

Recent Compounds for Immunosuppression and Experimental Therapies for Acute Graft-Versus-Host Disease

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ABSTRACT: Acute graft-versus-host disease (GVHD) is a major source of morbidity and mortality after allogeneic stem cell transplantation. Therapy of established acute GVHD depends heavily on corticosteroids, which have limited efficacy and are considerably toxic. It is still a matter of debate whether there is an alternative therapy to corticosteroids. Second-line treatment for acute GVHD after failure of steroids is not well substantiated due to the lack of controlled studies. This review examines the current treatment for acute GVHD, as well as novel therapeutics, such as cellular approaches (e.g., adoptive transfer of mesenchymal stem cells) and enhancement of regulatory T cells (e.g., photopheresis). These approaches avoid the toxicity of generalized immunosuppression and are likely to play a prominent future role in acute GVHD therapy.

IMAJ 2013; 15: 44–50

KEY WORDS: allogeneic stem cell transplantation, graft-versus-host disease (GVHD), bone marrow transplantation

Acute graft-versus-host disease remains one of the major barriers to improving outcomes after allogeneic stem cell transplantation. The pathogenesis of acute GVHD involves many consecutive and parallel pathways. A triphasic conceptual model introduced 20 years ago simplifies grasp of this complex network [1]: a) tissue damage from conditioning therapy, underlying diseases and prior infections, b) activation of host antigen-presenting cells and donor T cells resulting in differentiation, migration, and c) an effector phase in which T cells mediate tissue damage by releasing inflammatory cytokines. Once GVHD has occurred, all three phases of the cascade are activated. More recent investigation demonstrated the importance of regulatory mechanisms, including regulatory T cells.

GVHD = graft-versus-host disease

CD4+CD25+ Treg of host and donor origin can regulate alloimmunity after allogeneic bone marrow transplant and exert a beneficial effect in the setting of GVHD.

TREATMENT OF ACUTE GVHD

Glucocorticoids (prednisone or methylprednisolone at ≥ 1 –2 mg/kg for 7–14 days, followed by gradual dose reduction) have been considered the standard initial treatment for acute grade II–IV GVHD. Complete remission occurs in 35–50% of the patients on day 28 of therapy; more severe GVHD is less likely to respond to treatment [1]. Standard of care is not well established for the treatment of steroid-refractory acute GVHD. Divergent approaches in the initial therapy of acute GVHD have explored combination therapy with standard-dose glucocorticoids and additional immunosuppressive agents, as well as, conversely, steroid-sparing approaches, including sirolimus as a steroid-free approach to acute GVHD therapy [Table 1]. These strategies have had only limited success due to increased risk of infection and treatment-related mortality.

ANTIMETABOLITES (MYCOPHENOLATE MOFETIL, PENTOSTATIN)

A promising combination for initial therapy of acute GVHD consists of glucocorticoids and **mycophenolate mofetil**. MMF is an antimetabolite that results in non-competitive, reversible inhibition of inosine monophosphate dehydrogenase, blocking the de novo pathway of purine synthesis in T and B lymphocytes. It has been used in limited trials for the treatment of steroid-refractory acute GVHD. A response rate of 65% was reported with common toxicities of nausea, diarrhea, myelosuppression and opportunistic infections [2].

The Blood and Marrow Transplant Clinical Trials Network

Treg = regulatory T cells

MMF = mycophenolate mofetil

New immunosuppressive agents and/or strategies are required to improve management of acute graft-vs.-host disease and decrease its toxicities

