patients experience idiopathic pneumonia syndrome within 100 days of HSCT. Mortality is high and an immunologic mechanism similar to aGVHD has been implicated. The central nervous system has also been demonstrated to be a target of aGVHD in animal studies. Acute GVHD is frequently associated with hypogammaglobulinemia, impairment of thymic function, and propensity for infection.

Pitfalls in diagnosing aGVHD include the absence of reliable laboratory tests and heavy reliance on clinical and histological findings. Several promising biomarkers are under investigation and may yield diagnostic, predictive, and prognostic information.

Two systems are used most commonly to clinically determine the severity of aGVHD (**Table 2**). The Glucksberg system, which was proposed in 1974 and modified in 1994, grades severity from 1 (mild) to 4 (life threatening).[47,48] The CIBMTR Severity Index considers grades on a scale of A to D.[49] Severity at specific sites is stratified into 4 stages (Table 2). The clinical grading of aGVHD should not be confused with the histological grading reported.[50]

## PROPHYLAXIS

Prevention of GVHD focuses on targeting T cells; however, these T cells also mediate the allo-immune

**Table 2.** Systems for Determining Severity of Acute GVHD

| Organ Specific Severity | | |
|---|---|---|
| **Organ** | **Stage** | |
| Skin | 1 | Macular rash; <25% of body surface area |
| | 2 | Macular rash; 25%–50% of body surface area |
| | 3 | Generalized erythroderma |
| | 4 | Generalized erythroderma with bullous formation + desquamation |
| Liver | 1 | Bilirubin 2.0–3.0 mg/dL; AST 150–750 U |
| | 2 | Bilirubin 3.1–6.0 mg/dL |
| | 3 | Bilirubin 6.1–15.0 mg/dL |
| | 4 | Bilirubin >15.0 mg/dL |
| GI | 1 | Diarrhea >500 mL/day or >30 mL/kg or upper GI symptoms |
| | 2 | Diarrhea >1000 mL/day or >60 mL/kg |
| | 3 | Diarrhea >1500 mL/day or >90 mL/kg |
| | 4 | Diarrhea >2000 mL/day or >30 mL/kg or severe abdominal pain + ileus |
| **Modified Glucksberg Grading** | | |
| | Grade | |
| | I | Stage 1 or 2 skin without liver or GI involvement |
| | II | Stage 3 skin or stage 1 liver or GI involvement |
| | III | Stage 2 or 3 liver or stage 2–4 GI involvement |
| | IV | Stage 4 skin or liver involvement |
| **CIBMTR Severity Index** | | |
| | A | Stage 1 skin without liver or GI involvement |
| | B | Stage 2 skin or stage 1 or 2 liver or GI involvement |
| | C | Stage 3 skin, liver, or GI involvement |
| | D | Stage 4 skin, liver, or GI involvement |

AST = aspartate aminotransferase; CIBMTR = Center for International Bone Marrow Transplant Research; GI = gastrointestinal.

graft-versus-leukemia (GVL) effect that contributes significantly to malignant disease control after HSCT. GVL effect was first recognized in the 1970s when it was observed that patients with no or minimal GVHD experienced relapse more frequently.[51] T-cell-depletion from allograft was initially considered highly successful for prevention of GVHD; however, it fell out of favor due to increased rates of relapse attributable to impaired GVL effect, graft failure, and infections.[52] A more recent single center study of T-cell–depleted unrelated donor stem cell transplantation showed favorable disease-free survival for adults with hematologic malignancies.[53] Another option to remove T cells is the addition of antithymocyte globulin (ATG) to aGVHD prophylactic regimens among recipients of unrelated donor grafts after myeloablative conditioning.[54]

