



Published in final edited form as:

*Curr Opin Hematol.* 2009 November ; 16(6): 427–436. doi:10.1097/MOH.0b013e3283319a6f.

## Acute Graft versus Host Disease: New Treatment Strategies

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### Abstract

**Purpose of the review**—Graft-versus host disease (GVHD) remains a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT), despite improvements in our understanding of its pathophysiology as well as the generation of new monoclonal antibodies, immunomodulatory chemotherapy, cellular therapeutics and supportive care. Herein, we review therapies that have proven effective as well as newer agents that have recently improved GVHD response rates and survival following HCT.

**Recent findings**—Novel approaches to prevent or treat GVHD are often based on evidence from experimental models. Our understanding of the pathophysiology of GVHD may lead to the development of innovative strategies that target both soluble and cellular effectors. Among such agents are sirolimus, anti-TNF antibodies, anti-LFA-3–IgG fusion protein, extracorporeal photopheresis, mesenchymal stem cells and regulatory T cells.

**Summary**—Obstacles to the improvement of HCT include the tight linkage between GVHD toxicity and the beneficial graft versus leukemia effect (GVL), as well as the impairment of immune reconstitution by immunomodulatory drugs leading to life-threatening infections. The design of newer phase I/II clinical trials are underway. Future therapies are likely to include modulation of cell types that play key roles in the GVH process, including regulatory T cells, dendritic cells, NKT cells and B cells.

### Keywords

Allogeneic hematopoietic cell transplantation (HCT); graft versus host disease (GVHD); immunomodulatory drugs

### Introduction

Graft versus Host Disease (GVHD) is the principal complication of allogeneic HCT that limits the wider application of this therapeutic approach to patients with high-risk hematologic malignancies. The pathophysiology of acute GVHD is complex and can be considered in a framework of three sequential phases. In Phase I, the recipient conditioning regimen damages host tissues and causes release of pro-inflammatory cytokines. As a consequence, host antigen presenting cells (APCs) mature, acquiring adhesion and co-stimulatory molecules. In Phase II, host APCs activate mature donor T cells which subsequently proliferate and produce additional cytokines. Phase III involves inflammatory and cellular effectors that trigger additional inflammatory responses and together mediate target tissue damage [1\*\*,2\*]. Novel agents can act at different points of these three phases, and most current therapies are not specific to any single phase.

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## Prevention of GVHD

The most widely used GVHD prophylaxis following full intensity conditioning includes a combination of a calcineurin inhibitor (e.g. cyclosporine, tacrolimus, sirolimus) with “short course” methotrexate (MTX). This standard regimen was first described in 1986 by Storb et al. [3] and several clinical trials have shown superiority in reducing the incidence of GVHD and improving survival using this combination compared to either agent alone [4-6]. A recent meta-analysis of prophylaxis regimens for GVHD further supports the use of cyclosporine-MTX or tacrolimus-MTX over cyclosporine alone [7\*]. Tacrolimus and cyclosporine both interrupt the T-lymphocyte signaling pathway via inhibition of calcineurin, an activator of Nuclear Factor of Activated T cell (NFATc). In many centers tacrolimus has replaced cyclosporine; several studies have shown that tacrolimus-MTX is superior to cyclosporine-MTX in reducing acute GVHD although long-term survival is not affected [5,8].

Several other immunosuppressive agents are also used as GVHD prophylaxis. Sirolimus, mTOR (mammalian Target of Rapamycin), an inhibitor of activated T cells via coupling to FK binding protein 12 (FKBP12), may also expand and maintain of CD4<sup>+</sup>CD25<sup>hi</sup>FOXP3<sup>+</sup> regulatory T cells (Tregs) [9,10]. Furthermore, sirolimus may inhibit functions of dendritic cells, which are important in the initiation of GVHD [11-14].