Table 1. Summary of clinical trials for treatment of acute GVHD

Agent	Indication	n	Episodes of aGVHD	Results	Ref
Sirolimus	Steroid-refractory aGVHD	21	Grade III:10, grade IV:11	24% CR, 33% PR; significant toxicities including TMA	[6]
	Steroid-refractory aGVHD	34	Grade I:3, grade II:15, grade III:8, grade IV:8	76% ORR (44% CR, 32% PR); manageable toxicities	[7]
	De novo aGVHD (w/out systemic steroids)	32	Grade I:4, grade II:24, grade III:4	50% sustained CR; favorable toxicity profile	[8]
Pentostatin	Steroid-refractory aGVHD	23	Grade II:7, grade III:11, grade IV:5	63% CR, 13% PR; 26% alive at 1 year	[4]
	De novo aGVHD (plus steroids)	42	Grade I:4, grade II:26, grade III:11, grade IV:1	38% CR at day 28	[3]
Mycophenolate mofetil	De novo aGVHD (plus steroids)	45	Grade I:3, grade II:25, grade III:16, grade IV:1	60% CR at day 28	[3]
Etanercept	De novo aGVHD (plus steroids)	46	Grade I:8, grade II:25, grade III:12	26% CR at day 28	[3]
	De novo aGVHD (plus steroids)	61	Grade II:41, grade III-IV:20	69% CR at week 4	[9]
Infliximab	Steroid-refractory aGVHD	11	Grade II:1, grade III:4, grade IV:6	63% ORR (18% CR at week 8, 45% PR)	[10]
	Steroid-refractory aGVHD	32	Grade II:4, grade III:8, grade IV:20	59% ORR (19% CR, 40% PR); high infection rate	[11]
Daclizumab	Steroid-refractory aGVHD	20	Grade I:1, grade II:1, grade III:12, grade IV:6	29–50% ORR; high infection rate	[12]
	Steroid-refractory aGVHD	43	Grade I:1, grade II:22, grade III:12, grade IV:8		[13]
	Steroid-refractory aGVHD	12	Grade III:1, grade IV:11		[14]
Basiliximab	Steroid-refractory aGVHD	17	Grade II:3, grade III:12, grade IV:2	71–83% ORR; high infection rate, high rates of aGVHD flares	[17]
	Steroid-refractory aGVHD	23	Grade II:11, grade III:12		[18]
	Steroid-refractory aGVHD	34	Grade III:6, grade IV:28		[19]
Alemtuzumab	Steroid-refractory aGVHD	18	Grade III:6, grade IV:12	17/18 responses (gut & liver) at week 4; high infection rate	[22]
Extracorporeal photopheresis	Steroid-refractory aGVHD	59	Grade II:36, grade III:13, grade IV:10	70% CR; 86.55 & 30% CR in grade II, III & IV aGVHD; 4 year survival benefit for CR, 59 vs. 11%	[23]
Mesenchymal stem cells	Steroid-refractory aGVHD	55	Grade II:5, grade III:25, grade IV:25	71% initial response (55% CR, 16% PR); 2 year survival benefit for CR, 53 vs. 16%; no infusional toxicity	[27]

aGVHD = acute graft-versus-host disease, CR = complete response, PR = partial response, ORR = overall response rate, TMA = thrombotic microangiopathy

(BMT CTN) reported the results of a randomized phase II multicenter trial evaluating the efficacy of four agents, including MMF, each in combination with glucocorticoids, as initial therapy for acute GVHD [3]. Patients were randomized to methylprednisolone 2 mg/kg/day and etanercept, MMF, denileukin or pentostatin. At day 28 complete remission rates were 26%, 60%, 53% and 38%, respectively. The corresponding rates of severe infections were 48, 44, 62 and 57%, and the 9 month overall survival rates were 47, 64, 49 and 47%, respectively. Patients who received MMF for GVHD prophylaxis (30–34%) were randomized only to a non-MMF arm, creating an allocation bias. As pretreatment with MMF affects GVHD responsiveness, the allocation bias raised the concern that patients with less responsive acute GVHD were preferentially allocated to non-MMF arms and biased the results in favor of MMF. Despite this caveat, the efficacy and toxicity data of this BMT CNT trial indicated that MMF and glucocorticoids is possibly the most promising of the four regimens and it was therefore selected for comparison against glucocorticoids alone in an ongoing phase III trial (<http://clinicaltrials.gov/ct2/show/NCT01002742>).

Pentostatin is a purine analog that inhibits T cell numbers

and function by inhibiting adenosine deaminase. It was well tolerated in steroid-refractory acute GVHD patients [4] with a suggestion of increased risk for cytomegalovirus and other viral infections. The complete response rate was 63%, but only 26% of patients were alive at the one year follow-up.

mTOR INHIBITORS

Sirolimus (also called rapamycin) is a macrocyclic triene antibiotic with immunosuppressive, antitumor and antifungal properties. It binds uniquely to FKBP12 and forms a complex with the mammalian target of rapamycin (mTOR) that inhibits several biochemical pathways, resulting in T cell immunosuppression. Recent data show that sirolimus also promotes expansion of donor-derived regulatory T cells [5].

Sirolimus has undergone limited evaluation for treatment of steroid-refractory acute GVHD. A pilot phase I trial by Benito et al. [6] evaluated the safety of sirolimus as treatment for steroid-refractory grade III-IV acute GVHD (n=21). Only 11 patients completed the anticipated 14 day course due to lack of improvement and because of toxicity, including hematologic toxicity, renal failure and severe thrombotic microangiopathy. Despite this limitation, 5 of 21 patients (24%) achieved complete remission, suggesting a potential role for sirolimus

BMT CN = Blood and Marrow Transplant Clinical Trials Network

in the salvage of steroid-refractory acute GVHD. In a more recent retrospective study [7], treatment with sirolimus was continued indefinitely at the discretion of the treating physician rather than for a fixed duration. An encouraging complete response rate of 44% was observed. Additionally, in most cases, sirolimus allowed a reduction of glucocorticoid exposure. Other toxicities, such as thrombotic microangiopathy and hyperlipidemia, were manageable and did not cause associated grade IV toxicities.

