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REVIEW

# Pathophysiology, prevention, and treatment of acute graft-versus-host disease



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**Abstract:** Acute graft-versus-host disease (aGVHD) is an immunologically mediated inflammatory reaction, which continues to be a major cause of morbidity and mortality in patients undergoing allogeneic hematopoietic stem cell transplant. Although the occurrence and severity of this disease may be devastating, there is a proven immunologically mediated antitumor activity that accompanies the disease process, which has a beneficial effect on outcome. Animal models of graft-versus-host disease (GVHD) have given us a conceptual model that has allowed a better understanding of the pathophysiology and offers a framework for understanding the complex interactions between antigen-presenting cells, donor T-cells, and cytokines in the development of aGVHD. It has also given us a model that allows testing of various strategies for prevention and treatment. New, innovative approaches for treatment and prevention of aGVHD including better donor selection with the use of sophisticated human leukocyte antigen typing, use of T-cell depletion, reduced-intensity transplant regimens, and improved pharmacologic immunosuppression have improved outcomes by decreasing the incidence and severity of aGVHD. However, the limitation of these strategies is that effective treatment and prevention of aGVHD is often accompanied by a concomitant rise in relapses, graft failure and infections, and ultimately no improvement in overall survival. Investigators are working on understanding the difference between GVHD and graft versus tumor effect, as this would be the key in improving outcomes for our patients. In this review, we will discuss the pathophysiology of aGVHD along with the preventative and treatment strategies.

**Keywords:** acute GVHD, GVHD, acute graft-versus-host disease, bone marrow transplant

## Introduction

Over the last five decades, allogeneic hematopoietic stem cell transplant (HSCT) has become the treatment of choice for many hematologic malignancies, immunodeficiency disorders, hemoglobinopathies, genetic disorders, and aplastic anemia. The outcomes of transplantation have improved due to improvements in supportive care, high-resolution human leukocyte antigen (HLA) typing that allows for better donor matching, and an increased use of reduced-intensity transplant regimens. In spite of increasing understanding and application of the procedure in various hematologic diseases, the efficacy of the procedure is limited by graft-versus-host disease (GVHD). This immunologically mediated inflammatory reaction that often accompanies allogeneic transplantation remains a major cause of mortality and morbidity in patients undergoing allogeneic HSCT.

GVHD had historically been divided into acute and chronic, with a somewhat arbitrary 100-day boundary separating the two entities. With clinical experience and

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better understanding of the physiology involved, the HSCT community for the past few years has realized the limitations of this classification. In 2005, the National Institute for Health consensus statement on classification of GVHD was adopted as a guideline to define and differentiate acute graft-versus-host disease (aGVHD) and chronic GVHD.[1] This classification is shown in Table 1.

The incidence of aGVHD in patients undergoing allogeneic HSCT is 40%–60%.[2–5] The median time from transplant to diagnosis of aGVHD depends on the intensity of preparative regimens utilized ranging from 17 days for full-intensity myeloablative regimens[6] to 3 months for reduced-intensity regimens.[7]

The main factors that increase the risk of developing aGVHD include degree of HLA disparity, advanced age of donor and/or recipient, sex mismatch, and intensity of the preparative regimen.[5,8] In addition, a recent meta-analysis showed that the increased use of peripheral blood stem cells in place of marrow stem cells might contribute to an increased risk of aGVHD.[9] Previous studies, however, have suggested no difference in the incidence of aGVHD based on the source of stem cells.[10–12]

## Pathophysiology of aGVHD

In 1966, Billingham postulated that development of GVHD requires three factors:[13] 1) the graft must contain immunologically competent cells, 2) the host must possess important alloantigens that are lacking in the donor graft, so that the host appears foreign to the graft and can, therefore, stimulate it antigenically, and 3) the host must be incapable of mounting an effective immunologic reaction against the graft.

Our understanding of the pathophysiology of aGVHD has been aided by established animal models. HLA, the most immunogenic protein in humans, is expressed by genes encoded by the 'major histocompatibility complex' (MHC). The degree of disparity in HLA gene expression is the strongest predictor for aGVHD, and for this reason, the vast majority of transplants are performed from fully matched HLA-related or unrelated donors.[8,14–16] However, aGVHD still occurs in up to 40%–60% of such transplants implicating polymorphic genes outside of the MHC, referred to as minor histocompatibility antigens (mHAs), which may be disparate between the host and the recipient.[17–20]

Murine models suggest that aGVHD can be conceptualized as occurring in a three-step process. The first step is the release of inflammatory cytokines from tissue damaged due to the administration of high-dose chemotherapy and/or radiation therapy prior to the transplant.[21] It has been postulated that the damage caused by the conditioning regimen causes inflammation leading to release of cytokines including tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1) which in turn cause activation of host antigen-presenting cells (APCs). The second step involves the infusion of mature donor lymphocytes contained within the graft into an environment of inflammatory molecules and activated host APCs. This inflammatory environment favors expansion and activation of donor lymphocytes when contact is made with host and donor APCs expressing disparate host antigens (mHA in the HLA-matched transplants). The third step is tissue damage caused by these expanded effector T-cells, which work in unison with cytokines and chemokines to further amplify the immunologic insults on the host target tissue. The degree to which step one contributes to the development of aGVHD can be debated. For instance, tissue injury is not a prerequisite for aGVHD as it may develop in situations in which conditioning is not used, such as following an infusion of donor lymphocytes or in transfusion-associated GVHD. Furthermore, the evidence is not clear that a reduction in tissue damage through the use of reduced-intensity conditioning (RIC) regimens lowers the risk for aGVHD when compared to ablative regimens.[22,23] Nonetheless, the three-step model for aGVHD offers a framework for understanding the complex interactions between APCs, donor T-cells, and cytokines and the development of aGVHD.

