We hypothesize that *in vitro* cultured CTL without RA lack the ability to cause GVHD in part due to deficient LPAM expression. We used an established murine GVHD model in which B6SJL Sp/LN cells were stimulated against DBA splenocytes with IL-2 and IL-7 with and without addition of RA (100 nm). Day 14 comparison of CTL and CTLRA revealed comparable CD4 and CD8 populations. CTL with and without RA showed CD8 LPAM expression of 58% and 0.8% and CD8 CCR9 53% and 10% respectively. *In vitro* cytotoxicity was comparable between CTL and CTLRA: 41% vs 51% (n = 3, P = .30). Both CTL groups had comparable *in vitro* migration towards SDF (P = ns) but CTLRA had increased migration towards TECK; 17.3% vs 4.6% (n = 4, P = .01). For *in vivo* homing, 10^7 labeled cells from each CTL with (CFSE) and without RA (TRITC) were coinjected intravenously and analysed after 16 hours. CTLRA had increased homing to Peyers patch and MLN compared to CTL without RA [homing index (CTLRA/CTL) 2.3 and 2.5 respectively]. This finding is exaggerated in the radiated host [homing index (CTLRA/CTL) 15 for PP and 11 for MLN]. CTL and CTLRA (5×10^6 cells each) were injected intravenously with or without C57BL/6J BM into irradiated (600 rads) B6D2F1 recipients (6 groups; radiation control, CTL, CTLRA, BM control, CTL + BM, CTLRA + BM). Mice were followed for clinical GVHD scores and CBC and histopathologic GVHD scores (liver, skin, lung, small and large intestines) were obtained. Both CTL groups without BM rescue developed lethal BM aplasia around day 24; however, histopathologic GVHD scores were similar (Table 1). CTL and CTLRA groups with BM rescue had full hematopoietic recovery, yet had similar histopathologic GVHD scores (BM control; 3.9, CTL + BM; 5, CTLRA + BM: 3.9, P = ns). Our data demonstrate that both CTL and CTLRA cause a lethal hematopoietic GVH reaction which could be abrogated by parent BM. Despite high LPAM and CCR9 expression, significant *in vitro* migration to TECK and *in vivo* homing to gut associated lymphoid tissues, RA treated CTL did not cause significant GVHD in gut, liver or skin. This suggests that defective gut homing alone may not be sufficient to explain the attenuated GVHD from cultured CTL (Table 1).

Table 1. *Data from Day 24 Sacrifice*

Parameter (Mean)	Radiation Control	CTL	CTLRA	*P* Value*
Hb (g/dL)	12.2	3.8	3.1	.0001
WBC × 10^6/L	1000	300	300	.07
Platelets × 10^3/L	667 200	50 200	29 500	.003
Combined GVHD histology score	4.9	4.4	4.2	.30

*CTL or CTLRA vs radiation control group.

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EARLY TREATMENT WITH CD4+CD25+ REGULATORY T CELLS PROVIDES PROLONGED SUPPRESSIVE EFFECTS WHICH CONTROL EVOLVING BUT NOT ESTABLISHED GRAFT-VERSUS-HOST-DISEASE

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In our previous study with Treg trafficking, bioluminescence imaging (BLI) indicated a persistence of signal consistent with a prolonged survival of Treg in vivo following allogeneic bone marrow transplantation. In the current study, we evaluated the duration of Treg suppression and the impact of Treg on evolving and established GvHD. Lethally irradiated Balb/c (H2d) hosts received 5×10^6 T-cell depleted bone marrow cells from wild-type FVB mice (H2q) on day 0. On day 2, 3×10^6 splenocytes, or 1×10^6 purified CD4+/CD8+ T cells (Tcon) from luciferase+ transgenic mice (FVB) were infused to induce GvHD. Treg, purified from wt-FVB mice, in a 1:1 dose ratio with Tcon, were infused either on day 0, 2, 9, or 23 post-transplantation. BLI was used to localize and quantify the proliferation of Tcon in the absence or presence of Treg. Signal intensity, measured by photons/second/mouse, was significantly decreased in animals which received Treg at day 0, 2, or 9 ($P < .05$). Importantly, the greatest reduction in signal intensity occurred when Treg were given prior to the induction of GvHD by Tcon. This reduction was associated with a significantly lower clinical score for GvHD. Studies in which Treg are given up to 10 days prior to the addition of Tcon show similar findings. At day 23, when clinical GvHD was fully established in mice which received Tcon, the addition of Treg did not alter the increasing BLI signal level or the clinical course such that all animals died of GvHD (P = .38). Lymphoid reconstitution was not affected by the addition of Treg prior to the induction of GvHD. In dose titration studies whereby Treg are given two days prior to the induction of GvHD, a 10-fold dose reduction in Treg was sufficient to significantly reduce Tcon proliferation and suppress clinical GvHD. We next assessed the duration of Treg suppressive effect by inducing GvHD on day 7, 14, 19 with luc+ Tcon following the infusion of Treg on day 0 of allogeneic BMT. Treg provided protection from the Tcon challenge at all 3 time points, leading to improved survival ($P < .05$). We conclude that Treg provide prolonged protection due to their ability to proliferate in vivo in an allogeneic setting, permitting a significant reduction in the number of Treg needed for adoptive transfer to induce a clinical response. In addition, we conclude that Treg suppress the early proliferation of Tcon, allowing them to prevent and control evolving but not established GvHD.

