spleen and CD4+ Th17 cytokine (IL-17) elevation in the thymic compartment of recipients that received S-59 treated T cells (mean-28.44pg/ml versus 1.45pg/ml, p = 0.0059). In-vivo tracking of S-59 treated T cells demonstrated the disappearance of these cells in the peripheral blood, spleen, bone marrow and thymus within 48 hours of transplantation. Nonetheless, we noted that recipients of S-59 treated T cells had significantly less acute GVHD and better overall survival (p = 0.0001). In-vitro co-cultures of stem cells (HSC) with S-59 T cells demonstrated significant increase in multilineage CFU formation(p < 0.0001). In summary, our experiments indicate that there is an initial dominance of inflammatory and CD4 Th1 cytokines immediately post transplantation. Co-transplantation of S-59 treated T cells shifts the effector CD4 T cell profile to resemble a Treg phenotype. Thus, S-59 treated T cells appear to exert an important immunomodulatory effect to ameliorate GVHD and improve survival after MHC-mismatched allogeneic transplantation. In addition these T cells exert a direct proliferative effect on the HSCs.

Table 1. Cytokine Profile

	Peripheral blood	Bone marrow	Thymocyte
TGF-β [S59]	8482.7 pg/ml	7062.1pg/ml	2926.9pg/ml
TGF-β [control]	1.1pg/ml	301.55 pg/ml	46.5 pg/ml
IL-10 [S59]	419.0 pg/ml	174.0	30.4pg/ml
IL-10 [control]	<1pg/ml	82.1pg/ml	33.44pg/ml

p value- * 0.0001, **0.0006

497

SIROLIMUS INDUCES COMPLETE REMISSION OF ACUTE GRAFT-VERSUS-HOST DISEASE WITHOUT SYSTEMIC GLUCOCORTICOIDS

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While glucocorticoids have been considered essential in primary therapy of acute graft-versus-host disease (aGVHD), complete remission from aGVHD is achieved in the minority of cases. Sirolimus is a potent immunosuppressive agent which may provide a novel approach to primary aGVHD therapy. In a retrospective analysis, we examined the efficacy of sirolimus as sole primary therapy for aGVHD. A total of 32 allogeneic peripheral blood stem cell recipients (78% unrelated donors, 16% with 1-2 antigen mismatch) with biopsy confirmed aGVHD were treated with sirolimus at a median of 30 days (range 15 – 106) after allogeneic hematopoietic cell transplantation (HCT). Median age was 60 years (range 28–73). Patients were at high risk for relapse of primary malignancy, with 23/32 not in remission at time of HCT. Sirolimus was delivered orally to achieve serum levels of 4-12ng/mL. Therapeutic serum levels of sirolimus were achieved in all cases, including those with GI involvement. All patients had tacrolimus based aGVHD prophylaxis; target tacrolimus level was reduced to 3-7ng/mL once sirolimus was initiated. Complete remission (CR) was defined as complete resolution of all aGVHD manifestations sustained for at least 4 weeks without the addition of glucocorticoids or other systemic immune suppressive agents. At time of primary sirolimus therapy, 53% had skin involvement, 66% GI, and 16% hepatic. Overall grade was 1 (12%), 2 (75%), and 3 (13%). Sixteen (50%) achieved sustained CR of aGVHD following sirolimus treatment. CR did not significantly differ according to organ involvement or overall aGVHD severity. With median follow up time of 16 months (range 6-26 months), one year OS was 56% (95% CI 38-74%). Accounting for competing risk, the cumulative incidence of relapse at one year was 37% (23-60%), and NRM was 20% (10-42%). The cumulative incidence of any grade chronic GVHD per NIH consensus criteria was 55% (39-79%). Thrombotic microangiopathy occurred in three cases, which responded to dose reduction or elimination of calcineurin inhibitor. Sirolimus demonstrates activity that rivals that of high dose glucocorticoids in the primary therapy of aGVHD.