An ideal GVHD prophylaxis will prevent aGVHD as well as cGVHD, have minimal toxicity, permit early hematologic and immunologic reconstitution, and not interfere with GVL effect. Several regimens have been used for prevention of aGVHD. There is no consensus about which regimen to use, the dose, or schedule, and these choices mostly depend on institutional preferences. Historically, monotherapy with methotrexate or cyclosporine A was the most commonly used regimen for GVHD prevention.[55] However, a decrease in the incidence of grade II to IV GVHD and improvement in survival was observed with a combination of methotrexate and cyclosporine compared to monotherapy.[56] Tacrolimus (FK-506), a newer calcineurin inhibitor, in combination with methotrexate was shown to reduce the incidence of grade II–IV aGVHD following HSCT from related and unrelated donors.[57,58] It did not receive uniform acceptance due to lack of survival benefit with tacrolimus/methotrexate as compared to cyclosporine/methotrexate. Moreover, doses of methotrexate had to be omitted in approximately a third of patients due to toxicity, and effectiveness of preventing aGVHD was far from ideal. Therefore, the search for other regimens continued.

The combination of calcineurin inhibitors with mycophenolate mofetil (MMF) demonstrated a similar incidence of aGVHD, survival, lower toxicity, and earlier engraftment. However, concerns remain regarding its effectiveness in preventing severe aGVHD, especially among recipients of unrelated donor grafts.[59] MMF with cyclosporine or tacrolimus continues to be a promising regimen for prophylaxis against aGVHD in HSCT following nonmyeloablative conditioning.[60] Combination tacrolimus and sirolimus, an mTOR inhibitor, has also demonstrated promising results.[61] An ongoing phase III trial, BMT CTN 0402, is comparing the combinations of tacrolimus/sirolimus versus tacrolimus/methotrexate following HSCT from MRDs.

Since 2008, Luznik and colleagues have been developing an innovative method to promote tolerance in alloreactive host and donor T cells, leading to effective improvement in GVHD after HSCT. This method is based on high-dose post-transplantation cyclophosphamide (PTCy) and consists of an in vivo selective depletion of alloreactive T cells.[62] In a 68-patient cohort, aGVHD of grades II–IV and III–IV occurred in 34% and 6% of patients, respec-

tively, and cGVHD developed in 15%.[63] PTCy as GVHD prophylaxis was developed initially for haploidentical BM transplants, but recently the PTCy concept was applied in the setting of allogeneic BM from HLA-matched donors after myeloablative and reduced-intensity conditioning.[64] PTCy was used as a single agent for prophylaxis of GVHD, and the incidence of GVHD was remarkably low: 40% aGVHD grades II–IV, 10% aGVHD grades III/IV, and 10% cGVHD, showing the effectiveness of the approach.[63]

Exciting results have been reported with 2 new strategies. Maraviroc, a CCR5 antagonist that blocks T-cell chemotaxis, dramatically decreased the incidence of gastrointestinal and liver GVHD.[65] At the same time, drugs that block the central cytokine pathway such as interleukin-1 receptor antagonist, infliximab and etanercept, failed to improve rates of aGVHD.[66,67]

Two BMT CTN randomized phase II trials just opened to address GVHD prophylaxis in reduced-intensity and myeloablative conditioning. BMT CTN 1203 will compare 3 prophylaxis regimens in patients receiving reduced-intensity conditioning: tacrolimus/methotrexate/bortezomib, tacrolimus/MMF/cyclophosphamide, and tacrolimus/methotrexate/maraviroc for prevention of both aGVHD and cGVHD while allowing an effective GVL response and prompt immunologic reconstitution. The BMT CTN 1301 trial will compare 3 prophylaxis regimens in patients receiving myeloablative conditioning: tacrolimus/methotrexate, tacrolimus/MMF/cyclophosphamide, and CD34 selection.

## TREATMENT

Treatment of aGVHD depends on the severity of disease, sites involved, and risk-benefit ratio against reducing GVL effect. For grade I aGVHD, adjustment in GVHD prophylaxis and/or addition of topical steroids might be sufficient. GVHD prophylaxis should be restarted for patients who are no longer receiving it. For grade I aGVHD of skin, topical steroids (triamcinolone 0.1% or hydrocortisone 0.1%) are the preferred initial treatment. Topical tacrolimus can be considered a second-line therapy for cutaneous aGVHD. Nonabsorbable steroids, such as budesonide or beclomethasone, should be used for mild aGVHD of the upper GI tract. In a phase III trial, addition of oral beclomethasone to a short duration of systemic steroids reduced treatment failure and mortality among patients with mild aGVHD of the GI tract.[68]