## Preventative/prophylactic strategies

Various strategies have been studied to decrease the risk of aGVHD post-transplant. The focus has been to develop 1)

**Table 1** Classification of GVHD based on 2005 NIH consensus statement[1]

| Classification | Timing of symptoms | Acute GVHD features | Chronic GVHD features |
|---|---|---|---|
| **Acute** | | | |
| Classic | <100 days | Present | Absent |
| Persistent/recurrent/late | >100 days | Present | Absent |
| **Chronic** | | | |
| Classic | No time limit | Absent | Present |
| Overlap syndrome | No time limit | Present | Present |

**Abbreviations:** GVHD, graft-versus-host disease; NIH, National Institute for Health.

better donor selection, 2) better preparative regimens, 3) T-cell depletion (TCD) from the graft, and 4) optimal pharmacologic intervention post-transplant.

## Donor selection

Donor selection plays an important role in the development of aGVHD, and evaluating the risks of various donors becomes an important strategy to lessen the incidence of aGVHD. As mentioned earlier, HLA matching becomes the most important factor in determining the risk of aGVHD. It is well recognized that the incidence of aGVHD is increased in HSCT from HLA-nonidentical donors compared to HLA-identical donors.[24] A higher incidence of aGVHD is also seen in unrelated HLA-matched donors compared to HLA-matched sibling donors.[25] Since aGVHD occurs even in fully HLA-matched related and unrelated donors, the phenomenon is thought to be mediated by mHAs.[17,20] The influence of other donor characteristics is still present, although not as important as HLA. Sex mismatching has been shown to increase aGVHD in male recipients from female donors.[5,26] Women with pregnancies may be alloimmunized to the mHA from the fetus[27] and mount an anamnestic response in the donor's body on recognition of overlapping mHA. Increased donor age has been shown to be a determinant of the incidence of aGVHD.[28,29] The Center for International Blood and Marrow Transplant Research (CIBMTR) published an analysis in 2005, which showed an increased risk of aGVHD in ABO mismatched pairs.[30] Cytomegalovirus (CMV) seronegativity of the donor has also been shown to decrease the incidence of aGVHD in seronegative recipients.[5] However, in light of the limited HLA-matched donor availability, these other risk factors are of secondary importance compared to HLA matching.

## Conditioning regimen

The role of preparative regimen in the development of aGVHD has been discussed earlier. Most studies have shown that increased intensity of preparative regimens also increased the risk of aGVHD. In 1990, Clift et al reported that the rate of aGVHD was lower in patients who received a lower dose of total body irradiation with similar aGVHD prophylaxis.[31] Based on the preclinical murine models, RIC regimens were developed to decrease the treatment-related mortality of HSCT as well as the incidence of aGVHD. Comparisons of patients who underwent RIC versus myeloablative conditioning prior to HSCT showed 30%–40% relative reduction in the incidence of aGVHD for patients receiving RIC.[32,33] The decision with regard to choosing between RIC and myeloablative regimens is often based on not only the risk of aGVHD but also the condition and disease of the recipient.

## T-cell depletion

In 1958, Uphoff demonstrated that 'secondary disease' (a term used for GVHD) did not develop with the infusion of fetal liver/spleen tissue into lethally irradiated animals as these tissues lacked mature T-cells.[34] These findings were substantiated and built upon by other researchers in the field.[35,36] Subsequently, antisera against mature lymphocytes were developed and used for ex vivo TCD prior to transplantation in animal models, across histocompatibility barriers without significant GVHD.[37,38] Based on these studies, clinical trials in human HSCT investigated the use of TCD using various preparations of antibodies. The initial studies with antibodies directed against T-cells alone did not reduce the risk of aGVHD in humans likely due to the fact that TCD was inadequate.[39,40] The addition of complement resulted in a 2–3 log reduction in the T-cell numbers (compared to murine antibodies alone), which decreased the incidence of aGVHD to 20% in HLA-matched sibling HSCT.[41,42]

Marmont et al reported outcomes on 731 patients who underwent an ex vivo TCD-related HSCT for hematologic malignancies. Although TCD decreased the rate of aGVHD (relative risk (RR): 0.45; $P < 0.0001$), it also increased the risk of graft failure (RR: 9.29; $P < 0.0001$) and leukemia relapse. In patients with either first-remission acute leukemia or chronic myeloid leukemia (CML) in chronic phase, leukemia relapses were 2.75 times more likely after T cell-depleted transplants ($P < 0.0001$) compared to a T-replete transplant. Overall, TCD increased the risk of treatment failure (RR: 1.35; $P < 0.0003$) and decreased leukemia-free survival.[43] Wagner et al reported a large, randomized trial that investigated the role of ex vivo TCD in unrelated donor HSCT. Although the study showed a lower incidence of grade II–IV GVHD (39% vs 63%, $P < 0.0001$) in the TCD arm, there was no significant difference in overall survival. As in the previous trial, the lack of improvement in overall survival was contributed to higher relapse rates in the TCD arm for patients with CML in chronic phase. In addition, the frequency and severity of CMV and *Aspergillus* infections were also higher in the TCD arm.[44] The Italian group reported two trials with in vivo TCD using rabbit antithymocyte globulin (Thymoglobulin). All patients received cyclosporine (CSA) and methotrexate (MTX) as GVHD prophylaxis. In the first trial, Thymoglobulin was given at a dose of 7.5 mg/kg over 2 days. The development of aGVHD, infections, and survival were similar in the Thymoglobulin group compared

to the control group. In the second trial, Thymoglobulin was given at a dose of 15 mg/kg over 4 days. The rate of grade II–IV aGVHD was lower (37% vs 79%, $P = 0.001$) in the Thymoglobulin group; however, there was no difference in the treatment-related mortality due to an increase in infectious deaths in the TCD arm. No overall survival benefit was observed in these two trials.[45]

