

# Acute Graft-Versus-Host Disease

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## Abstract

Acute graft-versus-host disease (aGVHD) continues to be a problem in allogeneic transplantation. Recent advances in the understanding of the induction and propagation of GVHD have led to new transplant strategies and treatment of aGVHD. This overview covers the basic pathophysiology, prophylaxis and treatment of this disorder.

Acute graft-versus-host disease (aGVHD) is one of the most common complications of bone marrow transplant (BMT), peripheral blood stem cell transplant (PBSCT), and donor lymphocyte infusion (DLI). aGVHD is a reaction of the donor's immune system against the tissues of the recipient and was first described in irradiated mice once they received spleen cells. Mice that survived the aplasia died of a secondary disease (the primary disease being the radiation toxicity) with diarrhea, liver abnormalities, skin changes, and weight loss. Interestingly, the same investigators went on to describe the graft-versus-leukemia effect, which is the immune reaction against a tumor by the graft.<sup>1</sup> In humans, the infusion of bone marrow as a therapeutic tool goes back to the mid-20th century, when it was successfully used to rescue patients after a nuclear catastrophe.<sup>2,3</sup> US researcher E. D. Thomas was among the early investigators attempting to use BMT for the therapy of malignant disorders.<sup>4</sup> The early allogeneic transplants were complicated by aGVHD, and it was evident that this complication was going to prove a major hurdle in transplantation.<sup>5-12</sup> Billingham summarized the requirements for the induction of GVHD. For many years, the immunologic requirements for the induction of GVHD were understood to be the following:

- a) the graft must contain immune competent cells,
- b) the recipient should not be able to destroy the graft, and
- c) the recipient must express antigens not present in the donor.<sup>13</sup>

It is known that the induction of aGVHD is influenced by many factors such as type of graft used (bone marrow or peripheral blood stem cells), HLA typing, conditioning regimen, GVHD prophylaxis employed, etc.<sup>14-27</sup> Acute GVHD develops in 30–60% of recipients of sibling matched allografts, and its mortality (direct or indirect) can reach 50%.<sup>28-30</sup>

Generally, aGVHD presents before day 100 after transplant. However, it cannot be defined based on this criterion alone as it can be seen after day 100. This disease should be defined based on a clinical and pathological basis, and not solely on the day of its appearance.

## Pathophysiology

Our understanding of the pathophysiology of acute GVHD has improved greatly in the last few years. Perhaps the best

## Keywords

Bone marrow transplant, peripheral blood stem cell transplant, donor lymphocyte infusion

way to describe the biology of this problem is with the 3-step model of aGVHD.<sup>31</sup> These are:

- a) conditioning regimen damage,
- b) donor T-cell activation, and
- c) effector phase.

In the first 2 steps, T cells recognize the host tissues that were damaged during the conditioning, and during the effector phase, these lymphocytes attack the tissues or they recruit other effector cells. Cytokine dysregulation also plays an important role in the effector phase.<sup>32-35</sup>

### **Conditioning Damage**

With current conditioning regimens, chemotherapy with or without radiation is administered to the patients with the idea of either killing the tumor or causing immunosuppression. This insult will cause damage in several tissues, including the liver and gut. Gut damage is very important in the pathophysiology of this disorder.<sup>36,37</sup> Once the conditioning regimen is delivered, the mucosa becomes permeable and causes increase in cytokine release such as tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-1.<sup>38</sup> These cytokines cause up-regulation of adhesion molecules and major histocompatibility complex (MHC) antigens improving the recognition of host antigens by T cells.<sup>39,40</sup> The increase in permeability allows the introduction of bacterial products into the circulation. Of primary interest are capsular lipopolysaccharides (LPS).<sup>41</sup> LPS will stimulate immune cells to produce cytokines that will augment the GVH reaction. Some experimental approaches have shown to protect the intestine from this original insult retaining T-cell function such as keratinocyte growth factor, *Lactobacillus rhamnosus* GG, LPS antagonism, and IL-11.<sup>41-45</sup> However, IL-11 proved toxic to administer posttransplant, and a randomized trial failed to show an improvement in GVHD.<sup>46</sup> Also, it is unknown if “yogurt” will help to decrease the incidence of aGVHD.<sup>47</sup>

### **Donor T-cell Activation and Expansion**

After the conditioning regimen and the graft are given to the host, T cells will interact with antigen-presenting cells (APCs) for antigen presentation. T cells recognize these antigens (bound to MHC molecules) and once presented by APCs, costimulatory molecules provide help with activation of the T cells.<sup>48-50</sup> In experimental models, the blockade of the costimulatory molecules inhibited both acute and chronic GVHD.<sup>51</sup> Both CD4+ and CD8+ T cells can initiate the GVH reaction as the alloantigen composition of the host will determine which subset of lymphocytes will be activated.<sup>31</sup>

