myeloablative (19% vs. 24%, P=0.349) and nonmyeloablative conditioning (13% vs. 9%, P=1.000). PFS at 1 year was slightly higher for ATG1 (52.8% vs. 45.5%,P = .955), while OS at 1 year was not different between the two groups (53.7% vs 55.4%,P = .779).

Conclusion: A small decrease in the prophylactic ATG dose (1.5mg/kg) had a significant clinical impact. An increased incidence of severe lethal acute GVHD was observed with 4.5mg/kg dosing despite a greater degree of HLA-matching. The 6.0mg/kg dosing prevented grade IV acute GVHD and associated mortality. Further research is warranted to find the optimal dosing regimen prior to allogeneic stem cell transplantation.

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Vitamin D Supplementation Decreases Grade 3-4 Acute GVHD in Allogeneic Transplants with Normal Baseline Levels

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Background: Previous studies have shown that patients receiving AHSCT are likely to have sub-optimal Vit D levels. Vit D has also been shown to have significant immunomodulatory effects, including polarization of T cell population towards Th2 expression and decreasing allogeneic T-cell proliferation while increasing T-reg cell function. Alloreactive T cells upregulate the Vit D receptor and repletion of Vit D in vitro significantly reduces this response. Thus it is hypothesized that patients with VIT D deficiency at onset of AHSCT would have greater incidence and severity of GVHD and that early replacement may lessen the risk of GVHD. In this study we examine this relationship.

Methods: A retrospective evaluation of 290 adult AHSCT at the University of Michigan between January 2007-December 2010. All patients had Serum 25-hydorxyvitamin D (25OHD) levels at admission for AHSCT. Deficient levels were defined to be below 29 ng/ml and normal levels as 30 ng/ml or higher. The incidence and grade of GVHD were defined as maximum overall grade and acute GVHD was defined as starting within the first 100 days. The primary standard GVHD prophylaxis regimens were Tacrolimus/MTX for full intensity and Tacrolimus /MMF for reduced intensity transplants.

Results: Of all AHSCT 77% had Vit D deficiency at onset of transplant with no difference between related donors (RD) and unrelated donors (UD). Vit D supplementation was started within the first week of transplant and occurred in 83% who were Vit D deficient at baseline and 36% of those having normal levels at baseline. In patients with baseline Vit D deficiency there did not appear to be an impact of Vit D supplementation on the incidence of Grade 2-4 GVHD (44% vs 46%) or Grade 3-4 GVHD (17% vs 19%). However in patients who had baseline Vit D levels of 30 ng/ml or above there was a significant decrease in the incidence of Grade 3-4 GVHD in the group who received Vit D supplementation versus those who did not (9% vs 27%, X^2=2.99,P = .042) and a trend towards a decrease in Grade 2-4 GVHD (35% vs 54%, X^2=2.11,P = .073). This is despite having more Unrelated Donor transplants in the Vit D supplemented group (65% vs 46%).

Conclusion: AHSCT patients have high rates of Vit D deficiency. Ironically this study suggests that Vit D supplementation starting during transplant may significantly decrease the incidence of Grade 3-4 GVHD in patients who have baseline levels >30 ng/ml but not those severely deficient at baseline. This could imply that higher amounts of Vit D supplementation may be required to adequately effect T cell response. Future trials should evaluate the role of increasing Vit D supplementation and determine if an optimal Vit D level is required to decrease the incidence and severity of acute GVHD.

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Contribution of the PD–1–PD–L Pathway to Chronic Graft–Versus–Host Disease