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INTERLEUKIN-2 (IL-2) AND GRANULOCYTE-MACROPHAGE STIMULATING FACTOR (GM-CSF) FOR TREATMENT OF RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANT (ASCT)

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Donor lymphocyte infusion (DLI) is commonly used for relapse after allogeneic stem cell transplant (ASCT). Immune activation with cytokines is an alternative to DLI. We report a retrospective analysis and first 6 patients (pts) of a prospective study evaluating the safety and efficacy of granulocyte-macrophage stimulating factor (GM-CSF) and interleukin-2 (IL-2) administered at the time of relapse after ASCT in pts with hematologic malignancies. Pts received subcutaneous GM-CSF at 250 μg/day on days 1-14 and IL-2 at 1×10^6 units/m^2/day on days 8-14. Pts were off of immunosuppressive therapy and had no prior history of graft versus host disease (GVHD) at the start of treatment. A total of 10 pts have received cytokine therapy with IL-2/GM-CSF for treatment of relapsed AML (6), ALL (2), CML (1), MDS (1). Median age was 45 (range 8-61). Stem cell source included: peripheral blood = 7, bone marrow = 2, umbilical cord blood (UCB) = 1. Donor sources were matched-related sibling = 3, matched-unrelated donor = 7 (UCB = 1). Seven pts had resistant relapse or primary resistant disease at time of ASCT. Median time from transplant to relapse was 4 months (range = 1-14 months). Two pts had failed DLI and 5 pts had received reinduction chemotherapy prior to IL-2/GM-CSF. Seven pts responded to IL-2/GM-CSF (CR = 6, PR = 1). Two pts remain disease free at 18 and 26 months post IL-2/GM-CSF. Six pts developed GVHD (4/6 responders). Two pts had GM-CSF discontinued due to increase in peripheral blood blasts. No other toxicities were related to IL-2/GM-CSF except for flu-like symptoms and bone pains. In conclusion, cytokine therapy with IL-2/GM-CSF is feasible and we continue accrual to determine if this cytokine regimen is an alternative to DLI for relapse after ASCT.

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ELEVATED B CELL ACTIVATING FACTOR (BAFF) IN PATIENT PLASMA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IS A POTENTIAL BIOMARKER FOR CHRONIC GRAFT VERSUS HOST DISEASE

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Previous studies have identified a highly significant correlation between specific antibody responses directed against recipient

H-Y minor histocompatibility antigens and the development of chronic graft versus host disease (cGVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). These findings suggest that donor B cells play a role in the development of cGVHD and clinical trials utilizing anti-CD20 monoclonal antibody, rituximab, have demonstrated clinical improvement in some patients with active resistant cGVHD. B cell activating factor, BAFF, is a member of the TNF cytokine family that binds to one of three cell surface receptors on B cells. Activation of these B cell receptors by antigen and BAFF results in rapid induction of proliferation and differentiation of germinal center B cells into either Ig-secreting cells or memory B cells. The finding that elevated BAFF levels in patients with autoimmune diseases are associated with disease severity and autoantibody production has led to clinical trials using anti-BAFF monoclonal antibody in these diseases. To test whether BAFF contributes to the activation of B cells in patients with cGVHD, we employed a sandwich ELISA to measure soluble BAFF in plasma from 27 patients after allogeneic HSCT. Mean BAFF levels for extensive (n = 8), limited (n = 8), resolved (n = 6) or no (n = 5) cGVHD groups were: 7.3 ng/ml, 5.37 ng/ml, 3.92 ng/ml and 2.02 ng/ml, respectively. The differences between the extensive or limited groups versus the no cGVHD group were statistically significant (each $P = .03$) suggesting that BAFF levels corresponded with cGVHD activity. B cell subsets in peripheral blood were also examined by flow cytometry to identify circulating memory B cells. Of four cGVHD patients analyzed thus far, individuals with active disease and high BAFF levels also had high numbers of CD27+ memory B cells in peripheral blood compared to patients with resolved cGVHD and low BAFF levels. Ongoing prospective, serial assessment of BAFF levels and B cell phenotype in larger numbers of patients will extend these results. We propose that BAFF may serve as a new biomarker for cGVHD, specifically identifying patients with active disease. If further studies identify a pathologic role for increased BAFF in cGVHD, anti-BAFF monoclonal antibodies may provide a new therapeutic approach for this serious complication of allogeneic HSCT.