### **Effector Phase**

Once the T cells have been stimulated against the host, they are directly or indirectly responsible for the tissue damage. Initially, this was believed to be a pure “T cell” phenomenon. However, other cells such as natural killer (NK) cells or large granular lymphocytes also participate.<sup>52</sup> These, along with the T lymphocytes, secrete cytokines that continue to stimulate other cells and damage tissues. The Fas/FasL pathway and the perforin/granzyme pathway play an important

role in the GVHD insult.<sup>31,34,53-58</sup> The exact mechanisms of tissue damage and targeting of skin, liver, and gut remain areas of intensive research.

### **Pathology and Histology**

Histologic confirmation of aGVHD is important as the manifestations of the disease are not specific. Other processes can affect the same organs and occur simultaneously with aGVHD. Problems such as veno-occlusive disease of the liver, “engraftment syndrome,” drug reactions, and viral infections (such as cytomegalovirus [CMV], gastritis, hepatitis, or colitis) are all seen early posttransplant. It is also important to stress that the biopsy needs to be associated with the right clinical picture to be diagnostic.

Cutaneous aGVHD is characterized by apoptosis at the base of dermal crypt and dyskeratosis. Liver aGVHD is characterized by lymphocytic infiltration of small bile ducts with nuclear pleomorphism and epithelial cell drop-out. The extent of both apoptosis and dropout cells serves to distinguish acute liver GVHD from the other causes of liver dysfunction.<sup>59,60</sup> In the gut, aGVHD is a pan-intestinal process. A retrospective study found that upper endoscopy has a higher yield of a positive biopsy result than a lower endoscopy. Histologically, single-cell necrosis or apoptotic bodies at the base of the crypts or glands are characteristic.<sup>61</sup> Severe disease can lead to the destruction of all crypts. Because CMV inclusions and chemotherapy can cause similar histology in the gut, the diagnosis of aGVHD is very difficult to make in patients with viral inclusions or before day 21 after chemotherapy is given. Other organs besides the liver, gut, and skin can be affected. In the lung, for example, a lymphocytic bronchitis with necrosis of the bronchial mucosa and often the submucosal glands has been reported, but is uncommon.<sup>12</sup>

### **Factors Influencing the Development of aGVHD**

Many factors can play a role in the risk developing aGVHD. These factors are described below.

#### **HLA Typing**

HLA matching is the greatest determinant of risk for the development of aGVHD. Its impact on survival was recently reviewed.<sup>62</sup> The incidence of aGVHD increases with the level of HLA mismatch.<sup>63-65</sup> Utilizing DNA testing, it is known that mismatch in HLA-A, -B, -C or -DRB1 increase the risk of GVHD and have a negative effect on survival,<sup>23-27</sup> and HLA-DRB1 has been associated with an increase on severe aGVHD.<sup>66</sup> However, the mismatch causes not just an increase in aGVHD but also higher rates of transplant toxicities and treatment failures.

#### **Minor Histocompatibility Antigens**

Minor histocompatibility antigens (mHA) are MHC-associated peptides, which trigger T-cell responses that mediate GVHD.<sup>67</sup> A new mHA named HA-8 has been associated with the development of aGVHD. Disparity donor-recipient in 72 patients resulted in 64% with grades II-IV aGVHD compared with 50% of patients without disparity ( $P=.04$ ), but no differences in outcome.<sup>68</sup> Other mHA disparity does not

**Table 1.** Staging and Grading of Acute Graft-Versus-Host Disease

Extent of organ involvement			
Skin		Liver	Gut
<b>Stage</b>			
1	Rash on <25% of skin <sup>a</sup>	Total bilirubin 2–3 mg/dL <sup>b</sup>	Diarrhea >500 mL/day <sup>c</sup> or persistent nausea <sup>d</sup>
2	Rash 25–50% of skin	Total bilirubin 3–6 mg/dL	Diarrhea >1,000 mL/day
3	Rash >50% of skin	Total bilirubin 6–15 mg/dL	Diarrhea >1,500 mL/day
4	Generalized erythroderma with bullous formation	Total bilirubin >15 mg/dL	Severe abdominal pain with or without ileus
<b>Grade<sup>e</sup></b>			
I	Stage 1–2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	-	Stage 2–3 or	Stage 2–4
IV <sup>f</sup>	Stage 4 or	Stage 4	-

<sup>a</sup> Use “rule of nines” or burn chart to determine extent of rash.

<sup>b</sup> Range given as total bilirubin. Downgrade by 1 stage if an additional cause of elevated bilirubin has been documented.