A glucocorticoid-free first-line treatment of acute GVHD with sirolimus is an attractive approach. Pidala et al. [8], in a retrospective series, showed that initial treatment of acute GVHD with sirolimus results in a response rate comparable to that of high dose steroids. Treatment of grade I-III acute GVHD affecting primarily skin and gut (n=32) resulted in a complete remission rate of 50% with a favorable toxicity profile. With a median follow-up of 16 months (range 6–26 months), one year overall survival was 56%. Despite these encouraging results, because of the small number of patients included in the study, particularly those with above grade II acute GVHD, it is difficult to reach any conclusions regarding a hypothetical superiority of sirolimus over glucocorticoids. Prospective controlled trials are needed to assess the impact of sirolimus on the response rate in acute GVHD and on overall survival.

CYTOKINE BLOCKADE

Inflammatory cytokines (tumor necrosis factor- α and interleukin-2) are important mediators of acute GVHD and may be critical targets for therapy. Biologic therapies targeting TNF α have been evaluated both as primary and as salvage acute GVHD therapies.

Etanercept is a soluble human TNF receptor fusion protein that competes for TNF α binding and renders it inactive. This mechanism of action combined with its relative ease of administration by subcutaneous injection and generally minor side effects make etanercept attractive as primary acute GVHD therapy. Levine and co-researchers [9] tested the combination of methylprednisolone (2 mg/kg/day for 7 days) and etanercept (0.4 mg/kg subcutaneously twice weekly for 8 weeks) as initial therapy for new-onset acute GVHD in a pilot study (n=20) followed by a phase II clinical trial (n=41). Etanercept and glucocorticoids were significantly more likely to achieve complete remission after 4 weeks of treatment as compared to an external control group (n=99) treated with glucocorticoids alone (69 vs. 33%, $P < 0.001$), and the response benefits persisted

at 12 weeks (77 vs. 50, $P < 0.001$). A difference in results was observed regardless of the stem cell donor or the conditioning regimen and/or the organ involved. The incidence of infections, malignancy relapse, and/or flare GVHD did not vary among compared groups. Combination therapy translated into a significantly improved survival at 6 months for unrelated recipients. Etanercept has had limited evaluation in steroid-refractory acute GVHD. It was used to treat 13 patients with steroid-refractory acute GVHD [2] with clinical responses in 6 patients (46%), including 4 complete responses.

Infliximab is a chimeric TNF α antibody that can neutralize circulating TNF α and lyse TNF α -producing cells. Various dose escalation trials indicate that infliximab exerts some activity in steroid-refractory acute GVHD. Small retrospective series suggest a response rate of 59–63%, with responses more likely in the skin and gastrointestinal tract [10,11], although it is hampered by a high mortality due to infectious complications, especially invasive fungal infections.

CD25, the alpha subunit of the human IL-2 receptor, which is expressed on activated lymphocytes, is also an attractive target.

Daclizumab, the humanized anti-IL-2 receptor monoclonal antibody, was evaluated in three small phase II studies [12–14]. Overall response rates in the steroid-refractory setting have ranged from 29% to 50%. Combination therapy with daclizumab plus etanercept for steroid-refractory acute GVHD has also been reported (n=21) [15], with a 67% overall response rate. However, long-term mortality due to infectious complications and chronic GVHD remained high (overall survival 19%). Based on these encouraging results a phase III double-blinded randomized trial [16] was initiated to determine whether the addition of daclizumab to initial corticosteroid therapy for acute GVHD improved outcome. The study was terminated due to significantly worse survival in the daclizumab arm.

Basiliximab is a chimeric human/mouse monoclonal IL-2 receptor antibody, with different pharmacokinetic features from those of daclizumab. Basiliximab has also been evaluated in steroid-refractory acute GVHD. Phase II studies [17–19] utilizing various dosing schedules in mainly matched donors report an overall response rate ranging from 71 to 83% (complete responses of 18–53%) due to different characteristics of the patient cohorts. A recent retrospective study [20] suggests that basiliximab was effective in the treatment of steroid-refractory acute GVHD (n=53) after unmanipulated HLA-mismatched/haploidentical stem cell transplantation (complete remission 69.8%, median time to complete remission 12 days). New infections developed in 39 patients

Mature results from high quality clinical trials are needed to direct best practice in acute GVHD control that optimizes efficacy while sparing toxicity

TNF α = tumor necrosis factor- α

IL = interleukin

(73.6%) within 2 months of the last dose of basiliximab. The probabilities of 3 year overall survival and event-free survival were 51.4% and 47.7%, respectively.