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Chronic graft-versus-host disease (cGVHD) remains a major cause of late phase mortality and morbidity after allogeneic hematopoietic stem cell transplantation. We investigated the roles of the PD-1 pathway in cGVHD using a well-defined mouse model (B10.D2 into BALB/c). First, we confirmed that CD4 and CD8 cells from the spleen and peripheral lymph nodes (PLN) of allogeneic recipients expressed significantly more PD-1 compared to those of syngeneic recipients on days 14, 21, 28, and 56 after transplantation ($p < 0.05$). Upon transferring PD-1 KO donor T cells of the B10.D2 background into allogeneic bone marrow transplantation (BMT) models, there was severe weight loss and 100% of the recipients died by day 23. To avoid early death and to examine the roles of the PD-1 pathway in cGVHD, we next administered six antibody doses to recipients of wild-type (WT) donors from day 14 of BMT, just prior to the development of cGVHD. Mice treated with anti-PD-1 showed 70% mortality by day 35 in contrast to only 10% mortality in those administered anti-B7H1 Ab or anti-B7DC Ab. All groups treated with anti-PD-1 Ab, anti-B7H1 Ab, or anti-B7DC Ab had significantly higher cGVHD scores than controls (anti-PD-1 Ab: 2.51±0.31, anti-B7H1 Ab: 2.31±0.15, anti-B7DC Ab: 2.01±0.42, vs. control: 1.18±0.27, $P < .05$). To further examine the roles of the PD-1 pathway in cGVHD, we next used B7H1 KO mice as recipients. Allogeneic B7H1 KO BMT recipients showed significantly more severe skin cGVHD than WT controls ($p < 0.05$). Histopathological examination of the skin showed significantly increased cGVHD damage in recipients of B7H1 KO donors (5.86 ± 0.85 vs. 8.38 ± 0.38, P < 0.05). Since B7H1 expression of hematopoietic cells is critical for donor Treg cell expansion, we then harvested cells from PLNs 14 and 28 days after BMT and analyzed cytokine expression levels. Recipients of B7H1 KO showed fewer Foxp3+ regulatory T cells in the early phase (day 14), whereas there was no difference in the frequency of Foxp3+ regulatory T cells in the late phase (day 28). We previously reported that Th1 and Th17 cells contribute to the pathogenesis of cGVHD using this mouse model. On day 28, IL-17+ IFNg+ T cells were detected significantly more frequently in recipients of B7H1 KO donors than those of WT recipients ($p < 0.05$). These results suggested that B7H1 expression in the recipient regulates the frequency of IL-17+ IFNg+ T cells and Foxp3+ regulatory T cells. Finally, we established chimeras that expressed B7H1 in hematopoietic cells, but not in parenchymal tissue by transferring WT BM cells into B7H1KO recipients. Chimeric

mice had significantly poorer cGVHD scores than control mice (WT BM cells into WT recipients), suggesting the role of host parenchymal tissue cell expression of B7H1 in cGVHD. Taken together, the PD-1 axis, especially B7H1 expression on recipients, regulates the frequency of IL-17+ IFNg+ T cells and contributes to the pathogenesis of cGVHD.

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Post-Transplant Cyclophosphamide (PTC) As Sole Graft Versus Host Disease (GVHD) Prophylaxis in Patients Undergoing HLA Matched Sibling Donor Stem Cell Transplant (SCT) for Severe Aplastic Anemia (SAA)

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Between September 2010 and June 2012, 15 patients with SAA underwent HLA identical sibling donor SCT using Fludarabine 180 mg/m2 IV over 6 days and Cyclophosphamide 100 mg/kg IV over 2 days. Five patients had in addition a single fraction of Total Body irradiation (TBI) 200 cGy. Cyclophosphamide 50 mg/kg/day IV on day +3 and +4 was the sole GVHD prophylaxis. G-CSF mobilized peripheral blood stem cells (PBSC) was the graft source. Ten males and 5 females with a median age of 25 years (range: 8 − 42) had SCT. Median PBSC cell dose infused was 9.5 x 106 CD34/Kg (range: 5.4 − 17.2). Thirteen engrafted (86.6%) with median neutrophil and platelet engraftment of 15.4 days (range: 15-17) and 16.6 days (range: 12-32) respectively. Grade II–IV GVHD seen in 3 patients (23%) at 42, 49 and 68 days post SCT. Two responded to combination of cyclosporine and prednisolone while one patient with grade IV GVHD expired 64 days post SCT. Of 11 evaluable patients, 4 (36.3%) developed chronic GVHD which was limited in all. Two patients with de novo chronic GVHD were managed with prednisolone alone. Overall 7 patients (46.6%) have not required any immunosuppression after SCT while 3 have required immunosuppressive therapy for 114, 127 and 225 days respectively At a median follow up of 11 months (range: 1 − 22), 11 (73.3%) are alive and well including 7 patients who did not require any immunosuppressive therapy following SCT. The use of post transplant cyclophosphamide as GVHD prophylaxis following sibling donor transplant for SAA is associated with low rates of GVHD. A large number (46%) did not require any immunosuppression post SCT. Larger studies are required to understand the utility of this prophylaxis in sibling donor transplants for aplastic anemia.