<sup>c</sup> Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Gut staging for pediatric patients was not discussed at the Consensus Conference. Downgrade by 1 stage if an additional cause of diarrhea has been documented.

<sup>d</sup> Persistent nausea with histologic evidence of GVHD in the stomach or duodenum.

<sup>e</sup> Criteria for grading given as minimum degree of organ involvement required to confer that grade.

<sup>f</sup> Grade IV may also include lesser organ involvement but with extreme decrease in performance status.

have an influence on the development of aGVHD. In another group, 22 polymorphisms in 11 immunoregulatory genes including cytokines, mediators of apoptosis, and host-defense molecules by polymerase chain reaction using sequence-specific primers in 160 related myeloablative transplants were studied.<sup>69</sup> An intronic polymorphism in the TNF gene (TNF 488A) was associated with the risk of aGVHD (odds ratio [OR] 16.9), grades II to IV aGVHD (OR 3.3), chronic GVHD (OR 12.5), and early death posttransplant (OR 3.4). Recipient Fas -670G and donor IL-6 -174G were independent risk factors for aGVHD. Recipient IL-10 ATA and Fas -670 genotype were independent risk factors for chronic GVHD. Recipient IL-1 $\beta$  +3953T was associated with liver aGVHD, and Fas -670G was associated with major infections.

#### **Killer-Cell Immunoglobulin-Like Receptor Typing**

Cell surface receptors that can inhibit or activate NK cells to lyse target cells have been described, and include the killer-cell immunoglobulin-like receptor (KIR).<sup>70</sup> KIRs recognize groups of HLA class I alleles, and their interaction can inhibit the NK cell. However, if there is a KIR mismatch, the NK cells can kill the target cells. It has been observed that a graft-versus-tumor (GVT) effect can be seen even without GVHD with the KIR match.<sup>70-72</sup> This area is currently a subject of active research.

#### **Donor and Host Factors Other Than HLA Type**

Besides HLA typing, other donor and host factors can play a role in the development of aGVHD. Obviously, vigorous removal of cells involved in GVHD will prevent initiation of the disease; therefore, T-cell depletion from marrow grafts decreases the risk of aGVHD.<sup>73</sup> Age of the recipient is also very important: the older he or she is, the higher the risk of developing the disease.<sup>74,75</sup> Donor parity and sex mismatch, and CMV positivity have also been associated with an increased risk of aGVHD and decreased survival in some but not all studies.<sup>76-80</sup> Increased levels of LL-13 have been associated with the development of aGVHD.<sup>81</sup>

#### **Source of the Graft**

Perhaps few issues are as controversial as graft source, a risk factor for the development of aGVHD.<sup>16,22</sup> Several studies have addressed this issue. Of the 8 randomized trials published,<sup>15,19,82-87</sup> only 1 reported a statistically significant increase in grades II–IV aGVHD with the use of PBSCs when compared to bone marrow (BM; 52% vs 39%).<sup>87</sup> However, day-100 transplant-related mortality, disease-free survival and relapse rates were no different between both groups.<sup>87</sup> The meta-analysis published by Cutler et al<sup>22</sup> clearly found that both acute and chronic GVHD (for acute: relative risk [RR] was 1.16, 95% confidence interval [CI] 1.04–1.28,

$P=.006$ ; RR for chronic 1.53, 95% CI, 1.25-1.88,  $P<.001$ ) are more common after PBSC than after BM infusions. However, there was a trend toward a decrease in the rate of relapse after PBSC transplant (RR = 0.81; 95% CI, 0.62 to 1.05).<sup>22</sup> Also, higher doses of PBSC were associated with higher incidences of aGVHD, which was not seen when BM was used.<sup>88,89</sup> Interestingly, the use of granulocyte colony-stimulating factor after transplantation seems to increase the risk of aGVHD in patients receiving BM but not PBSC.<sup>90</sup> Umbilical cord stem cells have also been a source of stem cells, mainly in children and young adults due to cell yield. As children tolerate mismatches better than adults, interpretation of risk in this group is difficult. It seems that the rate of aGVHD is lower with this approach, but no randomized studies exist to clarify this issue.<sup>91-93</sup> The immunology of transplantation using cord blood needs clearer definition, with the hope that what is learned can be applied to other mismatch situation.