The combination of sirolimus and tacrolimus has resulted in rapid engraftment, a low incidence of acute GVHD, reduced transplant-related toxicity, and improved survival in phase II trials [15,16]. The Bone Marrow Transplant Clinical Trials Network (BMT-CTN) is currently conducting a prospective phase III trial of sirolimus-tacrolimus versus tacrolimus-MTX following HLA-matched, related peripheral blood stem cell transplantation. Recent reports of sinusoidal obstruction syndrome/veno-occlusive disease have been associated with sirolimus [16,17].

Mycophenolate mofetil (MMF) is the prodrug of mycophenolic acid which is a selective inhibitor of inosine monophosphate dehydrogenase, an enzyme critical to the de novo synthesis of guanosine nucleotide. MMF inhibits T cell proliferation, and is now commonly used in combination with a calcineurin inhibitor for GVHD prophylaxis, although the optimal prophylaxis regimen following reduced-intensity HCT is not well established [18-22].

Multiple factors influence the strategies to prevent GVHD in individual patients, including risk of relapse, organ dysfunction, patient performance status, and risk of infections. A recent study of international HCT registry data from 1995 to 2002 reported risk factors for grade II-IV acute GVHD in 1,960 adults after HLA-identical sibling myeloablative transplant for leukemia [23\*]. The cumulative incidence of grade II to IV acute GVHD was 35% (95% CI, 33% to 37%). In multivariable analyses, factors significantly associated with grade II to IV acute GVHD were total-body irradiation versus busulfan peripheral blood versus bone marrow, recipient age 40 and older, CML versus AML/ALL, white/Black versus Asian/Hispanic race, Karnofsky performance score less than 90, and recipient/donor cytomegalovirus-seronegative versus either positive.

For recipients of HLA-mismatched donor grafts, many centers have previously attempted to decrease the risk of GVHD by ex-vivo T-cell depletion. This approach has been limited, however, by an increased incidence of relapse as well as life-threatening infections [24]. Anti-thymocyte globulin (ATG) or alemtuzumab have been used to deplete T cells in vivo. ATG is a polyclonal antibodies mixture either of horse or rabbit origin that are directed against multiple epitopes of human T cells [25-29]. Alemtuzumab is a monoclonal antibody specific for CD52 antigen, which is present on all human leukocytes [30-34]. These

approaches can reduce acute GVHD but they increase the same complications observed with ex-vivo T-cell depletion [35-37]. With respect to long-term overall survival, the benefit of these prophylactic regimens compared to a calcineurin inhibitor + MTX remains uncertain.

## Treatment of acute GVHD

Acute GVHD can include the skin, liver and gastrointestinal (GI) tract and the current grading system (grades I-IV) for acute GVHD is based upon the extent of target organ involvement [38]. Significant (grade II or greater) GVHD is usually treated with high dose methylprednisone, typically starting at 2 mg/kg/day. Durable responses occur in less than half of patients with grade II to IV GVHD [39-42], and the most important predictor of long-term survival in patient with acute GVHD is the primary response to the first line of treatment [42]. Higher doses (> 2 mg/kg/day) have failed to show any advantage [43]. Because steroid-resistant GVHD has such a poor outcome, many clinicians prefer aggressive primary treatment of GVHD, even though protracted therapies with steroids incur multiple complications. Treatment with high dose steroids often continues for 7 days or more, with a progressive taper over 8 weeks or longer depending on the clinical response. In this light, a recent retrospective study from Seattle is particularly interesting. Low-dose prednisone (1 mg/kg/day) was compared to standard dose (2 mg/kg/day) for initial treatment of acute GVHD [44\*]. The non-relapse mortality and overall survival were similar between regimens, with a reduced risk of invasive fungal infections and shorter hospitalizations in the low-dose prednisone group.

