with myeloid cells, can be used as an adjuvant therapy which may help address some of the infectious complications of BMT, an approach that is being tested in clinical trials. The fact that MP can be cryopreserved and can be used effectively without being MHC matched to the host enables this approach.

We have reported in preclinical models that we can use MP to induce specific and lasting tolerance for solid organ transplants. In our model, mice (typically BALB/c) are preconditioned and reconstituted with autologous or allogeneic hematopoietic stem cells (HSC) and allogeneic MP. MP-matched skin grafts are placed simultaneously and their fate is followed long-term. We have established that MP induce specific tolerance, MP-matched skin grafts are accepted, while grafts unmatched to the MP, HSC or host are rejected. MP from Rag2-/-IL2rg-/- mice that are incapable of producing B cells, T cells or NK cells, establish that only organ-graft matched myeloid cells are essential for this process. Tolerance can be induced by 100,000 MP in the context of allogeneic (third party) HSC transplantation. However, we have previously reported that the same number of MP only protects skin grafts in approximately 80% of the recipients that have been given autologous HSC (Transpl Immunol 31:112-118,2014).

Here we report that while tolerance induction by MP is more efficient when combined with allogeneic HSC, increasing the cell dose ensures the same outcome with autologous HSC. Injection of 40,000 or more Linneg/lo, Sca-1neg, c-Kit+ MP induces robust tolerance in recipients of allogeneic HSC. However, similar results can be obtained in the context of host-autologous HSC by using slightly higher cell doses (200,000 or more MP are needed to protect all skin grafts).

We conclude that organ matched MP can induce robust tolerance for simultaneously or subsequently placed solid organ transplants, without the need for long-term high level, multi-lineage engraftment. The specific properties of MP (they are clinically available, they can be cryopreserved, they can be used without matching and MP can be expanded ex vivo) makes these cells of particular interest. They offer a new approach to the use of BMT to help in improving solid organ transplantation.

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Prevalence of Vitamin D Deficiency in Allogeneic HCT Recipients and Its Association with Graft Versus Host Disease

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Purpose: Vitamin D deficiency has been associated with chronic Graft Versus Host Disease (GVHD), and insufficient Vitamin D levels have been shown in patients who have undergone hematopoietic cell transplant (HCT). This pilot study tests the hypothesis that Vitamin D deficiency is prevalent and is associated with acute GVHD (AGVHD) in the immediate 90 days after HCT.

Methods: Fifty allogeneic subjects were selected from the HCT database based on available bio-samples pretransplant, 30 days, and 90 days post HCT, obtained during 2012-2013. Retrospective clinical data were also obtained. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine 25-OH vitamin D (D2, D3 and total) levels at the three time-points. Descriptive analysis was completed using PASW (IBM, V.20).

Results: 50 subjects (25 male, 25 female), mean age 41±11 years, 48% white (18% Asian, 34% other), were selected. AGVHD was present in 23/49 (~50%) of subjects and 73% had Vitamin D deficiency at baseline (20±8 ng/ml), significantly decreasing at 30 days (16±8 ng/ml, p=.048), and remained constant at 90 days (16±10 ng/ml). Majority of samples were below threshold for Vitamin D (<25 ng/ml, 77%), however no significant relationship to AGVHD was detected (p=.530). For sex, men had greater proportion with low Vitamin D (83% v 63%) and AGVHD (52% v 42%) than women, though not reaching significance (p=.193 & p=.571). For race, all Asian subjects were below threshold for Vitamin D (9/9) and 4 developed AGVHD (44%).

Conclusion: We identify a significant majority of subjects undergoing HCT have Vitamin D deficiency (73%), progressing 30 days post HCT with a disproportionate effect on sex and race. Although we do not detect an association between Vitamin D deficiency and AGVHD, an appropriately powered study is needed to determine whether a relationship exists. Supplementation and monitoring HCT patients for Vitamin D deficiency is warranted.

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Phenotype, Function and Expansion of Regulatory T Cells in the Cynomolgus Macaque (Macaca fascicularis)

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Regulatory T cell (Treg) therapy provides an alternative approach to chronic immunosuppression and has shown promising results in murine models. Translating this approach into the clinic depends on developing efficient methods with which to expand human or non-human primate Tregs *in vitro.*

At the Columbia Center for Translational Immunology (CCTI) we have started a pre-clinical bone marrow transplant program using the Cynomolgus macaque (CM) monkey model involving Tregs to promote tolerance to tissues and organs. We first characterized CM Tregs, which have similar phenotype when compared to human Tregs. 3.67% of CD4 T cells in the CM co-express high levels of CD25 and FoxP3 (n=15) and 3.53% express CD25 and are negative for CD127, a commonly used Treg marker. Similar to human Tregs, CM Tregs include naïve or resting Tregs (1.63%) and activated effector Tregs (2.4%) based on CD45RA expression (n=7). *In vitro* CM CD25hiCD127- (FoxP3+) Tregs were found to be similarly suppressive as human Tregs, supporting the clinical relevance of the CM model.

We subsequently aimed to expand polyclonal regulatory T cells (pTregs) *in vitro* for use in our transplant studies. We developed four protocols for the expansion of pTregs over 21-56 days. 50-100 thousand CD4+CD8-CD25hi or CD4+CD8-CD25hiCD127- Tregs were isolated from 20-30mL of blood and cultured with IL-2, αCD3, rapamycin and either donor PBMCs, artificial APCs (L-cells, murine fibroblast expressing human CD80, CD58 and CD32) or both. 1000-10,000 fold

expansion was reliably achieved when artificial APCs were used. In contrast, when donor PBMCs (without artificial APCs) were used as the source of APCs, 10-100 fold fewer Tregs were obtained. Regardless of protocol, pTregs successfully prevented the proliferation of bead (CD2CD3CD28) stimulated self PBMCs with up to 50% suppression at a 1:32 ratio of Tregs:PBMCs.