### Conditioning Regimen

The dose of radiation is also a risk factor in the development of aGVHD. The use of higher doses of total body irradiation has been associated with the development of this problem.<sup>94</sup> Also, in patients with aplastic anemia, the use of cyclophosphamide alone (compared with cyclophosphamide with limited field radiation and cyclophosphamide plus total body radiation) resulted in less GVHD than the other conditioning regimens.<sup>95</sup> The use of nonmyeloablative regimens has been associated with a low frequency of aGVHD.<sup>96</sup>

### Cytokine Polymorphisms

A study analyzing single-nucleotide polymorphisms in the genes for interleukin (IL)-1 $\beta$ , IL-1-receptor antagonist, IL-6, IL-10, and tumor necrosis factor- $\alpha$  in transplant recipients and their HLA-identical sibling donors was carried out. The recipient's IL-10 promoter region genotype was significantly associated with the risk of aGVHD. An analysis of all 993 transplant recipients showed that, compared with the C/C genotype, the IL-10-592A/A genotype was associated with a decreased risk of grade III or IV aGVHD (hazard ratio, 0.4; 95% CI 0.2-0.9,  $P=.02$ ) and death in remission (hazard ratio, 0.6, 95% CI 0.3-1.0,  $P=.05$ ).<sup>97</sup>

### Staging

Acute GVHD is staged by the number and extent of organ involvement. The current staging system was refined in 1994 after 8,249 patients were analyzed.<sup>98</sup> This system is helpful in further subdividing patients at different risk for complications and death (Table 1), and uses the "rule of nines" to determine the extent of skin involvement. Patients with grade IV disease are suffering from a life-threatening condition. The International Bone Marrow Transplant Registry developed another grading system as it was believed that there was interobserver variability in the grading of aGVHD patients.<sup>99</sup> The registry described 4 "risk categories," A-D. It is unknown if either grading system is better in terms of prospectively predicting the outcome of patients.<sup>100,101</sup>

### Clinical Manifestations

The clinical manifestations of aGVHD correlate very well with organ involvement. They include failure to thrive, jaundice and elevated bilirubin with or without hepatitis,<sup>102</sup> diarrhea with or without cramping, ileus, abdominal distention, nausea, vomiting, and maculopapular rash. Skin aGVHD is usually the earliest and most common manifestation and correlates with engraftment. In 1978 Beschorner et al reported a syndrome characterized by a lymphocytic bronchitis and pulmonary infiltrates, believed to be pulmonary aGVHD.<sup>12</sup> It was described as a lymphocytic bronchitis, with an infiltrate of dark lymphocytes, without plasma cells or neutrophils, throughout the muscularis, submucosa and mucosa. Single cell necrosis and vacuoles were common. Pulmonary aGVHD was consistently associated with disease elsewhere (ie, liver, gut, skin). As of today, pulmonary aGVHD still is a controversial entity, and no universal agreement exist regarding its definition.<sup>103</sup> Of patients with the disease, close to 80% of patients will have skin involvement, with around 50% having gut or liver. As other conditions can occur that resemble aGVHD and other conditions can coexist with aGVHD, it is important to establish the diagnosis, whenever possible, with a biopsy.

### Prophylaxis

Prophylaxis is more effective than treating aGVHD. In high-risk malignancies, GVHD prophylaxis has been avoided to favor a GVT effect. However, the results have been poor, suggesting that even patients with high-risk disease need to receive some form of prophylaxis.<sup>104,105</sup>

As mentioned earlier, there is no question that removing T cells from the graft reduces the incidence of aGVHD.<sup>73,106</sup> However, most studies have consistently shown higher rates of relapse, graft failure, and infections.<sup>107-109</sup> Therefore, many investigators do not favor this approach anymore.

Currently, the majority of centers will use methotrexate with a calcineurin inhibitor.<sup>110,111</sup> Methotrexate is a folic acid analog that inhibits dihydrofolate reductase and, therefore, interferes with purine synthesis and DNA replication.<sup>112</sup> It has been used as a single agent in aGVHD prophylaxis, in several regimens (long, mini, very-short, etc.). In adults, methotrexate is commonly used in combination with either tacrolimus or cyclosporine, rather than as a single agent. When given in combination, it is often used in the "mini-methotrexate" (5 mg/m<sup>2</sup> IV days 1, 3, 6, and 11) or "very-short methotrexate" (5 mg/m<sup>2</sup> IV days 1, 3, and 6) schedules. The mini-methotrexate is equivalent to more intensive schedules preventing aGVHD.<sup>113</sup> It is unclear if the dose in day 11 is needed; there are no prospective data available, so many centers continue to use this regimen.<sup>79,114</sup>