Local steroid therapy is also often commonly used in low grade GVHD, specifically of the skin and GI tract. For grade I skin GVHD, many centers use triamcinolone 0.1% ointment or cream to affected areas, and hydrocortisone 0.1% ointment or cream to affected facial lesions. For GI GVHD, oral non-absorbable steroids such as budesonide or beclamethasone dipropionate (BDP) have been used [45\*]. A small single-center study of oral budesonide in addition to systemic corticosteroids in patients with acute GI tract GVHD showed complete responses in 77% of patients compared to 32% in the historical controls [46]. A randomized, placebo-controlled trial of oral BDP for GI tract GVHD showed that oral BDP reduced flares of GVHD following a prednisone taper and resulted in superior survival at 1 year post transplant [47]. Other attempts to deliver the steroids to the target organ include intra-arterial administration of steroids for GI and hepatic GVHD [48,49]. A recent study of 17 patients with systemic, steroid-resistant GVHD reported 35% complete responses and 29% partial responses for this approach [50].

ATG is commonly used to treat steroid-refractory GVHD, but its use has been primarily limited by severe life-threatening opportunistic infections [51]. Response rates following ATG are highest in patients with skin involvement and lowest in those with liver involvement [51,52]. A prospective randomized trial comparing ATG and steroids at 5 mg/kg to steroids at 5 mg/kg alone as second-line therapy showed no significant difference between groups [53].

Various monoclonal antibodies to cell surface antigens on GVHD effector cells have also been used as treatment for the disease. There are case reports of resolution of acute GVHD with alemtuzumab [54,55]. Delayed immune reconstitution in these patients results in serious infections, particularly CMV infections [36,37,56]. Visilizumab, a humanized monoclonal anti-CD3 antibody, induces selective apoptosis of activated T cells after binding to CD3 [57,58], as does OKT3 [59]. Both agents induce 30- 50% overall response rates but life-threatening opportunistic infections remain a significant toxicity and no overall survival benefit has been reported.

A monoclonal antibody directed against CD147, a neurothelin expressed on activated T and B lymphocytes, monocytes and dendritic cells, has been tested in steroid-refractory GVHD. A pilot study showed a 41% overall response rate [60] but a phase II/III trial comparing anti-CD147 to ATG in 92 patients with steroid-refractory GVHD did not show any differences in either response or survival [61].

Several anti-IL-2-receptor (CD25) antibodies have been developed and studied extensively: inolimomab, a murine antibody specific for IL-2 $\alpha$  receptor [62,63]; basiliximab, a chimeric murine/human monoclonal antibody specific for IL-2R $\alpha$  [64-66] daclizumab, a humanized monoclonal antibody to the alpha subunit of the IL-2R [67]; and denileukin diftitox, a fusion protein combining sequences from CD25 with sequences from the diphtheria toxin leading to the toxic lysis of activated lymphocytes [68]. These agents have shown efficacy as second-line treatment, but infections remain the most significant problem associated with these drugs [62-67]. Denileukin diftitox, the only agent that is still currently under investigation, produced a 71% response rate for steroid-refractory acute GVHD in a phase I trial [68] but only a 27% response rate at day 100, in a phase II trial [69].

Alefacept is a fusion protein of the first extracellular domains of human LFA-3 and the Fc portion of IgG1 that blocks CD2–LFA-3 costimulation by selective binding to CD2 on memory effector T cells. Alefacept has been shown to be active in T cell mediated autoimmune disease, particularly in psoriasis [70]. Three small clinical studies have demonstrated response rates of more than 70% for steroid refractory GVHD [71-73].

A different strategy to treat GVHD is blockade of the inflammatory cytokine TNF $\alpha$ . Two anti-TNF $\alpha$  monoclonal antibodies have been used: infliximab, a chimeric monoclonal antibody that binds to TNF $\alpha$  and that lyses cells producing TNF $\alpha$ ; and etanercept, a recombinant DNA protein composed of TNF receptor II linked to the Fc portion of human IgG1. Infliximab resulted in a 19% complete response rate in 32 patients treated for grade II-IV steroid-refractory acute GVHD in a multicenter, retrospective analysis. These patients experienced high rates of infectious complications as would be expected with exposure to high dose steroids [74]. The combination of etanercept and steroids was recently tested as first-line therapy for patients with grade II-IV acute GVHD in a single-center trial. Complete response rates of approximately 70% were observed four weeks following treatment, although this finding has not yet been reproduced [75\*].