Steroids are the mainstay of treatment for clinically significant aGVHD. When systemic steroids are needed for treatment of mild aGVHD, a low dose can be considered. In a retrospective analysis of patients with mostly grade I/II GVHD, 1 mg/kg/day of prednisone-equivalent resulted in similar survival, NRM, and need for secondary therapy and decreased the risk of fungal infections, gram-negative bacteremia, and hospitalization.[69] However, most patients require the standard dose of 2 mg/kg/day methylprednisolone or equivalent.[70] Higher doses of steroids have failed to improve outcomes.[71] Tapering of steroids should be initiated as soon as significant improvement occurs in aGVHD manifestations. The steroid dose is usually decreased by 0.2 mg/kg/day every 3 to 5 days, although a slower taper is pursued below 30 mg/day.

Protocols define steroid refractory aGVHD as (1) absence of improvement 7 days after the initiation of corticosteroids for skin GVHD or 72 hours after its initiation for GI and liver GVHD, or (2) progressive disease after 4 days of treatment. Progressive disease is an increase in organ stage by at least 1.[72,74] Although complete response has been objectively defined in different studies, time-points for assessment of complete response vary significantly. Similarly, definition of partial response also varies significantly. In many studies, partial response is defined as any reduction in overall grade of GVHD. These definitions and recommendations of the American Society for Blood and Marrow Transplantation have recently been reviewed by Martin et al.[70] Currently, the BMT CTN GVHD studies evaluate complete response or lack of it 28 days after initiation of treatment. Less than 50% of patients achieve complete response to steroids; a second-line treatment is warranted but less effective, leading to a poor prognosis.[73,74] Due to poor outcomes after treatment failure with steroids, several studies have tested combination of steroids with another immunosuppressant for initial treatment of aGVHD; however, this approach has not yet yielded any improvement in response.[73]

Several agents are used for treatment of steroid-resistant aGVHD, and the choice between agents is mostly a matter of institutional preference due to lack of prospective phase III data. If patients did not develop aGVHD while on prophylaxis with MMF, it can be an option. In a phase II trial comparing combination of steroids with MMF, denileukin diftitox, pentostatin, or etanercept, MMF demonstrated the highest response rate for steroid refractory aGVHD and had equal efficacy for skin, GI tract, and liver.[75] However, the BMT CTN phase III trial comparing the combination of MMF and steroids versus steroids alone showed futility. Addition of ATG to steroids has also been tried, without any improvement in response, transplant-related morbidity, or survival.[71] Several other agents including sirolimus and monoclonal antibodies against CD3 (visilizumab, muromonab-CD3), CD52 (alemtuzumab), CD25, IL-2Rα (basiliximab, daclizumab), and TNF-α (etanercept, infliximab) have shown promising responses in small trials that were not confirmed in larger trials.[76]

**Table 3.** Clinical Features of Chronic GVHD According to NIH

| | Diagnostic Features | Distinctive Features |
|---|---|---|
| Skin | Poikiloderma, lichen planus-like features, morphea-like features<br>Lichen sclerosis-like features | Depigmentation |
| | | Dystrophy, onycholysis |
| Nails | | |
| Mouth | Lichen-type features, hyperkeratotic plaques<br>Restriction of mouth opening from sclerosis | Xerostomia, ulcers, pseudo-membranes |
| Eyes | — | Keratoconjunctivitis sicca |
| | | New-onset dry, gritty or painful eyes |
| Genitalia | Lichen planus-like features, vaginal scarring, or stenosis | Erosions, fissures, ulcers |
| Gastrointestinal tract | Strictures or stenosis in the upper to mid third of the esophagus | — |
| Lung | Bronchiolitis obliterans diagnosed with lung biopsy | Bronchiolitis obliterans diagnosed with PFTs and radiology |
| Muscles, fascia, joints | Fasciitis, joint stiffness, or contractures secondary to sclerosis | Myositis |