It is not clear if tacrolimus or cyclosporine better prevents aGVHD. These agents have been compared in the related and unrelated settings.<sup>20,21</sup> In the unrelated setting, 180 patients were randomized to receive either combination. Tacrolimus and methotrexate decreased the risk of aGVHD when

compared to methotrexate and cyclosporine (56% vs 74%,  $P=.0002$ ), without changing toxicity, infections, chronic GVHD, or unfortunately, disease-free survival.<sup>20</sup> In the related setting, the results are difficult to interpret. Three hundred twenty-nine patients were randomized, 165 to tacrolimus-methotrexate and 164 to cyclosporine-methotrexate. The incidence of grade II–IV aGVHD was lower in the tacrolimus-treated group, with an absolute difference between groups of 12.5% (95% CI for the difference  $-23.9$  to  $-1.2$ ,  $P=.01$ ). However, the incidence of grade III–IV was similar between the 2 groups. There was no difference in chronic GVHD. Patients who received cyclosporine-based therapy had a better survival. This difference was limited to patients with advanced disease, as patients with non-advanced disease had similar outcome. Unfortunately, patients were not stratified for disease status. The authors concluded that tacrolimus-methotrexate is superior to cyclosporine-methotrexate for the prevention of aGVHD.<sup>21</sup> The addition of steroids to methotrexate-cyclosporine has also not resulted in a higher survival rate, perhaps due to increased infections, so it is not recommended.<sup>115</sup> Moreover, steroids may increase the risk of infections.<sup>116</sup> Sirolimus has been added to tacrolimus and methotrexate in mismatched-related or -unrelated donor transplantation.<sup>117</sup> Out of 41 patients, 13%, 8%, and 5% developed aGVHD grades II, III, and IV, respectively.

The combination of methotrexate, cyclosporine, methylprednisolone, and hydroxychloroquine was tested in the unrelated setting.<sup>118</sup> Hydroxychloroquine is an immunosuppressive agent that interferes with antigen presentation and has activity in GVHD. Fifty-one consecutive unrelated donor transplant patients received the 4 drugs. Grade II–IV aGVHD was diagnosed in 56% of patients, and grade III–IV aGVHD was seen in 17%. When compared with a matched cohort of patients reported to the International Bone Marrow Transplant Registry, patients receiving hydroxychloroquine had comparable cumulative incidences of grade II–IV aGVHD, but lower incidences of grade III–IV aGVHD and better GVHD-free survival.

Because of the toxicity associated with posttransplant methotrexate, there is ongoing interest in identifying regimens without this agent. The combination of sirolimus-tacrolimus was studied after matched-related donor peripheral blood stem cell transplantation.<sup>111</sup> Thirty patients enrolled in a phase II study had very promising results. Grade II aGVHD occurred in 3 patients (10%), and no patient developed grade III or IV. Neutrophil and platelet engraftments were not delayed, occurring on days 14 and 13, respectively. All patients survived to hospital discharge and toxicity was mild. Four patients developed thrombotic microangiopathy, and 3 patients developed hepatic veno-occlusive disease. Chronic GVHD occurred in 11 patients. Relapse-free and overall survival were 93% and 97%, respectively, at 100 days, and 71% and 67% at 1 year.

Another combination being evaluated is mycophenolate mofetil-tacrolimus.<sup>119</sup> Thirty-four patients (median age

7 years, range 0.5–21) received this prophylaxis. This study is difficult to interpret as 22 patients received umbilical cord cells (while 6 BM and 9 PBSC). Twelve patients developed grade  $\geq$ II aGVHD, and 8 grade III–IV.

Alemtuzumab (Campath, Berlex), a monoclonal antibody directed against CD52 has been used as prophylaxis for aGVHD with good preliminary data when used for T-cell depletion in vivo.<sup>120</sup> In a retrospective analysis of 129 patients with chronic lymphocytic leukemia who received a nonmyeloablative PBSCT with fludarabine and melphalan, 78 received alemtuzumab and cyclosporine and 51 received methotrexate and cyclosporine for prophylaxis.<sup>121</sup> Patients receiving alemtuzumab had a higher incidence of CMV reactivation (85% vs 24%,  $P<.001$ ) and a significantly lower incidence of aGVHD (21.7% vs 45.1%,  $P=.006$ ) and chronic GVHD (5% vs 66.7%,  $P=.001$ ). Eighteen patients in the alemtuzumab group required donor lymphocyte infusions to achieve disease control. At last follow-up, there were no differences in disease status between the groups regarding complete or partial responses. No significant differences were observed in event-free or overall survival between the 2 groups. Other studies have shown similar results.<sup>122,123</sup> These studies are examples for the need to balance GVHD control with tampering GVT effect.

There has been renewed interest in the use of antithymocyte globulin (ATG; Thymoglobulin, Sangstat).<sup>124-127</sup> Despite a modest reduction in aGVHD, the high risk of infections erase any benefit caused by the lowest aGVHD incidence.<sup>124</sup> However, even when survival is unchanged, less extensive chronic GVHD is seen.<sup>124</sup> Other studies showed improvement in transplant-related mortality and aGVHD incidence.<sup>127</sup> Therefore, the exact role of ATG as prophylaxis is still controversial. It must be noted that the different studies did use ATG preparations that are not equipotent, making a direct dose-response comparison impossible.

