with an observed FEV1 < 90% or FEF 25-75 < 75% were recorded, and compared to the recorded date of BOS diagnosis. Fisher's exact test was applied to determine statistical significance where applicable.

Results: For all BOS patients, the mean time from transplantation to diagnosis of BOS was 478 days (95% CI 357-599d) compared to a mean time of 169 days (95% CI 100-238d) when patients had either an FEV1<90% or FEF25-75 <75%. The mean difference was 298 days (95% CI 176-420d, $P = .00003$). Patients with cGVHD and no BOS had similar decline in PFT in only 22/44 patients, compared to 100% of those with BOS. Serum IGF-1 values were > 4,000 (25^{th} percentile) in 12 of 13 patients with BOS ($P = .04$). Overall, 12 of 13 patients had biomarkers elevation prior to spirometric diagnosis of BOS.

Conclusions: Application of the BOS 0-p criteria to HSCT patients may identify patients at risk for BOS at an earlier time point. The use of the IGF 1 as a serum biomarker may also contribute to identify patients at risk, although further studies are needed to investigate whether this marker can differentiate between patients with BOS and those with other lung diseases.

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Double Haploidentical Hematopoietic Stem Cell Transplantation Enhances Anti-Tumor Activity After Transplant

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Haploidentical (HI) HSCT with low dose donor T-cell infusion provides a survival advantage in tumor bearing mice when compared to parent F1 or MHC-matched transplant models. We suggest that MHC difference in HI-HSCT generates early T-cell clonal activation against the unshared MHC haplotype, which eliminates residual tumor cells that express the unshared MHC haplotype. However, alteration in MHC antigen expression is a significant contributor to tumor escape from graft-versus-tumor (GVT) activity. Recent haploidentical transplant data revealed that uniparental disomy, the loss of the HLA haplotype, is a clinically relevant mechanism of tumor escape that leads to post-transplant leukemia relapse. Murine renal cell carcinoma, RENCA cell line normally expresses only $H2K^d$as a MHC molecule. Therefore, in our haploidentical transplant model, T cell clonal activity is usually restricted against $H2K^d$molecule only. For circumventing the single haplotype expression of tumor model, we transfected this cell line with a $H2K^b$expressing vector, pAcGFP-NeoR-$H2K^b$, and generated stable clones with G418 selection. The clone that has more than 95% $H2K^b$expression used for in vivo experiments. Both tumor cell lines, i.e. parental and transfected clone, had similar in vivo tumor growth acceptance and growth rate. We then used two different haploidentical donors that were targeting different MHC haplotypes. Lethally irradiated B6D2F1 ($H2K^{b/d}$) mice were transplanted with T cell depleted bone marrow (TCD-BM) from either B6C3F1 ($H\text{-}2K^{b/k}$) (single haplo-1; SH1), or C3D2F1 ($H2K^{k/d}$) (single haplo 2; SH2) or both donor mice with low-dose ($1x10^5$) T-cells. In some experiments, animals were also injected either $H2K^d$or $H2K^{b/d}$ expressing RENCA-TGL cells for the evaluation of GVT activity Bone marrow (BM), spleens and thymi were harvested from recipients of single and double HI-HSCT at day 35 and showed similar cellularities. Interestingly, spleen and bone marrow had similar chimerism from both donors in DH-HSCT. There were no early transplant mortality, graft failure, weight loss and GVHD scoring difference among the double or single-haploidentical transplant recipients. In two other sets of experiments, we followed the tumor growth and the survival of tumor bearing mice after transplant. The recipients of DH-HSCT showed a better survival and GVT activity than the recipients of SH-HSCT in RENCA-TGL ($H2K^{b/d}$) bearing tumor model. These observations confirmed that MHC targeting plays a prominent role in tumor surveillance, and immune targeting the unshared MHC haplotype with haploidentical transplant induce remarkable survival advantage.

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Prevention of Murine Sclerodermatous Chronic Graft-Versus-Host Disease by Rapamycin

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Background: The most widely used mice model of chronic graft-versus-host disease (cGvHD) is an MHC-matched bone marrow transplantation model of sclerodermatous cGvHD. A limitation of that model is that mortality is relatively low, making difficult to study the impact of potentially therapeutic compounds.