Two new anti-TNF antibodies have been approved by the Food and Drug Administration: adalimumab, a fully human monoclonal anti-TNF antibody that has been recently tested in both Crohn's disease [76,77] and juvenile rheumatoid arthritis [78]; and certolizumab pegol, a pegylated humanized Fab' fragment of an anti-TNF- $\alpha$  monoclonal antibody that has also used to treat Crohn's disease [79] and rheumatoid arthritis [80]. The cytotoxic effects of the newer anti-TNF agents are mechanistically different, e.g. infliximab and adalimumab exert complement-dependent cytotoxicity and induce apoptosis in Jurkat T cells that express transmembrane TNF $\alpha$ , while etanercept showed considerably lower levels of both activities [81,82].

Pentostatin is a purine analog that reduces DNA synthesis by inhibiting adenosine deaminase. In a phase I trial, pentostatin led to a 78% overall response rate in steroid-refractory acute GVHD, although infectious complications were frequent [83].

Sirolimus has shown some efficacy in the treatment of both acute and chronic GVHD [84,85] with a twenty-four percent complete response rate observed in patients with steroid-refractory acute GVHD [84].

MMF produced a 65% response rate when it was used with a calcineurin inhibitor and prednisolone to treat steroid-refractory acute GVHD [86]. Several small studies have confirmed these promising results [87-89]. Interests in MMF increased further following the results of a phase II, randomized, four-arm trial of 180 patients with newly diagnosed acute GVHD. Patients were treated with standard corticosteroids plus one of the four new agents: MMF, etanercept, denileukin diftitox and pentostatin. MMF produced the best results in terms of toxicity profile, response, survival, incidence of chronic GVHD, and infections [90\*\*]. A randomized phase III study to validate these findings is currently being planned. Should be noted that in a multicenter randomized trial, the addition of MMF to the initial systemic treatment regimen for chronic GVHD led to no difference in the cumulative incidence of chronic GVHD and resulted in a hazard ratio of death of 1.99 (95% confidence interval, 0.9 - 4.3) compared to the control arm [91].

Extracorporeal photopheresis (ECP) is increasingly used in chronic GVHD and is also used in some centers as an adjunct therapy for acute GVHD in an effort to minimize corticosteroid exposure. This technique exposes peripheral blood mononuclear cells to photo-activated 8-methoxypsoralen (8-MOP) and ultraviolet A radiation, which covalently binds and cross-links DNA, initiating apoptosis [92,93]. Experimental studies suggest ECP increases number of regulatory T cells in both humans and mice [94\*,95]. The mouse model is of interest as it showed that the transfer of cells treated with ECP reverses established GVHD by increasing donor regulatory T cells and reduces the number of donor effector lymphocytes even though they had never been directly exposed to 8-MOP and ultraviolet A radiation [94\*]. A recent phase II study of 59 patients receiving ECP for treatment of steroid-refractory GVHD demonstrated complete response rates of 60%, with a 59% overall survival at 4 years [96]. Other small studies showed complete response rate of 52% to 83% [97-99]. Response rates may depend on the length of time from diagnosis and the grade of GVHD at the time of treatment [98,99].

The infusions of mesenchymal stem cells (MSC) represent yet another approach for the treatment of acute GVHD. In a phase II study, thirty of 55 patients (55%) with steroid-resistant GVHD showed complete responses to MSC infusions [100\*]. These positive results were not reproduced, however, in a second study where only 2 out of 13 patients (15%) with steroid-refractory GVHD responded [101]. Clinical studies have not shown an increased risk of severe infections, and a randomized phase III trial is currently ongoing. MSC have been almost impossible to detect after infusion when administered in vivo, and thus little is known regarding their migration, their mechanism of action, or their persistence. In vitro, MSC suppress the responses of numbers of cell types including T cells, antigen-presenting cells, natural-killer cells, and B cells [102]. Experimental models have suggested a potential risk of malignant transformation [103,104].