### Treatment of aGVHD

The backbone for the therapy of aGVHD is the use of steroids. They are superior to cyclosporine and ATG.<sup>128-132</sup> Steroids have multiple mechanisms that theoretically can explain their effectivity: they are lympholytic and inhibit the release of inflammatory cytokines. However, not all patients need therapy. For instance, a patient with stage I–II skin-only aGVHD may benefit from observation or topical steroids. More advanced disease needs systemic therapy. Because of the availability of an IV formulation, methylprednisolone is commonly used as a first-line agent. When the dose of 2 mg/kg/day was compared to 10 mg/kg/day, the higher dose was not superior, and the concern for more infections with the higher doses was well founded.<sup>133</sup> Once therapy has been started, a rapid taper is effective controlling disease and minimizing side effects.<sup>134</sup> Perhaps a 25% decrease every 4 days is adequate. Once steroids are given, it is expected that 20–70% of patients will respond and 20–40% will have durable responses. Other therapies (TNF blockade, mycophenolate mofetil, photopheresis, haploidentical mesenchymal

stem cells, etc.) have either not been found to add benefit to the steroids or have not been compared directly to steroid therapy in large, randomized trials.<sup>135-138</sup>

Supportive care is also a critical issue for these patients. Prophylactic antibiotics, pain control, gut rest, and sunscreen are all important, and nutritional support is sometimes indicated.<sup>139-141</sup> Octreotide is sometimes helpful in controlling the diarrhea.<sup>142</sup> Also helpful in controlling gut aGVHD is oral beclomethasone, which has very few systemic effects but improves gastrointestinal symptomatology.<sup>143</sup>

### **Steroid-Refractory aGVHD**

As mentioned, less than 50% of patients with grades II–IV aGVHD will achieve durable responses after initial therapy.<sup>144-146</sup> Steroid therapy will produce sustained responses in only 41% of patients receiving a matched-related allogeneic BMT.<sup>130</sup> In the unrelated donor transplant setting, results are more disappointing with only 24% improving after steroid therapy.<sup>147</sup> If a patient fails to respond to these drugs, his or her prognosis is dismal and is stage dependent.<sup>128,144</sup> These patients will succumb to infections, aGVHD, and possibly chronic GVHD.

An area of debate is when to declare a patient steroid refractory. There are not universally accepted criteria for this definition, therefore, different studies may include slightly different populations. Van Lint et al<sup>133</sup> identified that patients who did not respond after 5 days of steroids are at high risk for complications and mortality. Many agents have been tried in steroid refractory aGVHD, the majority of them with disappointing results.

### **ATG**

Traditionally, ATG has been used as first line for steroid refractory aGVHD.<sup>130,144</sup> However, the benefit in patients treated with this modality was mainly seen in patients with skin-only disease or minimal gut or liver involvement.<sup>144,148</sup> The Johns Hopkins experience using ATG (10–15 mg/kg every other day for 7 doses) as salvage therapy was disappointing.<sup>144</sup> ATG induced complete responses in 14%, and 26% did not respond while 17% progressed on therapy. Survival was dismal for patients with grades II, III, and IV aGVHD, with median survivals of 4.1, 3.6, and 2.7 months, respectively. Ninety-five percent of patients died of infections (majority), GVHD, and multiorgan failure, and 5% died of relapsing disease. Those responding had a short disease-free survival. Other investigators have reported similar results.<sup>149</sup> The recent improvements in the therapy for fungal infections, a common cause of infections in these patients, may impact the overall survival with this agent.

### **Sirolimus**

Sirolimus (rapamycin) was tried in 21 patients at 4 or 5 mg/m<sup>2</sup>/day for 2 weeks (with or without a 15 mg/m<sup>2</sup> as loading dose on day 1).<sup>150</sup> Twelve patients responded (5 complete and 7 partial responses); however, 9 had to discontinue the drug due to toxicity including myelosuppression (thrombocytopenia and neutropenia), seizures, and hemo-

lytic-uremic syndrome. One major limitation of sirolimus is that it is only available orally, making it impractical for patients with gastrointestinal disease. Six responders and 1 nonresponder were long-term survivors (400–907 days posttransplant), with 3 later developing chronic GVHD.