In a Phase III randomized trial, patients received either anti-Jurkat ATG-Fresenius ((ATG-F) 20 mg/kg, given on days 3, 2, and 1 prior to transplantation) in combination with CSA and MTX; or CSA and MTX. Although the TCD treatment resulted in less overall aGVHD (grade II–IV), the rate of severe aGVHD (grade III–IV), early mortality, and overall survival were not different between the two arms. In addition, an increase in the rate of CMV and herpes simplex virus infection was noted with TCD.[46]

Alemtuzumab is a monoclonal antibody directed at CD52, which is a protein present at the cell surface of mature lymphocytes. It utilizes antibody-dependent cell-mediated cytotoxicity and complement fixation for cytotoxicity to lymphocytes.[47] Alemtuzumab, when used in vivo before graft infusion, is highly effective with reported incidences of 20% for grade II–IV aGVHD.[48,49] The use of alemtuzumab is limited by the increase in infections and relapse. However, there are no prospective, randomized trials to assess the role of alemtuzumab in prophylaxis of aGVHD. In summary, the majority of trials have shown that TCD reduces the incidence of aGVHD but often at the expense of higher relapse rates, higher graft failure rates, and higher infectious rates resulting in little impact on overall survival.

## Pharmacologic immunosuppression

Historically, pharmacologic immunosuppression has been the most common approach to prevent aGVHD after allogeneic and unrelated transplantation. GVHD prophylaxis was initiated with single-agent therapy; however, randomized trials showed the advantage of using more than one agent for the prevention of aGVHD. Table 2 provides a summary of the randomized trials of various GVHD prophylactic strategies.

MTX is an antimetabolite which inhibits dihydrofolate reductase affecting purine and thymidylate synthesis. It was one of the first drugs to be tested as a prophylactic agent to prevent aGVHD, as it was thought to be cytotoxic to the rapidly proliferating activated T-cells. In addition, it was shown to induce tolerance after transplantation in matched canine models.[50] MTX was used initially as a single agent for prevention of aGVHD. It was given in the intravenous formulation starting at 15 mg/m$^2$ on day 1, followed by 10 mg/m$^2$ on days 3, 6, and 11, and thereafter weekly till day 100.[51] Due to the cytotoxic activity of MTX, the major toxicities were mucositis and delayed engraftment resulting in prolonged hospitalization. Deeg et al then modified the regimen to be given as 15 mg/m$^2$ on day 1, followed by 10 mg/m$^2$ on days 3, 6, 11, 18, and 25, and thereafter for every 2 weeks till day 100.[52]

CSA was initially tested as an antifungal agent and was serendipitously found to cause immune suppression. It is thought to prevent activation of T-cells by blocking the calcium-dependent signal transduction pathway, which is activated when the T cell receptor is engaged. Single agent CSA was compared to MTX for the prevention of aGVHD, and studies showed no statistically significant differences in the incidence of aGVHD or overall survival between these two agents. However, the studies demonstrated shorter hospital stays and quicker engraftment in the CSA group.[52,53] Additional studies comparing the two agents confirmed these results with less mucositis in the CSA group.[54]

In 1986, Storb et al reported the results of a randomized trial, comparing the combination of CSA and MTX versus CSA alone for the prevention of aGVHD in patients undergoing HLA-related donor HSCT for acute myeloid leukemia and CML. MTX was administered at 15 mg/m$^2$ on day 1 followed by three doses of 10 mg/m$^2$ given on days 3, 6, and 11. The results showed a statistically significant benefit in favor of the combination arm for both the incidence of aGVHD (33% vs 54%, $P = 0.014$) and overall survival (80% vs 55% at 1.5 years, $P = 0.042$).[3] CSA was also combined with methylprednisolone (MP) and compared to single agent CSA for prevention of aGVHD. Although the trial showed statistically significant improvement in the incidence of grade II–IV aGVHD (60% vs 73%, $P = 0.01$) in favor of the combination arm, this trial did not demonstrate a significant improvement in overall survival.[55]

Tacrolimus is a macrolide antibiotic, which is extracted from a soil fungus.[56] It was found to be immunosuppressive, with a mechanism of action similar to that of CSA. Based on several Phase II trials which showed efficacy of the combination of tacrolimus and MTX for the prevention of aGVHD,[57,58] two large, randomized trials compared tacrolimus and MTX to CSA and MTX for the prevention of aGVHD in related and unrelated marrow transplantation. In the first trial, 329 patients undergoing HLA-matched sibling transplants received MTX on days 1, 3, 6, and 11 (15, 10, 10, and 10 mg/m$^2$, respectively) and randomized to either CSA (3 mg/kg/day) or tacrolimus (0.03 mg/kg/day) starting at day 1 and tapered off by day 180