The results of selected recent studies of therapies for GVHD are summarized in Table 1. There are currently no criteria to identify patients who are likely to benefit from these second line agents. The diagnosis of acute GVHD is still based on clinical criteria that may be confirmed by biopsy of one of the three target organs (skin, gastrointestinal tract, or liver). The severity of acute GVHD is graded from I to IV using a standardized system that evaluates the involvement of three principal target organs. Thus, new blood tests with predictive value for long-term survival such as the four biomarker panel [105,106\*] may be useful to further identify high-risk groups and their outcomes (Figure 1). Such blood tests could facilitate the decisions to introduce more intensive therapy at an earlier time for patients presenting with a high-risk biomarker panel. It may also guide the speed of steroid taper in patients with low-risk panels. Such biomarkers may also help identify patients who are not likely to respond to traditional treatment and who are at very high risk for subsequent



morbidity and mortality. If validated, biomarkers could thus result in immunosuppressive treatment plans tailored to patients in several risk strata.

## Future strategies

Novel approaches to prevent or treat GVHD are often based on evidence from experimental models. Recently, several groups have explored the potential of regulatory T cells (Tregs) in such models. In mice, naturally occurring Tregs develop in the thymus and express the Forkhead Box Protein P3 (FOXP3) gene as well as CD4 and CD25 surface markers. These cells have a vital role in preventing autoimmunity and pathology that is inflicted by uncontrolled immune responses [107]. In mouse BMT models, donor derived Tregs can suppress the proliferation of conventional T cells, prevent GVHD and preserve GVL effects [108-113]. Furthermore, viral immunity is preserved in the presence of Tregs after allogeneic HCT [114]. Clinical trials of this promising approach are ongoing, but isolation and expansion of human Tregs is challenging and labor-intensive [115-117]. A recent study demonstrated that combined CD4+ donor lymphocyte infusion and low-dose recombinant IL-2 expands Tregs in vivo following allogeneic HCT [118].

The role of IL-17-producing CD4 T cells (Th17) in GVHD is controversial. Different groups have shown that Th17 cells reduce GVHD [119,120], but their presence does not appear to impact overall survival [120]. In addition, the differentiation of Th17 cells in vitro appears to cause lethal acute GVHD with severe cutaneous and pulmonary damage [121]. These differences may be due to variations among the models, and further analyses are required before Th17 cells are translated from the bench to the bedside.

T cells in murine models can be divided into naïve (CD62L+ CD44-), central memory (CD62L+ CD44+) and terminally differentiated effector/effector memory (CD62L- CD44-). Donor effector memory T cells (TEMs) do not cause GVHD [122-124] but they transfer functional memory [122] and mediate GVL [125] in murine studies. For human T lymphocytes, the markers that best distinguish these subsets are CD45RA and CCR7. In both CD4+ and CD8+ T cell subsets, a progression from naïve (CD45RA+ CCR7+) to central memory (CD45RA-CCR7+) to effector/memory (CD45RA- CCR7-) has been established [126,127]. Although the loss of CD62L expression also can divide memory T cell subsets, the loss of CCR7 appears to be more complete, particularly in the CD4+ subset [126]. Such T cell subpopulations may have relevance to clinical GVHD. High proportions of CD4+ and CCR7+ cells in the donor graft correlate with the incidence and severity of GVHD [128] and reference values for the CD4+ and CD8+ naïve and memory subsets within allografts have been established [129]. If validated, the selection and infusion of human TEMs could become a new approach to modulate GVHD.

Modulation of dendritic cells (DCs) may influence GVHD. A recent murine study demonstrated that exposure to granulocyte colony stimulating factor (G-CSF) shortly after HCT, in addition to a conditioning regimen with total body irradiation (TBI), significantly worsened GVHD. The mechanism underlining this effect is the stimulation of host dendritic cells (DCs) that subsequently activate donor natural killer T cells [130]. These data might explain the increased incidence of GVHD found in recipients receiving prophylactic G-CSF in the European BMT registry [131].