Contamination of CD8+ T cells was observed despite our efforts to gate out CD8 cells during sorting. Re-sorting of the Treg cultures early (day 14-21 post isolation) successfully eliminated the CD8s. Re-sorting inherently caused a significant loss of Tregs, leading to a longer culture period. Because Treg burnout was of concern, we assessed the function and phenotype of cells maintained in cultures >50 days and observed that Tregs with early high FoxP3 expression maintained function and phenotype.

Finally, demonstration of fitness of cryopreserved pTregs is necessary for clinical application. Our preliminary studies show that Tregs can be re-cultured and re-expanded without loss of suppression or phenotype. In summary, we have successfully established several protocols for Treg expansion in the Cynomolgus macaque that can be used in pre-clinical studies of transplant tolerance induction.

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High Rates of Early Donor Chimerism in Intermediate and Poor Risk Patients Undergoing Stem Cell Transplantation Using Reduced Toxicity Ablative Conditioning Regimen Incorporating Busulfan Pharmacokinetics

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The impact of early donor cell chimerism on outcomes of reduced-intensity conditioning SCT in myeloid disorders is ill defined. To explore the impact of measuring busulfan pharmacokinetics (Bu PK) in conditioning regimens on early donor chimerism, we undertook a retrospective analysis of patients with myeloid disorders who received four days of fludarabine and busulfan with or without measuring Bu PK at our center in the last 10 years.

Methods: Chimerism assay was performed using a quantitative fluorescence-based short tandem repeat–polymerase chain reaction (STR-PCR) with capillary electrophoresis for PCR product resolution.

Results: Thirty patients were identified and included in the study. All patients received fludarabine (40 mg/m^2/day x 4 doses), busulfan (3.2mg/kg/dose IV x 4 doses). Of these thirty patients, 7 had Bu PK measured. There were 21 male and 9 female patients with a median age of 62 years (range 48–72yrs). Median time to follow up was 13.3 months. Disease risk was considered advanced in 17 patients, intermediate in 3 and early in 10. All patients in the Bu PK group had advanced disease except one had early disease. Regarding cytogenetics, all patients in the Bu PK group had high or intermediate risk cytogenetics. Median blast number at time of SCT was 5%. Stem cell source included peripheral blood in all patients.

There were no primary graft failures. Total Donor cell Chimerism analysis in the Bu PK group showed 100% donor at both time points (days 30, 100) in all patients except in one who relapsed at day 30 (85.7%). While in the non-PK group only 7 out of 23 (30%) patients had complete chimerism at day 30 and day 100. Complete donor chimerism at day 100 in the non-PK group was 47.8% compared to 85.7% in the PK (p=0.18). Ten out of 23 patients (43.5%) in the non-PK group had decreasing donor chimerism by day 100, while in the PK group only one patient (14%) who relapsed had decreasing donor chimerism by day 100 with an odds ratio of 0.241 (95% Confidence Interval=0.025-2.357; p-value=0.22). None developed sinusoidal obstructive syndrome.

Conclusion: In this small cohort from a single center, we found that patients with myeloid disorders who received fludarabine busulfan for 4 days incorporating Bu PK had a trend for higher rates of early complete donor chimerism and less decreasing donor chimerism by day100 despite having intermediate or high risk disease at time of SCT. Longer follow up is needed for our patients to see if there is effect on relapse or survival but previous studies have showed that low or decreasing donor chimerism early after SCT is an independent risk factor for relapse and impaired survival. This is especially important in myeloid disorders. Bu PK may help target better level for inducing early total donor chimerism and donor chimerism may identify high-risk patient cohorts who may benefit from additional therapeutic interventions.

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Impact of Gender: Female Matched Related Donor Versus Male Matched Unrelated Donor on Peripheral Blood Allogeneic Stem Cell Transplant for Male Recipients

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The female donor/male recipient combination increases the risks of graft-versus-host disease (GVHD) and non-relapse mortality (NRM) after allogeneic stem cell transplantation (allo-SCT). To explore the impact of Female matched related donor (F-MRD) versus male matched unrelated donor (M-MUD) on outcome of peripheral blood allo-SCT in male recipients, we undertook a single center retrospective analysis of male adult patients transplanted at our center in the last 10 years. We excluded patients who received donor lymphocyte infusion post SCT.

Methods: Disease-free survival (DFS) and OS were calculated using the Kaplan-Meier estimate. Cumulative incidences (CI) were used for relapse (REL) and GVHD in a competing risks setting, NRM being a competing event for REL, and death for GVHD.

Results: Fifty-six patients were identified and included in this analysis. The median age at transplant was 57 years (19-73). Diseases were AML (n=21), ALL (n=7), NHL (n=9), Hodgkin's disease (n=1), myeloma (n=5), MDS (n=11), CLL (n=1), and CML (n=1). Conditioning regimens were myeloablative (MAC) (n=35), reduced toxicity ablative (n=17) or non myelablative (n=4). Donors were F-MRD (n=24) or M-MUD (n=32). Source of stem cells was peripheral blood in all patients. GVHD prophylaxis consisted of tacrolimus and methotrexate in MRD and tacrolimus, methotrexate and anti-thymocyte globulin in MUD. Of the female donors, 63% had previous pregnancies before donation, 8% had no pregnancies and 29% were unknown. Mean age of female donors was 48 while the mean age for male donors was 35.