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TREATMENT OF MURINE ACUTE GVHD WITH THE NOVEL PRO-APOPTOTIC BENZODIAZEPINE BZ-423

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Bz-423 is a novel 1,4-benzodiazepine with both cytotoxic and cytostatic effects on activated lymphocytes. Bz-423 has been shown to suppress lupus in murine models by eliminating pathogenic lymphocytes without altering normal immune function or overt toxicities. We hypothesized that Bz-423 might enhance the apoptotic deletion of alloreactive donor T cells and therefore reduce graft-versus-host disease (GVHD). We first investigated the effect of Bz-423 on activated T cells in vitro. A 40 μM concentration of Bz-423 induced apoptotic death in >90% of both $CD4^+$ and $CD8^+$ T lymphocytes stimulated with allogeneic dendritic cells (DC) or ConA in the presence of syngeneic DC. Purified resting T cells were significantly less susceptible to Bz-423-induced apoptosis (data not shown). We next examined the effect of Bz-423 in vivo utilizing a well-characterized (Balb/c→B6) murine GVHD model. B6 recipient mice were conditioned with 11 Gy total body irradiation and injected with 5.0×10^6 bone marrow and $4\text{-}5.0 \times 10^6$ purified T cells from either syngeneic (B6) or allogeneic (Balb/c) donors. In order to model the administration of Bz-423 as a treatment strategy rather than a prevention strategy, animals were not treated for the first seven days after BMT. Beginning 7 days following BMT and continuing for the remainder of the experiment, Bz-423 was administered i.p. at a dose of 60 mg/kg three times weekly. As shown in the table below, treatment with Bz-423 resulted in significantly better day +60 survival ($P = .001$) and reduced clinical GVHD scores ($P < .02$) among allogeneic BMT recipients. Bz-423 also reduced GVH-associated immunodeficiency ($P = .02$) and decreased liver and gut histopathology of GVHD ($P = .01$). Delay of the onset of treatment to day 14 post-BMT also resulted in a significant survival benefit ($P = .03$). These data demonstrate that Bz-423 can reverse acute GVHD and induce selective apoptosis of alloreactive donor T cells in this model. Pro-apoptotic benzodiazepines may provide a strategy for the treatment of acute GVHD (Table 1).

Table 1.

	Syn + Bz	Allo + vehicle	Allo + Bz	*P* Value[1]
Treatment onset: day +7				
Survival (day 60)	100% (n = 9)	8% (n = 13)	59% (n = 19)	.001
GVHD clinical score (day 60)[2]	1.5 ± 0.2	6.5 ± 0.0	3.1 ± 0.4	<.02
Pathology (day 74)[3]				
Liver	1.3 ± 0.6	13.7 ± 1.5	8.3 ± 2.5	.01
Intestine	3.0 ± 2.0	21.3 ± 6.0	10.0 ± 0.8	.01
Splenic reconstitution (day 74)[3]				
T cells (×10⁶)	20.6 ± 1.8	1.4 ± 0.02	3.6 ± 0.4	.02
B cells (×10⁶)	41.6 ± 12.6	2.5 ± 0.7	21.2 ± 15.6	.02
Treatment onset: day +14				
Survival (day 60)	100% (n = 3)	20% (n = 5)	71% (n = 6)	.03

[1]Calculated at the 95% confidence level, Allo + vehicle vs Allo + Bz-423; [2]reported as mean clinical score ± SE; [3]reported as mean value ± SD.

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OPPOSING EFFECTS OF ICOS ON GRAFT-VERSUS-HOST DISEASE MEDIATED BY CD4 AND CD8 T CELLS

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Inducible costimulatory molecule (ICOS), a CD28 family member expressed on activated CD4+ and CD8+ T cells, plays important roles in T cell activation and effector function. Here we studied the role of ICOS in graft-versus-host disease (GVHD) mediated by CD4+ or CD8+ T cells in allogeneic bone marrow transplantation (BMT). In comparison of wild type (WT) and ICOS-deficient T cells, we found that recipients of ICOS−/− CD4+ T cells exhibited significantly less GVHD morbidity and mortality. ICOS−/− CD4+ T cells had no defect in expansion, but expressed significantly less FasL and produced significantly lower levels of IFN-γ and TNF-α. Thus, ICOS−/− CD4+ T cells were impaired in effector functions that lead to GVHD. In contrast, recipients of ICOS−/− CD8+ T cells exhibited significantly enhanced GVHD morbidity and mortality. In the absence of ICOS signaling, either using ICOS-deficient donors or ICOS-ligand deficient recipients, the levels of expansion and Tc1 cytokine production of CD8+ T cells were significantly increased. The level of expansion was inversely correlated with the level of apoptosis, suggesting that increased ability of ICOS−/− CD8+ T cells to induce GVHD was resulted from the enhanced survival and expansion of those cells. Our findings indicate that ICOS has paradoxical effects on the regulation of alloreactive CD4+ and CD8+ T cells in GVHD.

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HIGHER CLEARANCE RATES OF MYCOPHENOLATE MOFETIL (MMF) IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT (AlloSCT) RECIPIENTS

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