**Table 2** Randomized trials for prophylaxis of acute GVHD

| Authors | n | Preparative regimen | Diagnosis | Type of donor | Prophylaxis | Acute GVHD (ii–iv) | OS |
|---|---|---|---|---|---|---|---|
| **Single-drug regimens** | | | | | | | |
| Deeg et al[52] | 75 | Cy/TBI | AML | MRD | CSA (n = 36) | 33%* | 58% at 3 years* |
| | | | | | MTX (n = 39) | 56% | 56% at 3 years |
| Storb et al[53] | 48 | Cy/TBI | CML | MRD | CSA (n = 25) | 42%* | 66% at 3 years* |
| | | | | | MTX (n = 23) | 46% | 62% at 3 years |
| Ringdén et al[54] | 59 | NA | Hematologic malignancies | MRD | CSA (n = 30) | 40% | 58% at 3 years |
| | | | | | MTX (n = 29) | 22%* | 69% at 3 years* |
| **One-drug versus two-drug regimens** | | | | | | | |
| Storb et al[3] | 93 | Cy/TBI | AML CR1 and CML | MRD | MTX + CSA (n = 43) | 33%** | 80% at 1.5 years** |
| | | | | | CSA (n = 50) | 54% | 55% at 1.5 years |
| Deeg et al[55] | 122 | Cy/TBI<br>BU/Cy/TBI<br>BU/Cy | AML, CML, ALL, lymphoma, and MDS | MRD | CSA (n = 60) | 73% | 26% at 3 years |
| | | | | | CSA + MP (n = 62) | 60%** | 23% at 3 years* |
| **Two-drug versus two-drug regimens** | | | | | | | |
| Ratanatharathorn et al[4] | 329 | Multiple regimens | AML, ALL, CML, CLL, MDS, NHL, HD, and MM | MRD | MTX + CSA (n = 164) | 44% | 57% at 2 years |
| | | | | | MTX + T (n = 165) | 32%** | 47% at 2 years** |
| Nash et al[59] | 180 | Multiple regimens | AML, ALL, CML, CLL, MDS, NHL, and HD | MUD, up to one-antigen mismatch allowed | MTX + T (n = 90) | 56%** | 47% at 2 years |
| | | | | | MTX + CSA (n = 90) | 74% | 42% at 2 years* |
| Bolwell et al[65] | 40 | BU/Cy | AML, CML, CLL, NHL, HD, and MDS | MRD | MTX + CSA (n = 19) | 37%* | 68% at 6 months* |
| | | | | | MMF + CSA (n = 21) | 48% | 52% at 6 months |
| Perkins et al[67] | 89 | BU/FLU | AML, CML, CLL, NHL, HD, MDS, and MM | MRD/MUD | MMF + T (n = 42) | 78% | 54% at 3 years |
| | | | | | MTX + T (n = 47) | 79%* | 42% at 3 years* |
| **Two-drug versus multidrug regimens** | | | | | | | |
| Storb et al[68] | 147 | Cy/TBI<br>Cy | AML, CML, AA, and MDS | MRD | CSA + MTX + PSE (n = 59) | 36% | 54% at 2 years |
| | | | | | CSA + MTX (n = 63) | 45%* | 53% at 2 years* |
| Chao et al[69] | 150 | VP16/TBI | CML, AML, and CR1<br>ALL CR1 | MRD/MUD | CSA + MTX + PSE (n = 75) | 9%** | 64% at 3 years* |
| | | | | | CSA + MTX (n = 74) | 23% | 59% at 3 years |
| Chao et al[70] | 186 | VP16/TBI<br>Cy/TBI | AML, CML, and CML | MRD | CSA + MTX + PSE (n = 90) | 18% | 65% at 2 years |
| | | | | | CSA + MTX (n = 96) | 20%* | 72% at 2 years* |
| Ruutu et al[71] | 108 | Cy/TBI<br>BU/Cy | AML, ALLL, CML, CLL, MDS, NHL, and HD | MRD | CSA + MTX (n = 55) | 56% | 51% at 6 years |
| | | | | | CSA + MTX + MP (n = 53) | 19%** | 60% at 6 years* |
| Ruutu et al[85] | 242 | Multiple regimens | Hemetalogic malignancies/ nonmalignant disorders | MRD/MUD | UDCA + CSA + MTX (n = 123) | Grade III–IV (5/123) | 71% at 1 year |
| | | | | | CSA + MTX (n = 119) | Grade III–IV (17/119) | 55% at 1 year** |
| Blazar et al[86] | 100 | Cy/TBI<br>BU/Cy | AML, ALL, CML, CLL, MDS, NHL, and HD | MRD | Palifermin + MTX + CSA/T (n = 69) | 38% | NA |
| | | | | | MTX + CSA/T (n = 31) | 40%* | NA |
| Finke et al[46] | 202 | Multiple regimens | AML, CML, ALL, and MDS | MUD | Rabbit ATG + CSA + MTX (n = 103) | 33% | 59% at 2 years |
| | | | | | CSA + MTX (n = 98) | 51%** | 51% at 2 years* |

**Notes:** *$P > 0.05$ (not significant); **$P \leq 0.05$ (significant).

**Abbreviations:** Cy, cyclophosphamide; BU, busulfan; TBI, total body irradiation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; CLL, chronic lymphocytic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; MM, multiple myeloma; MRD, matched related donor; MUD, matched unrelated donor; MTX, methotrexate; CSA, cyclosporine; PSE, prednisone; MP, methylprednisolone; ATG, antithymocyte globulin; T, tacrolimus; UDCA, ursodeoxycholic acid; GVHD, graft-versus-host disease; MMF, mycophenolate mofetil; CR1, complete remission 1; NA, not available; FLU, fludarabine; AA, aplastic anemia.

for the prevention of aGVHD.[4] The incidence of aGVHD was 32% in the tacrolimus arm versus 44% in the CSA arm ($P = 0.01$). Although the overall survival was inferior in the tacrolimus arm, it appeared that this was due to a higher than expected number of patients with advanced disease in the tacrolimus arm. A companion study comparing the same regimens for aGVHD prophylaxis was performed in unrelated transplantation, and similar to the results of the study in related transplantation, the rate of aGVHD was lower in the tacrolimus and MTX arms (56% vs 74%, $P = 0.0002$), with no significant difference in overall survival.[59]