Several small series have suggested a role for extracorporeal photochemotherapy (ECP) in steroid-refractory aGVHD, especially when started within 35 days from onset of symptoms.[77] ECP involves infusion of ultraviolet-irradiated, 8-methoxypsoralin-exposed lymphocytes collected from the patient through apheresis. ECP is thought to enhance apoptosis of effector lymphocytes and increase Tregs.[78] Mesenchymal stem cells (MSCs) stimulate Tregs and are being explored to treat aGVHD and promote engraftment after autologous as well as allogeneic HSCT. Although initial studies demonstrated promising results, it was not uniformly effective; further studies are warranted to determine a role for MSCs in aGVHD.[79,80] The application of ECP in cGVHD has been more extensively validated, as described below.

## CHRONIC GVHD

Chronic GVHD has a highly variable course. It can have very minor manifestations or can present as life-threatening disease, and it can involve a single organ/tissue or be widespread. It can present as an autoimmune disease (eg, Sjögren syndrome, scleroderma). Diagnosis aGVHD is based on both clinical and histopathologic findings for each organ system. We describe here the common definitions relevant for diagnosis of cGVHD as characterized by the NIH consensus criteria for clinical trials in chronic GVHD.[4]

### CLINICAL DIAGNOSIS

**Table 3** lists clinical signs and symptoms that establish the diagnosis of cGVHD without any further investigation or evidence of other organ involvement. Infections, drug reactions, recurrent or new malignancy related to immunosuppression, and even confluent erythema after sun exposure can frequently have similar manifestations. Therefore, obtaining histopathology is encouraged whenever the differential diagnosis is broad or diagnosis is unclear. If the hallmark signs described are absent, diagnosis may also be made by the presence of at least 1 distinctive clinical manifestation listed and biopsy confirmation or other relevant biological test. Also, the clinical features can be much more subtle than the standardized criteria for diagnosis of cGVHD, with a variety of less distinct manifestations. Moreover, some manifestations can occur in both aGVHD and cGVHD. Additional details of major organ-specific manifestations of cGVHD are described in the following sections.

#### Skin

Early lesions include most frequently xerosis or follicular prominence (rough bumpy texture) and less often ichthyosis or papulosquamous or pityriasis-like lesions. Later, lesions can appear as lichen-planus eruption and/or sclerotic features. The lichen-planus lesions are erythematous/violaceous flat-topped papules or plaques with inconspicuous squamous top. These features usually affect the dorsal aspects of the hands, forearms, and trunk,[81] but all body areas could be injured (**Figure 3**).

Sclerodermoid cGVHD includes different types of semiologic presentation, depending on the depth of epidermal and dermal injuries. These features are often associated with dyspigmentation. The lesions may have an inflammatory frame (lilac ring) and progress to coalescent plaques with ulceration induced by friction. Ultimately,

this poikiloderma feature may progress to adherence to the deepest layer of skin, leading to limited range of joint motion and thickened, tight, fragile skin with poor wound healing, inadequate lymphatic drainage, and skin ulcers with minor trauma.

Other skin modification may be confused with aGVHD, including erythema, maculopapular rash, and pruritus. Integument involvement is also present in up to half of patients with different grades of cGVHD,[81] possibly leading to nail loss.

### Mucous Membranes

Mucous membrane lesions are common (occurring in up to 80% of cGVHD patients[82]) and affect the mouth, genitalia, and eyes with dryness, atrophy, hypertrophy, lichenoid changes, lacy white patches, and erosion as well as ulcerative lesions. This spectrum of lesions can be incapacitating due to pain and significantly impact nutrition behavior, sexuality, and overall quality of life. Notably, xerostomia can increase dental decay and oral candidiasis. Moreover, previous studies reported that transplant recipients who develop cGVHD are at especially high risk for squamous cell carcinoma of the oral cavity and skin (relative risk of 2.35 in a case-controlled study conducted by CIBMTR) that justifies attentive clinical examination beyond each suspect lesion.[83]

### Liver

Diagnosis of hepatic cGVHD can be controversial due to its nonspecific biological features: cholestasis with increased bilirubin or alkaline phosphatase levels. It may also present as acute hepatitis. Note that these biologic abnormalities are not included in the list of signs that alone are sufficient for cGVHD diagnosis but are included in the organ scoring system for global severity assessment.