### **Monoclonal Antibodies**

A variety of monoclonal antibodies have been tested in this setting. Visilizumab, a humanized non-FcR-binding anti-CD3 monoclonal antibody was given to 17 patients in either multiple (6 patients received 7 doses of either 0.25 or 1 mg/m<sup>2</sup> every other day) or single doses (11 patients received 3 mg/m<sup>2</sup>).<sup>151</sup> In the multiple-dose schema, the 6 treated patients achieved 1 complete and 5 partial responses. However, all patients died at a median of 87 days of infections, multiorgan failure, and posttransplant lymphoproliferative disease (PTLD). A single-dose regimen resulted in 6 complete and 3 partial responses, with 7 long-term survivors (median survival >300 days). Infections, PTLD, and adult respiratory distress syndrome were the causes of death in this group. The major concern with this experimental medication is the induction of PTLD.

Another new therapy used in this disease is ABX-CBL.<sup>146</sup> This antibody is directed against CD147 (member of the immunoglobulin superfamily) that is upregulated in activated lymphocytes. Fifty-nine patients with steroid-refractory aGVHD were treated, and 51 were evaluable for response. Twenty-seven patients received 0.01, 0.1, 0.2, or 0.3 mg/kg/day, and 32 were given 0.2 or 0.15 mg/kg/day. Twenty-six of the patients responded with 13 complete and 13 partial responses. Forty-four percent of the patients survived more than 6 months. The main toxicity was severe myalgias. Causes of death included infections, GVHD, and multiorgan failure. Six months after therapy, 44% of patients were alive. However, a recently completed phase III trial comparing ABX-CBL to ATG found that the response rates and survival were similar.<sup>152</sup>

The chimeric mouse/human monoclonal antibody infliximab binds to cells producing TNF- $\alpha$ , allowing for not only the neutralization of TNF- $\alpha$  but also lysis of the cells producing the cytokine. Although this agent has been available for several years now, there is little in the literature on its use. Much of the information comes from case series rather than controlled trials. For example, the Johns Hopkins transplant group reported 11 patients with end-stage, steroid-refractory aGVHD treated with infliximab.<sup>153</sup> The survival was very poor. Two patients with severe diarrhea from gut GVHD resolved their symptoms after treatment with this drug. Only these 2 patients survived. Gut GVHD is more responsive to treatment with infliximab than liver.<sup>153-155</sup> Fungal infections seem to be a common problem in patients treated with this drug.<sup>153,154,156</sup>

As discussed earlier, alemtuzumab has been used for GVHD prophylaxis. A recent report of 3 patients with steroid refractory aGVHD treated with alemtuzumab resulted in impressive responses without affecting chimerism, but with CMV reactivation.<sup>157</sup>

Daclizumab, a monoclonal antibody against the IL-2R $\alpha$  chain has also been tried. In a study, 43 patients with aGVHD (steroid refractory or not) received daclizumab with methylprednisolone, with or without ATG. Results with an intensive scheduling showed complete responses in 47%.<sup>158</sup> Also, daclizumab based combinations appear to be superior to ATG/mycophenolate mofetil in a small group of patients.<sup>159</sup> However, a prospective study failed to confirm a benefit, as the results were disappointing, with poor responses and survival.<sup>160</sup>

### ***Denileukin Diftitox***

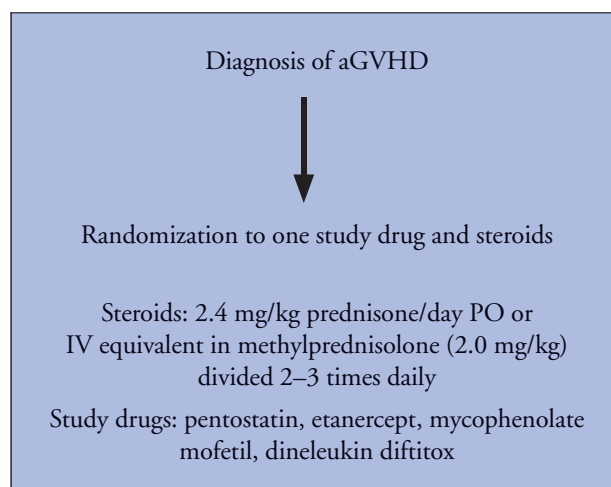
Researchers in Boston conducted a phase I study with denileukin diftitox.<sup>161</sup> This recombinant protein is composed of human IL-2 fused to diphtheria toxin with selective cytotoxicity against activated lymphocytes expressing the high affinity IL-2 receptor. Thirty patients with steroid-refractory aGVHD were enrolled. Seven patients received 9  $\mu$ g/kg IV on days 1 and 15; 18 received 9  $\mu$ g/kg IV on days 1, 3, 5, 15, 17, 19; and 5 received 9  $\mu$ g/kg IV days 1–5, 15–19. Hepatic transaminase elevation was the dose-limiting toxicity. Overall, 71% of patients responded with complete response (12/24) or partial response (5/24) of their GVHD. Eight of 24 patients (33%) are alive at a median of 7.2 months.