Although MTX has become a standard part of most immunosuppressive regimens for GVHD prevention, its two most common side effects, mucositis and delayed engraftment, make its use difficult. Several groups have investigated prophylactic regimens that do not contain MTX. Mycophenolate mofetil (MMF) is an antimetabolite that is hydrolyzed in the body to the active moiety mycophenolic acid. Mycophenolic acid inhibits inosine monophosphate dehydrogenase, blocking de novo purine synthesis, which results in an inhibition of both T- and B-cell proliferation as well as a decrease in antibody production.[60,61] Dog models showed that a combination of CSA and MMF prevented graft rejection and GVHD in transplants from DLA-identical litter mates.[62] Based on these preclinical models, various immunosuppressive agents have been combined with MMF for the prevention of aGVHD. Several single-center trials have reported Phase II results on the use of MMF with tacrolimus for the prevention of aGVHD. A recent study reported on 131 patients who received tacrolimus and MMF as prophylaxis for GVHD, after matched related donor (MRD) HSCT using myeloablative conditioning regimens. The cumulative incidence of grade I–IV aGVHD was 12% after 120 days.[63] In a nonrandomized study, tacrolimus and MMF were used as GVHD prophylaxis following nonmyeloablative conditioning and unrelated hematopoietic HSCT for patients with advanced hematologic diseases. MMF was tapered starting at day 28 and discontinued by day 50 after transplant, while tacrolimus taper was based on disease status at transplant. This study showed delayed onset of aGVHD, with an incidence of grade II–IV aGVHD of 54%.[64] A prospective, randomized trial compared CSA/MTX and CSA/MMF in the setting of myeloablative preparative regimens followed by HLA-MRD HSCT for patients with advanced hematologic malignancies. Patients on the CSA/MMF arm showed early engraftment (day 11 vs day 18, $P = 0.008$) and less mucositis (21% vs 65%, $P < 0.001$) compared to CSA/MTX but no difference in the incidence of aGVHD or overall survival.[65] In a retrospective analysis, CSA in combination with MMF or MTX after RIC HSCT from HLA-identical siblings showed similar incidence of grade II–IV aGVHD (48% vs 50%, $P$ = ns) with increased incidence of mucositis in the MTX arm (57% vs 23%, $P = 0.001$).[66]

Perkins et al have recently reported the results of a randomized Phase II trial that compared tacrolimus in combination with MMF or MTX as prophylactic regimens for related and unrelated transplantation in 89 patients. The incidence of aGVHD was 78% in the MMF/tacrolimus arm compared to 79% in the MTX/tacrolimus arm ($P = 0.8$). There was no difference in overall survival. The study also showed shorter hospital stays and less mucositis in patients who received MMF.[67] However, patients who received the MMF/tacrolimus combination had a higher rate of severe grade III–IV aGVHD (26% vs 4%, $P = 0.04$) in unrelated donors, suggesting this combination may not be adequate in unrelated transplantation.

The use of three drugs for the prevention of aGVHD has also been investigated. Storb et al reported a trial with 147 patients who received GVHD prophylaxis with the combination of CSA, MTX, and prednisone (MP) or CSA and MTX. The incidence of aGVHD was not decreased by the addition of prednisone, and overall survival was similar in the two groups.[68]

In another randomized trial, Chao et al also compared the incidence of aGVHD in patients undergoing allogeneic transplant, who received either CSA and MTX; or CSA, MTX, and prednisone.[69] They found there was a significantly lower incidence of aGVHD in the group receiving CSA, MTX, and prednisone (9%) compared to the group receiving CSA and MTX (23%) ($P = 0.02$). However, there was no difference in disease-free survival between the two groups at 3 years. Subsequently, when the same regimen was studied in patients with advanced hematologic malignancies, no difference in the incidence of aGVHD was seen between the three-drug and two-drug regimen (18% and 20%, $P = 0.6$).[70]

Ruutu et al used CSA and MTX with and without MP for GVHD prophylaxis in a prospective, randomized trial with 108 patients. Steroids were initiated at day 14 at a dose of 0.5 mg/kg, then increased to 1 mg/kg at days 21–34, and subsequently tapered. Initiation of steroids at day 14 was chosen to eliminate the potential interaction with MTX. Despite a significantly lower incidence of aGVHD (19% vs 56%, $P = 0.0001$), the study failed to show any survival benefit for the MP arm.[71]

Sirolimus is another macrolide, which was shown to have immunosuppressive activity. Structurally, sirolimus is similar to tacrolimus, containing a hemiketal-masked αβ-diketopipecolic acid amidic component.[72] Sirolimus has been shown to inhibit cytokine-driven growth of lymphoid cells,[73] decrease production of interferon γ (IFN-γ),[74] down-regulate CD28,[75] and increase regulatory T-cells.[76] Sirolimus was initially added to tacrolimus and MTX for the prevention of aGVHD. In a Phase II trial, 39 patients undergoing an allogeneic HSCT from mismatched related or unrelated donors after a myeloablative preparatory regimen received a combination of tacrolimus, sirolimus, and MTX. The incidence of grade II–IV aGVHD was 26%.[77] In a larger study of 91 patients who underwent HSCT from MRDs or MUDs with RIC regimen, the cumulative incidence of a grade II–IV aGVHD was 16%.[78]