### Gastrointestinal Tract

Table 3 shows the diagnostic features in the upper GI tract including esophageal web, stricture, or concentric rings that are documented by endoscopy. Anorexia, nausea, vomiting, diarrhea, weight loss, and failure to thrive are common manifestations of both aGVHD and cGVHD. However, pancreatic exocrine insufficiency may be due to cGVHD and often improves with enzyme supplementation. Wasting syndrome should be closely monitored as a manifestation of cGVHD related to many factors, including poor absorption, decreased caloric intake, and hypercatabolism.

### Lungs

Chronic GVHD of lungs is classically characterized as bronchiolitis obliterans syndrome (BOS), and its diagnosis is only biopsy proven. This complication is rare but critical because 5-year post-transplantation survival following BOS was only 10% in a 2003 retrospective study of 1789 allogeneic transplantations in Minnesota.[84] In a recent study evaluating the NIH consensus cGVHD scale in patients enrolled on the National Cancer Institute's cross-sectional cGVHD natural history study, the NIH lung score (3 versus 0–2) appeared as one of the strongest predictors of poor overall survival, both alone and after adjustment for other important factors.[85] BOS diagnosed by spirometry and radiologic testing requires at least 1 other distinctive manifestation in a separate organ system to establish cGVHD diagnosis. The most frequent symptoms are cough, dyspnea, abnormal chest signs with crackles, and wheeze.

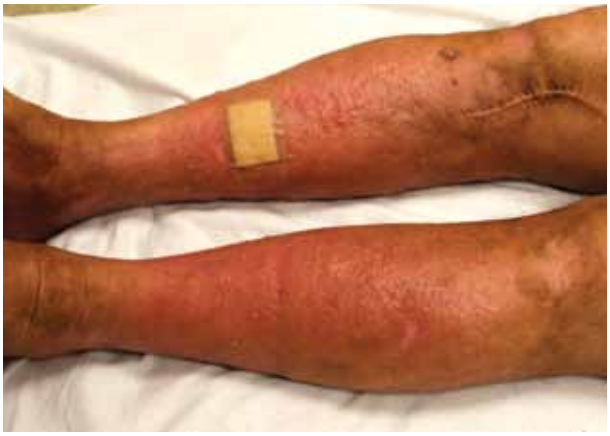

**Figure 3.** Cutaneous manifestations in chronic GVHD

### Immunohematologic Manifestations

An important complication associated with cGVHD is immunodeficiency, leading to susceptibility to wide ranges of opportunistic infections. Antimicrobial prophylaxis against *Pneumocystis jiroveci*, cytomegalovirus, and pneumococcus are crucial to prevent potential fatal infection. Chronic GVHD may have hematopoietic manifestations expressed through cytopenia, and these are poor prognostic factors. Thrombocytopenia (<100,000/μL) at the time of cGVHD diagnosis has been associated with a poor prognosis, as have lymphopenia (≤500/μL) and eosinophilia (≥500/μL).[86]

## ASSESSMENT AND SCORING

Staging of cGVHD presumes that its diagnosis, including overlap syndrome, has been confirmed through the use of the criteria described above. The NIH consensus development project on the criteria for clinical trials in cGVHD has reviewed staging of cGVHD. This document proposed a new clinical scoring system on a 4-point scale (0–3) with 0 representing no involvement, 1 mild involve-

ment (no significant impairment of daily living), 2 moderate involvement (significant impairment of daily living), and 3 severe impairment (major disability). Mild cGVHD involves only 1 or 2 organs (except lung) with a maximum score of 1. Moderate cGVHD involves at least 1 organ with a score of 2, or 3 or more organs with a score of 1 (or lung score 1). Severe cGVHD indicates a score of 3 in any organ (or score of 2 in the lung).