MONOCLONAL ANTIBODIES

Targeting T cells and antigen-presenting cells with **alemtuzumab**, a monoclonal antibody against CD52, has had some success in treating steroid-refractory acute GVHD. Alemtuzumab was evaluated in 20 patients with grade III-IV steroid-refractory intestinal acute GVHD [21]. Complete and overall response rates were 35% and 70%, respectively. Cytomegalovirus reactivation, bacterial infection and invasive aspergillosis were frequent complications. A more recent report [22] showed the efficacy of alemtuzumab for both intestinal and liver grade III-IV steroid-refractory acute GVHD in 18 patients and supported the idea that smaller doses were preferable due to less toxicity. Impressive responses were seen (17 of 18 patients responded to alemtuzumab), but all patients suffered from at least one infectious episode and only 6 patients were alive at a median follow-up of 108 weeks. The spectrum of opportunistic infections was remarkable and emphasizes the depth of immunosuppression induced by alemtuzumab.

Identification of biomarkers for acute GVHD with diagnostic and, possibly, prognostic significance may eventually make the treatment of acute GVHD preemptive rather than prophylactic

PHOTOTHERAPY

Phototherapy, primarily **extracorporeal photopheresis**, involving ex vivo therapy of T cells plus a psoralen sensitizer, exposed to ultraviolet light and subsequently reinfused, has been utilized to control refractory acute GVHD. The mode of action of ECP in GVHD remains poorly understood and may be of multifactorial nature. Some hypotheses propose alteration of host antigen presentation, effector lymphocyte apoptosis and enhancement of regulatory T cells [2]. In a phase I-II study of 59 patients [23] with steroid-refractory acute grade II-IV GVHD, ECP therapy resulted in complete remission rates in 70% of patients overall, with 86, 55 and 30% complete remission in grade II, III and IV acute GVHD, respectively. Complete resolution of GVHD was documented for 82% of the patients with cutaneous involvement, 61% for those with liver involvement and 61% for gut involvement. Complete responders had an overall survival benefit of 59% at 4 years compared with 11% for those who did not obtain a complete response. A recent phase II prospective multicenter study [24] assessed whether the addition of pre-transplant ECP (on cyclosporine and methotrexate) could decrease the expected incidence of

acute GVHD in patients undergoing standard myeloablative conditioning and related or unrelated donor allogeneic stem cell transplant. Patients (n=62) received ECP on 2 consecutive days within 4 days prior to the preparative regimen. The 100 day cumulative incidence of grade II-IV acute GVHD was 35%. Multivariate analysis revealed a significantly lower rate of grade II-IV acute GVHD developing in the ECP arm compared to historical groups. The adjusted probabilities of disease-free survival (74 vs. 63%) and overall survival (83 vs. 67%) at one year were significantly higher in the ECP arm than in the historical groups. In conclusion, the use of ECP before a standard myeloablative regimen is safe and may improve overall and disease-free survival, though longer follow-up, larger sample sizes and randomized comparisons to standard approaches are necessary.

A very recent prospective controlled study [25] examined whether ultraviolet B irradiation can be tolerated during an allogeneic SCT and whether depletion of epidermal dendritic

cells (Langerhans cells) by broadband UVB could improve acute (cutaneous) GVHD outcomes. Seventeen patients received six whole-body irradiations, every second day starting from day 1 after allogeneic

SCT with a reduced-intensity conditioning regimen. Only 2 of the 17 patients (12%) developed acute cutaneous GVHD grade II-IV, in contrast to 45% in historical controls with the identical reduced-intensity conditioning regimen. The irradiation protocol was well tolerated in all patients. UVB treatment decreased the number of Langerhans cells in the skin and also affected dermal dendritic cells. Strikingly, all nine patients with complete Langerhans cell depletion developed only grade I GVHD or no GVHD up to day 100. UVB-treated patients also had significantly higher 25-hydroxyvitamin D3 serum levels and higher numbers of circulating regulatory T cells. These results suggest that prophylactic UVB irradiation post-transplant is safe and should be further explored as a clinical strategy to prevent acute skin GVHD.