In the setting of RIC regimen, Ho et al combined sirolimus and tacrolimus and reported an incidence of aGVHD of 17%. The incidence was felt to be similar to a three-drug prophylactic regimen of tacrolimus/sirolimus/MTX (11%) used by the same group in a previous study.[79] A Phase II study of tacrolimus/sirolimus GVHD prophylaxis for MRD HSCT using different myeloablative conditioning regimens in 85 patients showed a cumulative incidence of aGVHD (grade II–IV) of 43%.[80] Others have shown similar efficacy with the combination of sirolimus and tacrolimus. Cutler et al reported on the incidence of aGVHD in 83 patients who received either related or unrelated transplants using sirolimus and tacrolimus.[81] The cumulative incidence of grade II–IV and III–IV aGVHD was 20.5% and 4.8%, respectively. They also indicated that the omission of MTX was associated with low transplant-related toxicity and an excellent 100-day survival rate of 95.2%. No differences in the aGVHD rate were noted between related and unrelated transplant patients. The same group also reported that sirolimus may lead to a higher than expected rate of venoocclusive disease when used with some of the more intense preparative regimens.[82]

Extracorporeal photopheresis (ECP) has recently been reported to be a strategy for prevention of aGVHD. The mechanism of action of ECP is not clear, although it may decrease CD8+ cytotoxic T-cells and increase regulatory T-cells.[83] Sixty-two patients, 31 (6/6 matched unrelated donor (MUD)), 30 (6/6 MRD), and 1 (5/6 MRD), underwent two rounds of ECP within 4 days of starting the preparative regimen. All patients received prophylaxis with CSA and MTX in addition to the ECP. The cumulative incidence of aGVHD was 30% in MRD HSCT and 41% in MUD HSCT.[84] ECP is being further investigated currently in a randomized Phase II study.

## Other interventions

Several strategies involving organ protection have been studied to reduce the incidence of aGVHD. The Nordic group reported a prospective, randomized trial on the addition of ursodeoxycholic acid (UDCA) to their standard prophylactic regimen of CSA and MTX. UDCA was administered from the start of the conditioning regimen until day 90 post-transplant. Patients who received UDCA had a significantly lower incidence of severe grade III–IV aGVHD ($P = 0.01$) and showed significant improvement in overall survival at 1 year (71% vs 55%, $P = 0.01$).[85] The improvement seen with the use of UDCA may be due to reducing the expression of various antigens involved in aGVHD.

As mentioned earlier, animal models suggested gastrointestinal (GI) injury may be the first step in the pathophysiology of aGVHD. Based on these data, the effect of keratinocyte growth factor (KGF) was studied in a Phase I/II placebo-controlled trial for the prevention of aGVHD. KGF is an epithelial growth factor which has demonstrated efficacy in the prevention of chemo or radiation injury to various epithelial cells. Although the administration of KGF was safe, it had no significant benefit when added to MTX and CSA or MTX and tacrolimus for the prevention of aGVHD.[86]

# Treatment strategies

## Primary therapy for aGVHD

Despite the various prophylactic measures used to prevent aGVHD, its incidence remains high occurring in 40%–60% of the patients undergoing HSCT. Corticosteroids have been the mainstay in treatment of aGVHD and are used in IV, oral, and topical formulations depending upon the severity and organ involvement. Randomized studies evaluating different agents for primary treatment of aGVHD are summarized in Table 3.

For isolated skin GVHD up to stage 2 (<50% involvement of skin with maculo-papular rash), treatment with topical steroids is acceptable. The recommended dose of systemic steroids for grade II–IV disease is 2 mg/kg/day of MP. Higher doses of steroid provide no further benefit as shown in a randomized trial comparing 2 mg/kg/day versus 10 mg/kg/day for treatment of aGVHD.[87] Hings et al randomized patients to long versus short taper of steroids after response to 2 mg/kg/day of MP on day 14 of treatment. The patients on long taper were on MP for median of 147 days,

**Table 3** Randomized trials for up-front treatment of aGVHD

| Authors | n | Treatment | aGVHD grade | | | | Overall response rate (CR + PR) | Overall survival |
|---|---|---|---|---|---|---|---|---|
| | | | I | II | III | IV | | |
| Cragg et al[2] | 96 | Prednisone (60 mg/m$^2$) (n = 46) | 22% | 74% | 4% | 0 | 73% (grade II–IV) | 50% at 2 years |
| | | ATG (15 mg/m$^2$ bid) + prednisone (20 mg/m$^2$) (n = 50) | 24% | 70% | 6% | 0 | 78% (grade II–IV)* | 40% at 2 years* |
| Van Lint et al[87] | 95 | MP 2 mg/kg (n = 47) | NA | NA | NA | NA | 68% | 63% at 3 years |
| | | MP 10 mg/kg (n = 48) | NA | NA | NA | NA | 70%* | 62% at 3 years* |
| Lee et al[90] | 102 | MP (2 mg/kg) (n = 49) | 20% | 55% | 25% (III–IV) | | 53% | 60% at 1 year |
| | | Daclizumab + MP (2 mg/kg) (n = 53) | 21% | 64% | 15% (III–IV) | | 51%* | 26% at 1 year** |
| Couriel et al[91] | 59 | MP (2 mg/kg) (n = 28) | 0 | 68% | 32% | 0 | 54% | 28% at 5 years |
| | | MP (2 mg/kg) + infliximab (10 mg/kg) (n = 29) | 0 | 65% | 31% | 3% | 30%* | 17% at 5 years* |
| Alousi et al[92] | 180 | Etanercept + MP (2 mg/kg) (n = 46) | 17 | 54 | 26 | 0 | 26% (CR) | 47% at 9 months |
| | | MMF + MP (2 mg/kg) (n = 45) | 7 | 56 | 36 | 2 | 60% (CR) | 64% at 9 months |
| | | Denileukin + MP (2 mg/kg) (n = 47) | 17 | 49 | 32 | 2 | 53% (CR) | 49% at 9 months |
| | | Pentostatin + MP (2 mg/kg) (n = 42) | 10 | 62 | 26 | 2 | 38% (CR) | 47% at 9 months |