All patients should be scored periodically to assess efficacy of treatment and to avoid missing subtle early signs before progression to a highly morbid form of cGVHD. Note that the quality of this assessment relies on clinical experience and that some items require multidisciplinary skills. The complete approach for the reliable method of GVHD assessment is also demonstrated in a 30-minute instructional video (found at http://www.fhcrc.org/en/labs/clinical/projects/gvhd.html) provided by Paul Carpenter, MD, from the Fred Hutchinson Cancer Research Center.[87]

Regarding prognosis, Arora et al reported in 2011 a cGVHD risk score in which 10 variables were identified as significant in terms of overall survival and NRM: age, prior aGVHD, time from transplantation to cGVHD, donor type, disease status at transplantation, GVHD prophylaxis, gender mismatch, serum bilirubin, Karnofsky score, and platelet count.[88] Thus, thrombocythemia of less than $100 \times 10^9$/L is consistently associated with increased risk of NRM among patients with cGVHD.

## PROPHYLAXIS

The main risk factor for developing cGVHD is previous aGVHD. However, calcineurin inhibitor–based approaches that successfully prevented aGVHD in some prospective trials have not had a major impact on cGVHD. A phase III study from BMT CTN used 2 GVHD prophylaxis regimens: tacrolimus/methotrexate, and cyclosporine/methotrexate. The incidence of cGVHD was 56% and 49% in the tacrolimus and cyclosporine groups, respectively (*P* value not significant).[58] A more recent study has shown that cGVHD incidence at 2 years was not affected by the type of GVHD prophylaxis but by the graft source: 53% and 41% in the PBSC and BM groups, respectively ($P = 0.01$).[88] Although ex vivo or in vivo donor T-cell depletion can considerably reduce aGVHD and cGVHD, the benefits of this approach are counterbalanced by increased risks of relapse, graft failure, post-transplantation lymphoproliferative disease, and infection.[89,90]

High-dose PTCy (post-transplantation cyclophosphamide) demonstrated promising results in cGVHD prophylaxis. These data were discussed above for aGVHD prophylaxis.

Based on data supporting a mechanistic role for B cells in human cGVHD, anti-CD20 strategy prophylaxis was evaluated in a phase II trial. Cutler and colleagues[91] showed that rituximab prevents steroid-requiring cGVHD when given after PBSC transplantation and improves overall survival compared with control.

## TREATMENT

Despite the association of cGVHD with reduced relapse of malignancy,[92] the protracted duration of cGVHD makes it the leading cause of impaired immunity, compromised functional status, and late treatment-related deaths. The goal of treatment is to achieve a fine balance between efficacy and toxicity. Efficacy refers here to preventing GVHD progression that may lead to irreversible disability or death.

According to the NIH consensus for cGVHD, symptomatic mild cGVHD can often be treated with local therapies alone (eg, topical steroids). However, in patients with cGVHD involving 3 or more organs or with a score of 2 or higher in any single organ, systemic immunosuppressive therapy may be considered.

### First-line Therapy

Corticosteroids represent the major component of first-line treatment in cGVHD. The standard initial dosage of glucocorticoids is 1 mg/kg/day. Sullivan and colleagues reported that treatment with corticosteroids late in the course of cGVHD resulted in a 23% probability of 3-year survival after HSCT, compared to 76% if treatment was administrated earlier.[93] Therefore, treatment should begin as soon as initial diagnosis and severity of cGVHD have been established. If prednisone or equivalent at a dose of 1 mg/kg/day is contraindicated (eg, poorly controlled diabetes, hypertension, osteoporosis, avascular bone necrosis, major psychiatric disturbance), patients may begin at 0.5 to 1 mg/kg/day.

Most patients are still treated with calcineurin inhibitors at the time of cGVHD diagnosis. The most widely used initial systemic treatment relies on prednisone in conjunction with calcineurin inhibitor therapy. However, it has been proposed to remove it in the last BMT CTN 0801 protocol comparing sirolimus plus prednisone to sirolimus/calcineurin inhibitor plus prednisone as first-line treatment of cGVHD. Whenever possible, patients found to have cGVHD should be entered on treatment protocols if any are available.