498

RAPAMYCIN DELAYS XENOGENEIC ACUTE GRAFT VERSUS HOST DISEASE (AGVHD) IN NOD/SCID/IL2RγNULL (NSG) MICE: IMPACT OF REGULATORY T CELLS

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Background: Rapamycin (RAPA), a mTOR inhibitor also termed sirolimus, is a potent immunosuppressive drug which might induce tolerance by inhibiting activated T cells but not regulatory (Treg) T cells. The aims of the current study were to establish a humanized model of aGVHD in NSG mice, and to assess the impact of RAPA and Treg infusion/depletion on morbidity from xenogeneic aGVHD.

Methods and Results: We first assessed the impact of the dose of IV infused human PBMC on GVHD mortality. After 2.5 Gy TBI irradiation NSG mice received 5×10^5 (n = 5), 1×10^6 (n = 5), 2×10^6 (n = 5) or 3×10^6 (n = 5) human PBMC. Mean survivals in each group were 36±7 days, 30±6 days, 24±5 days and 16±1 days, respectively. At time of death, mice presented clinical symptoms of aGVHD such as weight loss higher than > 20%, and showed massive CD3+ human T cells infiltration of their marrows, livers, spleens and lungs. Based on these results, we decided to use 2×10^6 PBMC for further experiments. We then investigated whether injection of RAPA or PBMC supplemented in Treg could mitigate aGVHD in this model. After receiving 2.5 Gy TBI NSG mice were transplanted with 2×10^6 PBMC and i.p. PBS (control arm), with 2×10^6 PBMC and i.p. RAPA (rapa arm), with 2×10^6 PBMC depleted in Treg and i.p. RAPA (Treg-depleted arm), or with 2×10^6 PBMC supplemented in Treg (1Treg:8PBMC) and i.p. PBS (Treg arm). Mice were daily monitored for survival and weighed at least once a week until sacrifice. Median survival was higher in the RAPA arm than in the control arm (96 vs. 24 days, P < 0.01), while mice given Treg-depleted PBMC plus RAPA had a median survival of 48 days (P < 0.01 in comparison with the rapa arm). In contrast, mice in the Treg arm had a median survival of 71 days (P < 0.01 in comparison with the control arm).

Conclusions: In summary, our data indicate that i.v. injection of 2×10^6 PBMC into irradiated NSG mice induced a severe xenogeneic aGVHD. RAPA administration increased survival but this effect was greatly affected by Treg depletion suggesting that RAPA administration prevented death from GVHD at least in part by promoting Treg.

499

STAT1 DEFICIENCY FAILS TO PROTECT AGAINST MURINE CHRONIC GVHD

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Donor Th1 cells, which play a key role in promoting acute GVHD, may also contribute to chronic GVHD. To further characterize the role of Th1 cells in cGVHD, we used a model developed by the Drobyski Lab whereby acute GVHD is initiated (B6 ⇒ BALB/c BMT; 21-day transplant interval), with subsequent adoptive transfer of post-BMT B6 T cells into syngeneic, immune-deficient hosts (B6129s7-Rag1; 45-day observation interval) for induction of auto-reactive, cGVHD. Because STAT1 is the signaling pathway for the Th1-polarizing cytokines IFN-α and IL-12, we used STAT1-deficient (KO) donor T cells to assess whether Th1 cell deficiency might yield reduced cGVHD; other transplant cohorts received T cells that were either wild-type (WT), STAT3-KO (loss of Th17 function), or STAT6-KO (loss of Th2 function). Clinical signs of acute GVHD (weight loss, hunched posture) was severe in WT, mild in STAT3-KO, and STAT6-KO T cells; in contrast, STAT1-KO T cell recipients showed no signs of acute GVHD. Chronic GVHD, which was manifested by weight loss and extensive skin disease (erythematous, scaling rash with alopecia; > 50% body surface area), occurred in 100% of recipients of WT and STAT1-KO T cells (5/5 and 10/10 cases, respectively); by comparison, extensive skin disease was not observed in recipients of STAT3-KO or STAT6-KO T cells (0/9 and 0/5 cases, respectively). As such, STAT1-deficiency appeared to reduce acute GVHD but not chronic GVHD. Further experiments were performed at the end of the cGVHD observation interval to confirm that recipients of