Aims: To develop a more severe model of cGVHD and to assess the impact of Rapamycin administration in that model.

Results: Lethally irradiated Balb/C mice were injected with $10x10^6$ bone marrow cells and $70x10^6$ splenocytes from B10.D2 donor mice. Twenty-one days later, all mice developed cGvHD. For the severe model, donor B10.D2 mice were injected with $0.5x10^6$ splenocytes from Balb/C twenty-one days before transplantation. All mice from the severe model (n=8) died a median of 32 days while 3 of 7 mice in the classical model survived beyond day 52. Mean survival was decreased in the severe model compared to the classical model (32 days versus 37 days; p=0.0185). Recipient mice in the severe group experienced higher weight loss, hair loss and skin fibrosis. Numbers of T lymphocytes (251.9 ± 151.4 versus 626.3 ± 532.8; p=0.0004) and $CD4^+$ T cells (61.57 ± 41.93 versus 125.0 ± 14.39; p=0.0008) per microliter of blood at day 21 were lower in the severe group than in the classical model. Moreover, number of regulatory T cells (Tregs) was decreased in the severe model (0.9870 ± 0.8864 versus 7.979 ± 6.753; p=0.0062).

We then investigated whether rapamycin administration could prevent GVHD in the severe model. All (n=8) mice treated with PBS (placebo) died a median of 32 days after transplantation, while 6 of 8 mice given 1mg/kg/day i.p. rapamycin survived beyond day 52 (p=0.0012). Number of Tregs/μl was higher at day 21 in rapamycin-treated mice than in mice given PBS (3.532 ± 1.195 versus 1.958 ± 0.8864; p=0.0796). Moreover, number of naïve $CD4^+$T (7.798 ± 4.192 versus 25.71 ± 5.185; p= 0.0276), effector memory $CD4^+$ T cells (EMT) (40.50 ± 3.180 versus 25.17 ± 7.881; p= 0.0392) and central memory $CD4^+$ T cells (CMT) (53.58 ± 3.180 versus 26.29 ± 7.881; p= 0.0060) were higher in rapamycin mice. Finally, proliferation of EMT (assessed by flow cytometry using Ki-67) ($34.70\% \pm 4.084$ versus $30.80\% \pm 2.003$; p=0.0221) and CMT ($33.25\% \pm 4.084$ versus $27.50\% \pm 2.003$; p=0.0183) was higher in PBS than in rapamycin mice.

Conclusion: We have developed a mice model of severe cGVHD. Interestingly, rapamycin prevented death from cGVHD in that model, perhaps through *in vivo* expansion of Treg.

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Depletion of Naïve T Cells From Peripheral Blood Stem Cell Grafts for GVHD Prevention

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Graft-versus-host disease (GVHD) frequently causes morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT). In HLA-identical HCT, GVHD results from donor T cell recognition of minor histocompatibility (H) antigens on recipient tissues. Complete T cell depletion (TCD) of donor hematopoietic cell products is more effective than pharmacologic immunosuppression for preventing GVHD, but is complicated by delayed immune reconstitution and consequent life-threatening infections. Approaches to HCT that preferentially deplete T cells responsible for GVHD and preserve pathogen-specific T cells may improve outcomes.

Mature $CD3^+CD8^+$ and $CD3^+CD4^+$ T cells can be classified into $CD45RA^+CD62L^+$ naïve (T_N) and $CD45RO^+$ memory (T_M) subsets, the latter of which includes effector memory (T_{EM}) and central memory (T_{CM}) T cells. Murine studies in MHC-matched and −mismatched models demonstrated that transplanting T_N cells causes severe GVHD, purified T_{CM} causes mild GVHD, and T_{EM} do not cause GVHD. *In vitro* studies have similarly demonstrated that human donor $CD8^+$ T cells specific for recipient minor H antigens are found predominantly within the T_N cell subset. In sum, these data suggest that selective $T_N$cell depletion may alter the incidence or severity of GVHD in human HCT.