Martin et al conducted a randomized trial in 151 patients to determine whether addition of MMF improves the efficacy of initial systemic treatment. The trial was closed prematurely due to a low probability of reaching the primary end-point (2-year survival without systemic immunosuppression), indicating that MMF should not be added to the treatment of cGVHD.[94]

Long-term treatment with high-dose prednisone is associated with high morbidity, including hypertension,

glucose intolerance requiring insulin administration, infections, avascular necrosis, osteoporosis, cataracts, psychiatric complications, and inhibition of growth and development in children. Thus, efficient corticosteroids withdrawal is an ultimate goal of cGVHD therapy.

Corticosteroids must be continued at the initial dose until objective evidence of improvement in manifestations of cGVHD is observed. Tapering begins within at least 2 weeks after the first evidence of improvement. After successful completion of steroid tapering to less than 0.5 to 1 mg/kg every other day, it is recommended that the dose of prednisone be held constant for 10 to 12 weeks until all reversible manifestations of cGVHD have resolved, after which a second taper may be attempted, avoiding the flare effect. Once the steroid taper is completed, the calcineurin inhibitor is then reduced carefully.

### SECOND-LINE THERAPY

Salvage treatment should be initiated within 4 weeks whenever clinical manifestations of cGVHD show evidence of progression or if daily administration of prednisone cannot be tapered from 1 mg/kg/day within 3 months. It is also critical to recognize that some manifestations of cGVHD might be either irreversible or require prolonged time periods to resolve, and the guidelines here are of limited use.

**Extracorporeal photopheresis.** ECP represents a reliable procedure for the management of refractory cGVHD, achieving overall responses of 75% in cutaneous lesions and 45% to 65% in other organ manifestations (lung, liver, or oral mucosa).[95] The investigators reported a steroid-sparing effect of ECP, and significant improvements in overall survival and quality of life have been reported in ECP responders.[95]

**Other strategies for salvage therapy of steroid-refractory cGVHD.** There is currently no standard of care for cGVHD patients who fail first-line steroid-based therapy, so enrollment in a clinical trial is always recommended. The most recent data concerning second-line treatments is discussed here.

Rituximab has been used for treating steroid-refractory cGVHD, and data demonstrate that the skin is the most responsive organ. Cutler et al[96] reported a decrease in median body surface area involved with sclerodermoid cGVHD from 35% to 25% after 2 cycles of therapy, followed by a further decrease to 20% at 1 year after initiation of rituximab.

An encouraging pilot study reported improvement in sclerodermoid cGVHD with imatinib in salvage therapy.[97] This kinase inhibitor was used for targeting the platelet-derived growth factor receptor. However, the characteristics of patients who may benefit from imatinib treatment remain to be determined in future prospective studies.[98]

Among the most prevalent treatment modalities available for refractory cGVHD, the immune-modulating approach proposed by Koreth et al should be highlighted. In their observational cohort study of 29 patients, daily low-dose IL-2 was associated with reversal of glucocorticoid-refractory cGVHD.[99] The results supported their hypothesis that IL-2 could enhance Tregs in vivo, thereby suppressing clinical manifestation of cGVHD.

## CONCLUSIONS AND FUTURE PERSPECTIVES

The National Marrow Donor Program projects that by 2015 the number of HSCT performed annually will increase to 30,000. This increase is primarily due to advancements made in finding a suitable donor for most recipients, especially with UCB transplants and haploidentical donors. With this development in HSCT, the major challenges of GVHD prevention and therapy are still high morbidity and mortality. The recent identification of prognostic biomarkers for GVHD may improve risk stratification of patients[100,101] and provide a platform for advances in preemptive treatment of high-risk GVHD. However, the "holy grail" of HSCT is a treatment that can control the GVHD without impairing the antitumor effect of the transplant. Developments of such drugs are realistic today through substantial progress made in our understanding of the immunobiology of GVHD, which will allow identification of new immunomodulating therapies.

**BOARD REVIEW QUESTIONS**

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