CELLULAR THERAPIES

- **Mesenchymal stem cells:** MSC are a population of phenotypically heterogeneous cells that can be isolated from many readily accessible tissues, including bone marrow, umbilical cord, placenta and adipose tissue, where they form part of the supporting stromal micro-environment. Extensive ex vivo and preclinical data suggest that subpopulations within MSC contribute to immunomodulation of the host without

ECP = extracorporeal photopheresis

SCT = stem cell transplant

UVB = ultraviolet B

MSC = mesenchymal stem cells

provoking immunologic responses from alloreactive T cells or other effector cells, as well as contributing to tissue repair. Recent data demonstrated that MSC also induce regulatory T cells [5]. These unique properties make MSC an ideal investigational agent for treating GVHD. Therapeutic trials with varied MSC dosing schedules and clinical end-points have showed mixed results. Kebriaei and Robinson [26], in a recent review, summarized the results of clinical trials using MSC for the treatment of GVHD. MSC have been studied most extensively in steroid-refractory acute GVHD. In the largest phase II non-randomized multicenter trial [27], 39 of 55 patients with steroid-refractory grade II-IV acute GVHD responded (30 complete responses) after one to five infusions of HLA-identical, haploidentical or third-party MSC. Complete responders had a 53% overall survival benefit at 2 years compared with 16% for those who did not achieve a complete response. Preliminary results from two multicenter controlled randomized phase III clinical trials for de novo acute GVHD (<http://www.osiris.com/clinical.php>) and steroid-refractory acute GVHD [26] have been reported. In both studies weekly or bi-weekly MSC (Prochymal[®], Osiris Therapeutics, Inc., Columbia, MD, USA) was administered for 4 weeks. Neither the steroid-refractory nor the newly diagnosed GVHD trials reached the primary end-point of durable complete response of at least 28 days. However, selected patients with steroid-refractory liver or gut acute GVHD were reported to have significantly improved response rates (81 vs. 68%, $P = 0.035$). In conclusion, clinical experience with MSC for the treatment of GVHD is encouraging but incomplete. More multicenter trials with well-defined end-points are necessary to better understand the therapeutic potential of MSC for GVHD. Finally, experience with MSC for the prevention of GVHD is also limited. In a small pilot open-label randomized controlled trial [28] of HLA-matched sibling allotransplantation with (n=10) and without (n=15) MSC for prophylaxis of GVHD, promising reductions in grade II-IV acute GVHD were noted in the MSC arm (11 vs. 53%), but a higher than expected relapse rate in the MSC arm raised concerns (60 vs. 20%). Additional clinical trials evaluating the safety and efficacy of MSC for acute GVHD prophylaxis are needed.

- **Donor regulatory T cell infusion:** Naturally occurring CD4+CD25+FOXP3+T regulatory cells tip the balance between auto- and tumor immunity. T regulatory cells also regulate allo-reactivity in vitro and in vivo. In murine models, adoptively transferred Treg prevent the development of acute GVHD without affecting the graft-versus-tumor response. Treg content in the graft or the donor's blood correlates with acute GVHD incidence after HLA-identical sibling stem cell transplant. Relapse-related mortality or infections did not increase in patients receiving grafts with

high Treg numbers [5]. Recently, the first clinical trial of adoptive Treg transfer for prevention of acute GVHD was reported [29]. In this trial 28 high risk heavily pretreated patients undergoing haplo-identical SCT received freshly isolated donor Treg on day -4 followed by transfer of highly purified CD34+ stem cells together with conventional T cells (Tcons). No post-transplantation GVHD prophylaxis was given. Rapid and stable engraftment was seen and only two patients developed grade II-IV acute GVHD. At a median follow-up of 11.2 months, no patient had developed chronic GVHD. Fourteen patients died during the study and only one patient died from relapse. When compared to a dataset of 152 patients receiving haplo-identical SCT without Treg transfer, this approach promoted lymphoid reconstitution and improved immunity to opportunistic pathogens. The study is small, the median follow-up is short and thus the data too premature to draw definite conclusions. However, donor Treg infusion (DTI) appears to be feasible and safe in terms of GVHD prevention. Randomized studies are now needed to test the preventive as well as the therapeutic potential of DTI and to evaluate optimized application schedules.

In addition to adoptive Treg therapies, the Treg compartment can also be modified by pharmacological intervention. Data from murine in vivo stem cell transplantation models support the observation of Treg-supportive effects of rapamycin (sirolimus), including increased generation of thymic Treg and infiltration into GVHD target organs. Treg expansion by rapamycin preserves the graft-versus-leukemia effect [5]. Rapamycin together with IL-2 [5] enables expansion of donor-derived natural Treg (nTreg) and conversion of CD25- T cells to induced Treg (iTreg), which potently inhibits development of GVHD in vivo. Thus, the combination of adoptive Treg transfers with in vivo application of rapamycin in combination with IL-2 to get higher yields of nTreg and iTreg represents a promising strategy for pharmacological modification of Treg for treatment or prevention of GVHD. It has also been shown that rabbit-derived anti-T lymphocyte immunoglobulin is a potent inducer of iTreg. Lopez and collaborators [30] and Feng et al. [31] showed that thymocyte-induced ATG (rabbit ATG, Thymoglobulin, Genzyme, Cambridge, MA, USA) converts human CD4+CD25-FoxP3- effector T cells into CD4+CD25+FoxP3+ iTreg that subsequently suppress the proliferation of autologous responder cells to external stimuli. Ruzek et al. [32] further demonstrated that anti-mouse thymocyte globulin induces Treg in mice that express several Treg markers (but not FoxP3) and inhibit GVHD. Similarly, another study has recently shown that Fresenius anti-T lymphocyte globulin (ATG-S, Fresenius,