The properties of myeloid DCs can be modulated by several agents in order to render them tolerogenic. Histone deacetylase inhibitors such as suberoylanilide hydroxamic acid (SAHA) have been shown to reduce development of GVHD in murine models by modulating indoleamine 2,3-dioxygenase-dependent DCs functions [132\*-134]. SAHA is currently in clinical trials to prevent GVHD. Another therapeutic approach to modulate DCs could be an antibody to the DC surface maturation antigen CD83; this antibody has been

shown to prevent acute GVHD in a humanized mouse model with severe congenital immunodeficiency [135].

As noted earlier, DCs are important initiators of GVHD. Plasmacytoid dendritic cells (pDCs) have gained recent interest because of their potential tolerogenic effect in an immature state. In one study a subset of host pDCs that express CCR9 receptor has been shown to suppress GVHD [136]. However, in another study, host pDCs have been shown to induce GVHD in manner independent of Toll like receptors [137]. Thus the role of pDCs in acute GVHD is still controversial and confirmatory studies about their functions will be required.

The role of B cells in acute GVHD is currently under investigation, particularly regarding their possible role in attenuating the disease. In a study of 254 HCT recipients from sibling donors, the number of donor B cells in the graft inversely correlated with the cumulative incidence of grade II-IV acute GVHD [138]. A retrospective study of several hundred B-cell lymphoma patients showed that those receiving rituximab, a chimeric monoclonal CD20 antibody, within the 6 months prior to allogeneic HCT had significantly lower TRM (relative risk (RR) = 0.68) and lower incidence of grade II-IV acute GVHD (RR = 0.72) compared to controls [139]. The authors speculated that recipient B cells might function as semi-professional APCs to stimulate donor T cells. Mouse studies show that host B cells produce IL-10 following TBI and attenuate acute GVHD after allogeneic BMT [140].

## Conclusions

The number of patients at high risk for GVHD is increasing, particularly as more allogeneic HCT are performed from unrelated donors and older patients. Our understanding of the complex pathophysiology of this disorder continues to increase, but few of these insights have thus far led to major therapeutic advances. Deleterious side effects of infectious complications and relapse of underlying malignancy remain barriers to successful new approaches. Several lines of investigation with cellular therapeutics and immunomodulating agents are currently in Phase II clinical trials. These promising therapies are most likely to achieve success when they are used early in the disease process, either as primary treatment or as prophylaxis.

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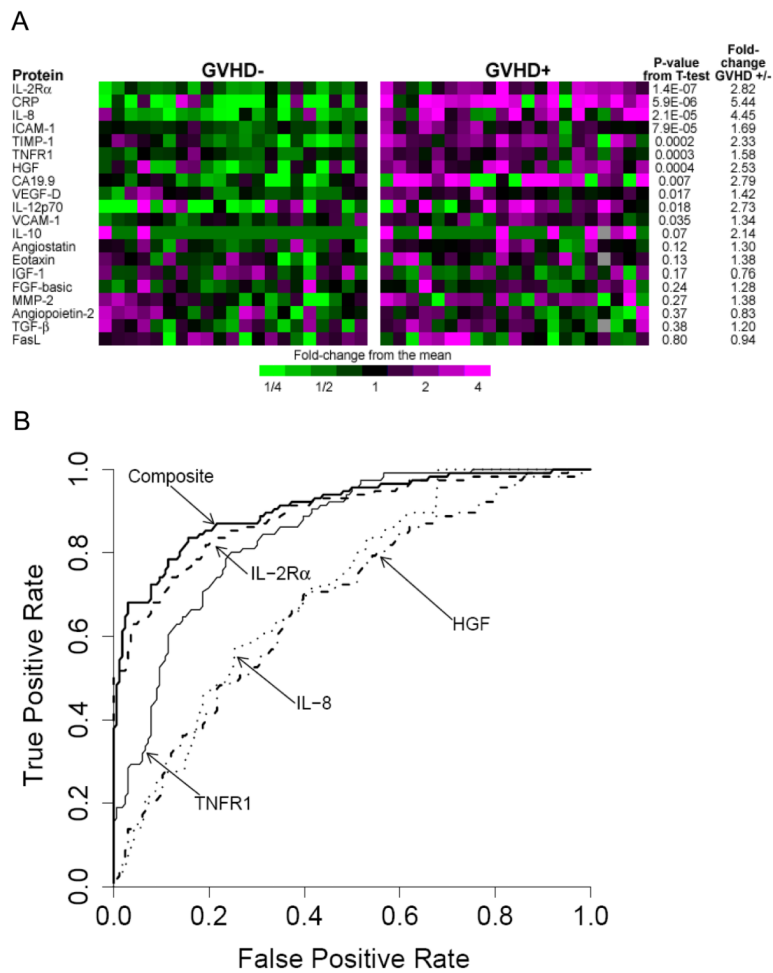