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Efficacy and Safety of Immunomodulation with Fast Withdrawal of Immunosuppression (FWI) and Donor Lymphocyte Infusions (DLI) for Prevention of Relapse in Children Receiving Allogeneic Hematopoietic Stem Cell Transplant (HCT) for Hematologic Malignancies

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Persistence of mixed chimerism (MC) following myeloablative HCT in pediatric hematologic malignancies is related to a high risk of relapse. We initiated a prospective study of FWI and DLI in patients with MC at 30 days post-transplant. We are reporting preliminary results on 43 enrolled patients with a mean age of 10±6.5(SD) years. Fifty-eight percent of patients had myeloid malignancies, 40% lymphoid malignancies and 2% had biphenotypic leukemia. Based on day +30 bone marrow and peripheral blood chimerism results, 26/43 (60%) patients were found to have MC, and were assigned to the intervention arm of the study consisting of FWI and DLI; 12 patients (28%) had full donor chimerism (FDC) or early graft-versus host disease (GVHD) and were assigned to observation arm, and 5 (12%) could not be assigned to either arm due to early death or relapse. FWI started at a median of day +50 (range 40-85), and ended at a median of day +75.5 (range 49-113). Following FWI, 9 patients (35%) converted to FDC. Of 17 patients who remained MC following FWI, 15 proceeded to DLI, 1 did not receive further intervention due to GVHD and one relapsed prior to DLI. Acute GVHD developed in 3/26 (12%) patients undergoing FWI and in 9/12 (75%) of patients in the observation arm ($P < .01$). Two patients undergoing intervention developed grade II aGVHD which resolved and 1 developed grade IV aGVHD that progressed to fatal cGVHD of the lungs. In the observation arm, 2 patients developed grade I, 5 developed grade II, 2 developed grade III, and 1 developed grade IV aGVHD. Chronic GVHD developed in 6 patients (2 in the intervention and 4 in the observation arm). One of 6 patients developed de novo cGVHD, following DLI. The incidence of acute and/or chronic GVHD was 15% in the intervention arm of the study. Toxic death rate due to GVHD was 4%. There were 11 events (3 treatment-related deaths and 8 relapses). Mean follow-up of living patients was 17.6±10 (SD) months. EFS for the entire cohort was 71±7(SD)% and was not significantly different between the observation arm and the intervention arm. Ten patients (23%) had evidence of disease by flow or cytogenetics, at the time of HCT. Based on chimerism results, 4 were assigned to the intervention arm, 3 to the observation arm, and 3 could not be assigned to any arm of the study due to early relapse or death. EFS was significantly lower in patients with positive disease prior to transplant than in those without evidence of disease (EFS 27±15% vs. 86±7%). Among 26 patients undergoing intervention, relapse was significantly more common ($P = .014$) in patients with positive disease pre-transplant. Our data indicate that post-transplant immunomodulation is safe and has overall low GVHD risk (15%). Our schedule of FWI was not adequate to prevent relapse in patients coming to transplant with persistent disease. We would recommend an earlier, (day 30) and more aggressive schedule of immunosuppression withdrawal for these patients.

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From Murine Model to Clinical Trial of Graft-Versus-GVHD, a Second Transplantation From Another Donor for the Rescue From Refractory Acute GVHD

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Background: GVHD is still a major obstacle in allogeneic transplantation despite the progress of immunosuppressive drugs and cell therapy such as mesenchymal stem cells. GVHD is caused by donor lymphocytes, mainly T cells,