DTI = donor regulatory T cell infusion

ATG = anti-T lymphocyte immunoglobulin

Biotech GmbH, Graefelfing, Germany) can generate CD4+CD25+FoxP3+ Treg cells in vivo that suppress mixed lymphocyte culture [33]. These results may pave the way for novel potential therapies; in addition, ATG may be synergized with another Treg inducer to obtain long-term tolerance against the activity of T cells in allotransplantation and GVHD.

- **Natural killer T cells:** NKT cells, of either donor or host origin, have the unique capacity to prevent acute GVHD by secreting large amounts of IL-4 [34], after activation via the T cell receptor, which recognizes glucolipids presented by the non-polymorphic antigen-presenting molecule, CD1d, which is present on both the donor and host antigen-presenting cells. NKT cell-secreted IL-4 is known to drive type 2 helper (Th2) polarization of conventional donor-derived T cells, attenuating their capacity to mediate GVHD [5]. In preclinical studies, the protection afforded against GVHD by the conditioning regimen of total lymphoid irradiation plus anti-thymocyte globulin in wild-type host mice was dependent on host NKT cells, since protection was lost in mice in which the CD1d gene was inactivated, causing developmental failure of NKT cells in the thymus and other lymphoid tissue [35]. After the wild-type mice were treated with total lymphoid irradiation, the NKT cells were the main source of host IL-4, which subsequently increased the production of IL-4 by donor T cells [34,35].

In a more recent murine SCT model, using total lymphoid irradiation and ATG as conditioning regimen, host NKT cells were shown to cooperate with the donor Treg to induce IL-4-dependent in vivo Treg expansion and prevent lethal GVHD [36]. Moreover, in a murine model of semi-allogeneic bone marrow transplant, transplantation of DX5+ cells, some 40% of which are NKT cells, led to amelioration of GVHD, improving survival and alleviating GVHD-related skin, bowel and liver injury. This effect was associated with modulation of effector cell subsets. In addition, the transplantation of peripheral NKT cells induced a Th2 shift in the cytokine paradigm [37]. In another study in mice, highly purified NKT cell infusion protected against the development of GVHD by limiting T cell-mediated secretion of pro-inflammatory cytokines such as interferon-gamma and TNF α , while the graft versus leukemia effect was preserved [38]. These observations underscore the therapeutic potential of donor NKT cells for immune modulation within the recipient, possibly in combination with DTI, supporting the ongoing efforts to use adoptive infusion of allogeneic NKT cells in clinical allogeneic BMT. However, donor NKT cells might under certain conditions also contribute to the pathogenesis of GVHD [39]. Thus,

NKT = natural killer T cells
BMT = bone marrow transplantation

further research is needed to determine how NKT cells can be optimally modulated to skew the T cell response towards Th2 cells, as they might also boost GVHD.

CONCLUSIONS AND PERSPECTIVES

Steroid-resistant acute GVHD is still a major challenge for transplanters. Substantial progress has been made recently in the identification of biomarkers (IL-2 receptor- α , TNF receptor-1, IL-8, hepatocyte growth factor, elafine, REG3 and T regulatory cells) for the diagnosis of acute GVHD that also predict response to first-line treatment and survival [40]. These findings may lead to the development of innovative strategies such as the preemptive treatment of acute GVHD. A risk stratification of GVHD that combines both biomarkers and clinical grade could ultimately guide the intensity and duration of GVHD treatment to minimize the toxicities related to glucocorticoids. Furthermore, the innovative combination of pharmacologic or biologic agents targeting specific immune processes, as well as cellular and phototherapy-based approaches will be critical to get beyond the limitations of current therapy.