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**Figure 1. From discovery to validation of plasma biomarkers of acute graft versus host disease** Panel A shows the heatmap of proteins levels measured sequential ELISA in the discovery set samples. Samples from 21 GVHD<sup>-</sup> patients (left) and 21 GVHD<sup>+</sup> patients (right) are represented. Eleven proteins gave a P values for differences between GVHD<sup>+</sup> and GVHD<sup>-</sup> patient plasma < 0.05. Panel B shows the receiver operating characteristic (ROC) curves of four individual discriminator proteins and the composite panel in the training set. Individual ROC curves for IL-2R $\alpha$ , TNFR1, HGF, and IL-8 and the composite panel (Reproduced with permission [106].)



**Table 1**  
**Selected studies of novel agents as secondary or first line therapy in acute GVHD**

Novel Therapy	Type of trial	Type of treatment	Sample size	Overall Response at 4 weeks	Survival at 3-6 months	Study	Reference
Denileukin difitox	Phase I	steroid refractory	30	71%	33%	Ho et al.	68
Denileukin difitox	Phase II	steroid refractory	22	41%	ND	Shaughnessy et al.	69
Denileukin difitox	Phase II randomized	Initial therapy	60	60%	49%	Alousi et al.	90
Etanercept	Phase II randomized	Initial therapy	60	48%	56%	Alousi et al.	90
Pentostatin	Phase I	steroid refractory	23	78%	ND	Bolanos-Meade et al.	83
Pentostatin	Phase II randomized	Initial therapy	60	62%	52%	Alousi et al.	90
MMF	Phase I/II	steroid refractory	48	72%	78%	Basara et al.	87
MMF	Phase I/II	steroid refractory	10	60%	70%	Krejci et al.	88
MMF	Phase I/II	steroid refractory	6	67%	64%	Takami et al.	89
MMF	Phase II randomized	Initial therapy	60	78%	64%	Alousi et al.	90
Sirolimus	Phase I/II	steroid refractory	21	57%	28%	Benito et al.	84
BDP	Phase II randomized	Initial therapy	129	71%	87%	Hockenbery et al.	46
ECP	Phase II	steroid refractory	59	68%	ND	Greinix et al.	96
ECP	Phase II	steroid refractory	23	52%	48%	Perfetti et al.	97
ECP	Phase II	steroid refractory	41 (children)	73%	100%	Calore et al.	98
ECP	Phase II	steroid refractory	15 (children)	0% for grade IV GVHD	ND	Berger et al.	99
MSC	Phase II	steroid refractory	55	71%	ND	Le Blanc et al.	100
MSC	Phase II	steroid refractory	13	15%	31%	von Bonin et al.	101
Alefacept	Phase I	steroid refractory	16	75%	ND	Shapira et al.	72

GVHD indicates graft versus host disease, MMF, mycophenolate mofetil, BDP, beclomethasone dipropionate, ECP, extracorporeal photopheresis, MSC, mesenchymal stem cell