**Notes:** *$P > 0.05$ (not significant); **$P \leq 0.05$ (significant).
**Abbreviations:** MP, methylprednisolone; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; NA, not available; aGVHD, acute graft-versus-host disease; PR, partial remission; CR, complete remission.

while those on short taper were on MP for median of 86 days. This trial did not show any difference in overall survival for both the groups.[88]

Although steroids have become the standard of care for the treatment of aGVHD, their effectiveness remains suboptimal. Two large, retrospective studies on the use of steroids for the primary treatment of aGVHD reported sustained complete remission (CR) rates of only 18% and 35%.[6,89]

Due to the poor overall response rates, several groups have looked at intensifying the immunosuppressive regimen by adding additional agents to steroids for the initial treatment of aGVHD. Daclizumab was studied by Lee et al in a randomized trial comparing steroids versus steroids plus daclizumab for the initial treatment of aGVHD. The trial enrolled 102 patients from 5 institutions.[90] Patients on the trial received HSCT from MRDs (40%) or MUDs (40%), with a minority of the patients on each arm receiving mismatched related/unrelated HSCT. The response rate was 53% in the steroid arm, compared to 51% in patients who received the combination of steroids and daclizumab ($P = 0.85$). The 100-day survival was worse in the combination arm (77% vs 94%, $P = 0.02$).

Infliximab was tested in a randomized Phase III trial as up-front treatment of aGVHD.[91] A total of 63 patients were randomized to either infliximab and MP (2 mg/kg/day) or MP (2 mg/kg/day). Sixty-seven percent of patients had grade II and 33% had grade III–IV aGVHD. At day 28, the response rate for infliximab + MP was 62%, while the response rate for MP was 58% ($P = 0.7$). Cumulative incidences of nonrelapse mortality and overall survival were not significantly different between the two groups.

A randomized trial of 96 patients evaluated the combination of steroids and ATG compared to steroids alone as initial therapy of aGVHD.[2] This trial showed partial and complete response rates of 76% patients in both the arms ($P > 0.8$). Complications with CMV reactivation and pneumonitis (both infectious and noninfectious) were higher in the ATG arm, with no significant difference in overall survival at 2 years.

Alousi et al,[92] on behalf of Bone Marrow Transplant Clinical Trials Network, investigated the role of etanercept,[93]

mycophenolate,[94] denileukin,[95] or pentostatin[96] plus corticosteroids for initial treatment of aGVHD. In this randomized Phase II trial, the day 28 complete response rates were 60%, 53%, 38%, and 26% for MMF, denileukin, pentostatin, and etanercept, respectively, suggesting the combination of steroids and MMF provides the best combination for treatment of aGVHD. A randomized Phase III trial which will compare MMF/steroids with steroids alone is ongoing. In summary, the trials to date have not shown benefit when further immunosuppressive agents are added to steroids for the treatment of aGVHD.

## Secondary therapy for aGVHD/steroid-refractory aGVHD

Criteria for diagnosing steroid refractoriness have not been formalized, but the study by Van Lint et al suggests that no response by day 5 of treatment with steroids is a reliable marker of poor outcome; patients who respond to 2 mg/kg/day of steroids by day 5 had a nonrelapse mortality of 27%, compared to a 49% nonrelapse mortality for day-5 nonresponders.[97] Unfortunately, there are no proven treatments based on Phase III trials for steroid-refractory GVHD. The bulk of the data from treatment of these patients often rely on small Phase II trials with different eligibility criteria making comparisons and treatment decisions difficult. The choice of secondary therapy is often based on pre-existing organ toxicity and prior GVHD prophylaxis of the patient.

### Antithymocyte globulin

ATG is the most common treatment of choice for steroid-refractory aGVHD based on an international practice survey by Hsu et al.[98] ATG has been shown to increase regulatory T-cells which have an important role in the development of tolerance.[99]

There are various ATG preparations (rabbit vs horse) with differing potency and different treatment regimens making comparisons between studies difficult. Arai et al retrospectively reviewed 69 patients who were treated with ATG for steroid-refractory aGVHD.[100] The criteria for adding ATG varied. In some patients, ATG was added when patients were refractory to frontline treatment. In other patients, ATG was administered during flares of aGVHD, which did not respond to reescalation of steroids. Patients received a total of seven doses of horse ATG of 10–15 mg/kg every other day. The overall response rate with ATG salvage, which included partial and complete response, was 42% and 24% for grade II and grade III–IV GVHD, respectively. MacMillan et al retrospectively analyzed data from 79 patients treated at their institution with horse ATG (15 mg/kg, twice a day for 5 days).[101] Steroid-refractory aGVHD was defined as worsening of GVHD within 4 days of initiation of steroids or failure to respond by 7 days of treatment with steroids. The overall response rate of day 28 was 54% (20% CR + 34% partial remission (PR)). Based on these and other studies, 20%–50% of patients will improve on ATG but responses appear most common with steroid-refractory skin GVHD as 60%–75% of these patients respond.[102] The main complications from therapy with ATG are related to infusion reactions and increased risk of viral and fungal infections.

### Biological therapies

Various antibodies have been studied for treatment of aGVHD including antibodies directed at T-cells (anti-CD2, anti-CD3, anti-CD5, anti-CD25, anti-CD 52, and anti-CD147) and inflammatory cytokines (etanercept and infliximab).