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References

1. Perez L, Anasetti C, Pidala J. Have we improved in preventing and treating acute graft-versus-host disease? *Curr Opin Hematol* 2011; 18 (6): 408-13.
2. Koreth J, Antin JH. Current and future approaches for control of graft-versus-host disease. *Expert Rev Hematol* 2008; 1 (1): 111.
3. Alousi AM, Weisdorf DJ, Logan BR, et al. Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network. *Blood* 2009; 114 (3): 511-17.
4. Bolanos-Meade J, Jacobsohn DA, Margolis J, et al. Pentostatin in steroid-refractory acute graft-versus-host disease. *J Clin Oncol* 2005; 23 (12): 2661-8.
5. Wolf D, Lilienfeld-Toal M, Wolf AM, et al. Novel treatment concepts for graft-versus-host disease. *Blood* 2012; 119 (1): 16-25.
6. Benito AI, Furlong T, Martin PJ, et al. Sirolimus (rapamycin) for the treatment of steroid-refractory acute graft-versus-host disease. *Transplantation* 2001; 72 (12): 1924-9.
7. Hoda D, Pidala J, Salgado-Vila N, et al. Sirolimus for treatment of steroid-refractory acute graft-versus-host disease. *Bone Marrow Transplant* 2010; 45 (8): 1347-51.
8. Pidala J, Tomblyn M, Nishihori T, et al. Sirolimus demonstrates activity in the primary therapy of acute graft-versus-host disease without systemic glucocorticoids. *Haematologica* 2011; 96 (9): 1351-6.
9. Levine JE, Paczesny S, Mineishi S, et al. Etanercept plus methylprednisolone as initial therapy for acute graft-versus-host disease. *Blood* 2008; 111 (4): 2470-5.
10. Jacobsohn DA, Hallick J, Anders V, McMillan S, Morris L, Vogelsang GB. Infliximab for steroid-refractory acute GVHD: a case series. *Am J Hematol* 2003; 74 (2): 119-24.
11. Patriarca F, Sperotto A, Damiani D, et al. Infliximab treatment for steroid-