We developed an effective process for engineering human peripheral blood stem cell (PBSC) grafts that depletes $CD45RA^+$ T_N and retains $CD34^+$ stem cells and functional $CD45RO^+$ T_M specific for diverse opportunistic pathogens. We initiated a clinical trial to evaluate selective depletion of T_N cells from allogeneic PBSC for the prevention of GVHD in patients with acute leukemia. Each of the first 22 patients has engrafted (median day 12), regimen-related toxicity has been acceptable, T-cell numbers recover faster than reported for TCD HCT (median 412 $CD3^+$ T cells/μl on day 28), and there is no increase in the rate of relapse, opportunistic infections, or EBV reactivation compared to patients treated with T cell replete PBSC grafts. Early onset gastrointestinal symptoms that are compatible with mild acute GVHD occur frequently, but rapidly respond to therapy, and most patients have successfully tapered immunosuppression. T cell responses to pathogens recover early after HCT. At a median of 14 months follow-up, overall and disease free survival are 80% and 75% respectively, and the frequency and severity of chronic GVHD is substantially reduced compared to recipients of T cell replete PBSC grafts.

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Adenovirus PCR-Positivity in Stool Precedes Intestinal GRAFT Versus Host Disease After Allogeneic Hematopoietic Stem Cell Transplantation

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Introduction: Acute graft versus host disease (aGvHD) is a common (20-50% of all HSCT recipients) and potentially lethal complication after allogeneic hematopoietic stem cell transplantation (HSCT). Risk factors for aGvhD include donor source, preparative regimen and the degree of HLA-mismatching. Currently, there is increasing evidence that (reactivation of) viral infection is a risk factor for aGvHD. We hypothesized that the presence of viruses in the gastrointestinal tract, including AdV, triggers the initiation phase of intestinal GvHD. Therefore we investigated the association between viral PCR-positivity in stool prior to HSCT and the occurrence of intestinal aGvHD.

Methods: We prospectively evaluated 27 consecutive pediatric allogeneic HSCT patients from January '09 until October '10. Primary endpoint was the development of intestinal aGvHD diagnosed according to Gluckbergs criteria and confirmed by histopathology. Follow-up ranged from 100 to 376 days or until death. Stool specimens were taken peri-HSCT and analyzed for entero-, -noro-, astro-, -parecho- and adenovirus, by real-time PCR. The association between fecal PCR- positivity and intestinal aGvHD was analyzed using Fisher's exact tests.

Results: Of the 27 patients that were evaluated, 6 (22%) developed intestinal aGvHD after a median of 64 days (range, 38-74). Four (15%) patients died due to transplant related complications or disease progression/relapse after 22-61 days. All patients with stool specimens positive for AdV (4/27, 15%) developed intestinal aGvHD (versus 2 / 23 (9%) patients without AdV in stool,$P = .001$, positive predictive value = 100%, negative predictive value = 91%). Four patients were positive for other viruses but none of these developed aGvHD. Interestingly, one patient became positive for AdV at 327 days post-SCT and developed chronic intestinal GvHD after 476 days. AdV persisted in patient stool for more than 140 days, and preceded systemic infection: AdV was first detected in plasma on day 409 and loads raised from 482 copies/ml to 1.4*10.4 copies/ml on day 500.

Conclusion: AdV in stool prior to HSCT was associated with intestinal acute GvHD. It supports the hypothesis that virally induced tissue damage leads to influx of inflammatory mediators and ultimately activation and influx of activated cytotoxic T-cells involved in GvhD. Currently we perform a prospective follow- up study. These results may impact monitoring and treatment (preventive and curative) guideline/protocols.

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Allogeneic T Cells up-Regulate Fatty Acid Metabolism and Can Be Targeted Through Metabolic Inhibition of Fatty Acid Oxidation

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Recent research has increased our understanding of lymphocyte metabolism *in vitro,* but the metabolism of lymphocytes activated *in vivo* remains poorly understood. To evaluate this important issue further, we explored the metabolism of proliferating, donor T lymphocytes seven days after the initiation of graft versus-host disease (GVHD) in an acute model of GVHD (B6 into B6D2F1). Donor T cells up-regulated mRNA for fatty acid (FA) transport proteins (e.g.