BTI-322, a rat monoclonal IgG2b directed against the CD2 antigen on T-cells and natural killer cells, blocks primary and memory alloantigen proliferative responses in vitro. It was tested in 20 patients with steroid-refractory aGVHD and showed a response rate of 55%.[103] OKT3 is a murine monoclonal antibody directed at CD3. When OKT3 was added to MP, there was an observed response rate of 53% compared to a 33% response in the control arm that used steroids alone. The difference was not statistically significant, and the dose of MP (10 mg/kg) was high.[104] Several complications were seen with the use of OKT3 including higher risk of viral infections, especially Epstein–Barr virus (EBV), and a higher incidence of cytokine storm due to TNF-α.[105] Visilizumab, an antibody directed at CD3 that does not bind to human Fc receptor, showed a response rate of 32% in steroid-refractory aGVHD. EBV reactivation occurred in 19/44 patients treated on the protocol.[106]

Anti-CD5 antibody conjugated with ricin cytotoxin has been studied in a Phase I–II trial with 34 patients. Response rate of skin, GI, and liver GVHD were 73%, 45%, and 28%, respectively.[107]

Activated T cell can be targeted by antibodies directed at CD25 (α-subunit of IL-2 receptor). Daclizumab, Inolimomab, basiliximab, and denileukin diftitox have been used to target activated T-cells to elicit a response in refractory GVHD. The use of daclizumab was reported to show improvement in 30%–50% of patients with steroid-refractory GVHD but came with a risk of increased infectious

complications.[108–110] Inolimomab (murine IL-2 receptor antibody) showed a response rate of 63% in steroid-refractory aGVHD.[111] Basilixumab (chimeric IL-2 receptor antibody) was shown to have an impressive 71% response rate in steroid-refractory aGVHD;[112] a later trial with 34 patients demonstrated overall response rates of 26%, 48%, and 84% in liver, GI, and skin GVHD, respectively.[113] Denileukin diftitox, a recombinant fusion protein which contains parts of IL-2 and diphtheria toxin amino acid sequence, showed a 71% response rate in steroid-refractory aGVHD in a Phase I trial.[95] Alemtuzumab was evaluated in a Phase II setting for 10 patients with steroid-refractory aGVHD and showed a clinical response rate of 55%, with complete resolution seen only in two patients.[114] ABX-CBL is a murine antibody directed at CD147. It was tested in a randomized trial against ATG, and similar response rates were observed for the two agents (56% vs 57%, $P = 0.91$). However, the survival rate of patients treated with ABX-CBL was inferior, although not achieving statistical significance (35% vs 45%, $P = 0.1$).[115]

Based on murine data that showed inflammatory cytokines such as TNF-α are important in the pathophysiology of aGVHD, various studies testing antibodies against these inflammatory molecules have been reported. The use of etanercept (fusion protein capable of neutralizing TNF-α) combined with steroids was reported to have a 75% complete response rate for up-front treatment of aGVHD.[93] Busca et al studied this drug in steroid-refractory GVHD and obtained responses in 46% of the patients.[116] Infliximab is a chimeric antibody directed against TNF-α, which was reported to have activity (response rates of 60%) in steroid-refractory GVHD, especially with the involvement of the GI tract.[117] However, when both of these anti-TNF compounds were tested in larger separate trials, their efficacy was somewhat lower.[91,92]

### Extracorporeal photopheresis

Several studies have now shown that ECP may play a role in treatment of aGVHD. In a pilot trial of 21 patients with steroid-refractory aGVHD, 60% responded to ECP. However, none of the patients with GI GVHD responded to this intervention.[118] Greinix et al, in 2006, reported the results of a Phase II trial of 59 patients with steroid-refractory aGVHD treated with ECP. They had a complete response rate of 82%, 61%, and 61% in patients with skin, liver, and GI GVHD, respectively.[119] A review, published in 2002, summarized the results on the use of ECP in aGVHD in 76 patients treated in 11 separate studies.[120] Of the 76 patients, 59, 47, and 28 presented with skin, liver, and GI manifestations of aGVHD, respectively. Treatment duration ranged from 1 to 24 months. Regression of skin manifestations was observed in 83% of the patients with a complete response in 67%. A complete regression of liver and gut manifestations was reported in 38% and 54% of the patients, respectively.

### Mesenchymal stem cells

Bone marrow–derived mesenchymal stem cells (MSCs) have been shown in animal models and in vitro testing to modulate immune and inflammatory responses and facilitate repair of connective tissue. In addition, they inhibit inflammatory cytokines, such as TNF-α and IFN-γ. These experimental data supported the concept of MSCs as therapeutically effective cells for treatment of aGVHD. Leblanc et al treated 55 patients with MSCs for steroid-refractory aGVHD and showed responses in 55%.[121] A response rate of 15% was reported by Von Bonin et al after utilizing MSCs for steroid-refractory aGVHD.[122] Kebriaei et al, in a small Phase II trial, reported on the safety and efficacy of a commercially available preparation of unrelated human MSCs formulated for IV delivery in patients with aGVHD.[123] Patients were given MSCs along with steroids with the onset of aGVHD. They reported that the cells could be administered safely, and 77% of patients had an initial CR to therapy. Given the safety and initial efficacy, these cells were then tested in a randomized placebo-controlled Phase III trial in steroid-refractory aGVHD. In spite of the initial reports, there was no statistical advantage in response rates or overall survival in patients who received the MSC preparation.[124]

## Summary

It is clear that outcomes of transplantation have improved markedly. Improvements have been due to many factors including better supportive care, high-resolution HLA typing that leads to better donor matching, and the increased use of reduced-intensity transplant regimens. However, aGVHD remains a major complication when using this procedure and continues to play a role in the morbidity and mortality associated with it. To expand the use of allogeneic transplant, better management strategies for the prevention and treatment of aGVHD are needed. Although many agents have been studied, few have shown promise in treating aGVHD. Certainly, more clinical trials are needed to better define options for the prevention and treatment of aGVHD.

## Disclosure

The authors report no conflicts of interest in this work.

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