- refractory acute graft-versus-host disease. *Haematologica* 2004; 89 (11): 1352-9.
12. Anasetti C, Hansen JA, Waldmann TA, et al. Treatment of acute graft-versus-host disease with humanized anti-Tac: an antibody that binds to the interleukin-2 receptor. *Blood* 1994; 84 (4): 1320-7.
 13. Przepiorka D, Kernan NA, Ippoliti C, et al. Daclizumab, a humanized anti-interleukin-2 receptor α chain antibody, for treatment of acute graft-versus-host disease. *Blood* 2000; 95 (1): 83-9.
 14. Willenbacher W, Basara N, Blau IW, Fauser AA, Kiehl MG. Treatment of steroid refractory acute and chronic graft-versus-host disease with daclizumab. *Br J Haematol* 2001; 112 (3): 820-3.
 15. Wolff D, Roessler V, Steiner B, et al. Treatment of steroid-resistant acute graft-versus-host disease with daclizumab and etanercept. *Bone Marrow Transplant* 2005; 35 (10): 1003-10.
 16. Lee SJ, Zahrieh D, Agura E, et al. Effect of up-front daclizumab when combined with steroids for the treatment of acute graft-versus-host disease: results of a randomized trial. *Blood* 2004; 104 (5): 1559-64.
 17. Massenkeil G, Rackwitz S, Genvresse I, Rosen O, Dorken B, Arnold R. Basiliximab is well tolerated and effective in the treatment of steroid-refractory acute graft-versus-host disease after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2002; 30 (12): 899-903.
 18. Schmidt-Hieber M, Fietz T, Knauf W, et al. Efficacy of the interleukin-2 receptor antagonist basiliximab in steroid-refractory acute graft-versus-host disease. *Br J Haematol* 2005; 130 (4): 568-74.
 19. Funke VA, de Medeiros CR, Setubal DC, et al. Therapy for severe refractory acute graft-versus-host disease with basiliximab, a selective interleukin-2 receptor antagonist. *Bone Marrow Transplant* 2006; 37 (10): 961-5.
 20. Wang JZ, Liu KY, Xu LP, et al. Basiliximab for the treatment of steroid-refractory acute graft-versus-host disease after unmanipulated HLA-mismatched/haploidentical hematopoietic stem cell transplantation. *Transplant Proc* 2011; 43 (5): 1928-33.
 21. Schnitzler M, Hasskarl J, Egger M, Bertz H, Finke J. Successful treatment of severe acute intestinal graft-versus-host resistant to systemic and topical steroids with alemtuzumab. *Biol Blood Marrow Transplant* 2009; 15 (8): 910-18.
 22. Schub N, Gunther A, Schrauder A, et al. Therapy of steroid-refractory acute GVHD with CD52 antibody alemtuzumab is effective. *Bone Marrow Transplant* 2011; 46 (1): 143-7.
 23. Greinix HT, Knobler RM, Worel N, et al. The effect of intensified extracorporeal photochemotherapy on long-term survival in patients with severe acute graft-versus-host disease. *Haematologica* 2006; 91 (3): 405-8.
 24. Shaughnessy PJ, Bolwell BJ, van Besien K, et al. Extracorporeal photopheresis for the prevention of acute GVHD in patients undergoing standard myeloablative conditioning and allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2010; 45 (6): 1068-76.
 25. Kutz M, Karrer S, Hoffmann P, et al. Whole-body UVB irradiation during allogeneic hematopoietic cell transplantation is safe and decreases acute graft-versus-host disease. *J Invest Dermatol* 2012; 132 (1): 179-87.
 26. Kebriaei P, Robinson S. Treatment of graft-versus-host disease with mesenchymal stromal cells. *Cytotherapy* 2011; 13 (3): 262-8.
 27. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 2008; 371 (9624): 1579-86.
 28. Ning H, Yang F, Jiang M, et al. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. *Leukemia* 2008; 22 (3): 593-9.
 29. Di Ianni M, Falzetti F, Carotti A, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* 2011; 117 (14): 3921-8.
 30. Lopez M, Clarkson MR, Albin M, Sayegh MH, Najafian N. A novel mechanism of action for anti-thymocyte globulin: induction of CD4+CD25+FoxP3+ regulatory T cells. *J Am Soc Nephrol* 2006; 17 (10): 2844-53.
 31. Feng X, Kajigaya S, Solomou EE, et al. Rabbit ATG but not horse ATG promotes expansion of functional CD4+CD25highFoxP3+ regulatory T cells in vitro. *Blood* 2008; 111 (7): 3675-83.
 32. Ruzek MC, Waire JS, Hopkins D, et al. Characterization of in vitro antimurine thymocyte globulin-induced regulatory T-cells that inhibit graft-versus-host disease in vivo. *Blood* 2008; 111 (3): 1726-34.
 33. Shimony O, Nagler A, Gellman YN, et al. Anti-T lymphocyte globulin (ATG) induces generation of regulatory T cells, at least part of them express activated CD44. *J Clin Immunol* 2012; 32 (1): 173-88.
 34. Lan F, Zeng D, Higuchi M, Huie P, Higgins JP, Strober S. Predominance of NK1.1+TCR alpha beta+ or DX5+TCR alpha beta+ T cells in mice conditioned with fractionated lymphoid irradiation protects against graft-versus-host disease: "natural suppressor" cells. *J Immunol* 2001; 167 (4): 2087-96.
 35. Lan F, Zeng D, Higuchi M, Higgins JP, Strober S. Host conditioning with total lymphoid irradiation and antithymocyte globulin prevents graft-versus-host disease: the role of CD1-reactive natural killer T cells. *Biol Blood Marrow Transplant* 2003; 9 (6): 355-63.
 36. Pillai AB, George TI, Dutt S, Strober S. Host natural killer T cells induce an interleukin-4-dependent expansion of donor CD4+CD25+FoxP3 T regulatory cells that protect against graft-versus-host disease. *Blood* 2009; 113 (18): 4458-67.
 37. Margalit M, Ilan Y, Ohana M, et al. Adoptive transfer of small numbers of DX5+ cells alleviates graft-versus-host disease in a murine model of semiallogeneic bone marrow transplantation: a potential role for NKT lymphocytes. *Bone Marrow Transplant* 2005; 35 (2): 191-7.
 38. Leveson-Gower DB, Olson JA, Segal EL, et al. Low doses of natural killer T cells provide protection from acute graft-versus-host disease via an IL-4 dependent mechanism. *Blood* 2011; 117 (11): 3220-9.
 39. Haragushi K, Takahashi T, Matsumoto A, et al. Host-residual invariant NK T cells attenuate graft-versus-host immunity. *J Immunol* 2005; 175 (2): 1320-8.
 40. Paczesny S, Krijanovski O, Braun TM, et al. A biomarker panel for acute graft-versus-host disease. *Blood* 2009; 113 (2): 273-8.

Capsule

Structure of the agonist-bound neurotensin receptor

Neurotensin (NTS) is a 13-amino-acid peptide that functions as both a neurotransmitter and a hormone through the activation of the neurotensin receptor NTSR1, a G-protein-coupled receptor (GPCR). In the brain, NTS modulates the activity of dopaminergic systems, opioid-independent analgesia, and the inhibition of food intake; in the gut, NTS regulates a range of digestive processes. White and collaborators present the structure at 2.8 Å resolution of *Rattus norvegicus* NTSR1 in an active-like state, bound to NTS8-13, the carboxy-terminal portion of NTS responsible

for agonist-induced activation of the receptor. The peptide agonist binds to NTSR1 in an extended conformation nearly perpendicular to the membrane plane, with the C terminus oriented towards the receptor core. These findings provide, to our knowledge, the first insight into the binding mode of a peptide agonist to a GPCR and may support the development of non-peptide ligands that could be useful in the treatment of neurological disorders, cancer and obesity.

Nature 2012; doi:10.1038/nature11558

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