### ***Mycophenolate Mofetil***

There is limited information on the use of mycophenolate mofetil in steroid refractory aGVHD. Kim et al<sup>162</sup> reported on 13 patients received mycophenolate mofetil after failing steroids, and 4 responded. There were responses in all organs. The 2-year survival was 33.3%. In another study with 7 patients with aGVHD refractory to steroids, they received the drug at a dose of 2 g/day (children at 600 mg/m<sup>2</sup>) for 25 days. Two patients responded but 4 patients required a 50–75% dose reduction.<sup>163</sup> In another report, 2 patients with aGVHD who did not respond to either steroids or ATG received 1 g/day of mycophenolate mofetil plus cyclosporine, and both responded to therapy.<sup>137</sup> A case report described a patient with chronic myeloid leukemia who was treated with mycophenolate mofetil after failing steroids, with dramatic improvement in his aGVHD but CMV reactivation.<sup>164</sup> A potential problem with mycophenolate mofetil in this setting is its gastrointestinal toxicity: nausea, vomiting and diarrhea, many times already present because of the disease. However, due to the promising results reported so far, further studies are underway.<sup>165,166</sup>

### ***Pentostatin***

Pentostatin, a nucleoside analog with potent immunosuppressive properties, has been studied in a phase I trial of steroid-refractory aGVHD.<sup>167</sup> It is an interesting agent with modest myeloid toxicity and kills lymphocytes by inhibiting adenosine deaminase, blocking the metabolism of 2'-deoxyadenosine.<sup>168</sup> Patients with biopsy proven, steroid-refractory, grade II or higher aGVHD, were enrolled. Twenty-three patients were recruited. Twenty patients were evaluable for response and these included 11 complete responders, 3 partial responders, 3 with mixed response (improvement in 1 organ with deterioration in another organ), and 3 with progressive disease (deterioration in at least 1 organ without improve-



**Figure 1.** Schema of the phase II randomized trial evaluating etanercept, mycophenolate mofetil, denileukin diftitox and pentostatin in combination with corticosteroids as initial therapy for acute graft-versus-host disease (aGVHD) by the Blood and Marrow Transplant Clinical Trials Network.

ment in others). Five patients died due to infection, including 2 patients who experienced late infections at the 2 mg/m<sup>2</sup> dose. Our data suggest that a dose of 1.5 mg/m<sup>2</sup> should be used in efficacy trials, and attention should be placed to opportunistic infections.

### ***Extracorporeal Photopheresis***

Extracorporeal exposure of peripheral blood mononuclear cells to the photosensitizing compound 8-methoxypsoralen and ultraviolet A radiation has been shown to be effective in the treatment of several T-cell-mediated diseases as it kills lymphocytes. Researchers from Austria treated 6 patients with aGVHD grades II to III not responding to cyclosporine A and prednisolone with photopheresis.<sup>136</sup> Therapy was tolerated without any significant side effects. After a median of 14 cycles, aGVHD resolved completely in 4 of 6 patients (67%) and partially in another 2 patients. In children, 7 of 9 responded to this therapy.<sup>169</sup> There are several potential difficulties with this therapy. First, the length of time for response in critically ill patients is very long. Second, size does matter, as young pediatric patients may be too small to undergo photopheresis. Despite the limitations, the response rate and low toxicity make this a very interesting approach.

### ***Mesenchymal Stem Cells***

Bone marrow's mesenchymal stem cells have immunomodulatory capabilities *in vitro*.<sup>170,171</sup> Le Blanc et al<sup>138</sup> reported a 9-year-old boy with leukemia that developed resistant aGVHD and improved after the infusion of mesenchymal stem cells from his haploidentical mother. There are preliminary data showing that mesenchymal stem cells can prevent acute and chronic GVHD.<sup>172</sup> Therefore, it is plausible to believe that mesenchymal stem cells can prevent this condition and, perhaps, treat it. However, the exact mechanism of how these cells cause immunomodulation is still unknown and the subject of intensive research.

## Conclusions

GVHD still is one of the most important features of allogeneic transplantation. As the understanding of the pathophysiology evolves, new therapeutic and prophylactic strategies are being developed. One of the most encouraging developments is the National Institutes of Health–sponsored Blood and Marrow Transplant Clinical Trials Network. This group of large transplant centers has identified GVHD as an area where large trials can be rapidly completed to speed the development of agents for the treatment of this disorder, and they are directing the study “Initial Systemic Treatment of Acute GVHD: A Phase II Randomized Trial Evaluating Etanercept, Mycophenolate Mofetil, Denileukin Diftitox, and Pentostatin in Combination with Corticosteroids” for the first-line therapy of aGVHD (Figure 1). Hopefully, the more rapid trials made possible by this group will result in the ability to move therapy forward for this disorder.

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