SR-GVHD, defined as lack of response or disease progression after at least 7 days of treatment with methylprednisolone at 2 mg/kg. Between 3/2003 and 6/2008, 20 patients with advanced SR-GVHD following allogeneic transplantation were treated with DD at OHSU, 7 after MRD and 13 after URD (six matched, one A antigen mismatch, and six allele mismatch) .GVHD prophylaxis consisted of cyclopsporine, methotrexate, and steroid (19) or cyclosporine plus MMF (1). Nineteen patients developed acute SR-GVHD overall grade III (7) and IV (12) while one patient developed chronic extensive SR-GVHD. Twelve patients received DD as first salvage therapy while 8 patients were treated with other agents prior to DD (6 had one and 2 had two prior salvage therapies including MMF and Etanercept). Planned treatment was DD at a dose of 600 mcg (9mcg/kg) on days 1, 3, 5, 15, 17, and 19. Median days to DD therapy after stem cell transplant was 38.Ten patients showed response (4 CR and 6 PR defined as at least a one overall grade improvement), six were non responders, and four patients died before completion of treatment. One patient remains alive. Causes of death included GVHD (8), opportunistic infection (5), respiratory failure (2), transplant-associated microangiopathy (2), ICH (1), and relapse (1). Median survival for the entire group was 30.5 days from the onset of DD (4–1839+) with median survival of 116 days for patients who received all 6 doses of DD. Despite measurable responses in 50% of patients, no survival benefit was observed. Profound immune suppression was significant among responders, contributing to opportunistic infection and death. Thus, DD may be most beneficial if used earlier in the course or in lower grade aGVHD. Responses can be achieved in advanced SR-GVHD with DD but novel approaches still remain the research target for these patients given poor overall survival .

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EVALUATION OF ALLOIMMUNE RESPONSES IN HUMANIZED NOD/SCID/IL2RG^{NULL} MICE FOLLOWING HUMAN CD34$^+$ STEM CELL TRANSPLANTATION

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Immunodeficient NOD/SCID/IL2Rg (yc)^{null} mice can be efficiently used to establish human lymphohematopoiesis or lymphohematological neoplasia in a murine environment. Thus, upon engraftment of human CD34$^+$ hematopoietic stem cells (HSC) or acute leukemia cells and allogeneic donor lymphocyte infusions these mice might become a powerful tool to provide an allogeneic HSCT model for investigating human graft-vs-host (GvH)- and graft-vs-leukemia (GvL)-immunity in vivo. In the current study, we engrafted NOD/SCID/yc^{null} mice with human CD34$^+$ HSC to provide a model for analyzing the role of patient-derived antigen presenting cells for the induction of GvH- and GvL-reactivity and to evaluate reactivity to human alloantigens of ex vivo engineered T cell allografts for improved immunotherapy. Following reconstitution of busulfan conditioned neonatal or (300 cGy) irradiated adult NOD/SCID/yc^{null} recipients with 1×10^5 to 1×10^6 purified CD34$^+$ adult HSC and weekly i.v. injections of Fc-IL7, human CD45$^+$ PBMNC were detected as early as early as 30 or 60 days (d) post reconstitution of newborn and adult mice, respectively. After 120d, T-, B-, and NK-cells, as well as monocytes and dendritic cells (DCs) were found in spleen (SPL) and bone marrow of both newborn and adult recipients by flow cytometry and immunhistological studies in e.g. SPL and skin. However, as reconstitution of newborns resulted in thymopoiesis of human CD3$^+$ cells and better lympho- and myelopoiesis as compared to adult mice, further studies were performed in HSC engrafted newborn recipients. Functional analyses of lymphocytes grown in humanized NOD/SCID/yc^{null} mice revealed that human CD3$^+$ T cells isolated from SPL and challenged ex vivo with i) murine NOD/SCID/yc^{null} derived DCs, ii) HLA-mismatched DCs or iii) HSC donor derived (autologous) DCs elicited alloresponses to human but not murine alloantigens(s). These results and further studies on ex vivo examined allo- and xenoimmunity of naïve HLA-matched and HLA-mismatched CD8$^+$ T cells transferred into HSC engrafted NOD/SCID/yc^{null} recipients will be presented to demonstrate T cell alloimmune responses in humanized mice and to show that our model can be used to evaluate residual GvH reactivity of CD8$^+$ donor T cells in HLA- matched or haploidentical settings. Finally, these studies should help to elucidate the impact of xenoreactivity on the induction of GvH immunity by human T cells in humanized mice.

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PROPHYLACTIC ADMINISTRATION OF EX VIVO CO-STIMULATED DONOR LYMPHOCYTE INFUSION (DLI) FROM RELATED AND UNRELATED DONORS AFTER REDUCED INTENSITY CONDITIONING FOR HIGH RISK HEMATOLOGIC MALIGNANCIES

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Enhancing the graft versus tumor (GvT) effect without graft versus host disease (GvHD) is critical to the success of reduced intensity (RIC) strategies for allogeneic transplantation. Despite establishing donor lymphohematopoiesis, relapse rates remain high. Standard DLI given at relapse or prophylactically to enhance donor chimerism has met with limited success in improving transplant outcomes. Co-stimulation of donor T-cells, using anti-CD3/CD28 coated beads to serve as artificial APCs, may reverse functional T cell tolerance, thereby restoring immune responsiveness and potentiate GVT. Unlike standard DLI, ex vivo co-stimulation may enhance T cell activity by removing and activating T cells outside of a tumor-induced immunosuppressive milieu. We report the preliminary results of a feasibility trial of ex vivo co-stimulated DLI from sibling and unrelated donors given 'prophylactically' at 4 and 6 months after RIC allogeneic transplantation for patients (pts) with high-risk hematologic malignancy.

Methods: 15 pts have undergone RIC with alemtuzumab, Fludarabine,Busulfan with peripheral blood stem cell transplantation (PBSCT) and planned activated DLI (pADLI) at 4 months (1×10^7 CD3+/kg) and 6 months (1×10^8 CD3+/kg) post-transplant in the absence of GvHD or relapse. All pts received tacrolimus and methotrexate as GvHD prophylaxis.

Results: 8 pts (AML, n = 6; ALL CR1 n = 2) received grafts from HLA-identical sibling donors, 7 (AML n = 5; MDS n = 1; ALL, n = 1) from matched unrelated donors. Donor hematopoiesis was established in all patients. Of the 11 infusions given to date, there has been no infusion-related toxicity, confirming the safety and feasibility of this strategy. 8 pts have received the first of 2 planned infusions of pADLI. Of the remaining 7 pts, 4 infusions are upcoming, 1 was precluded by early relapse, and 2 were precluded by early NRRM. Of the 8 pts who received their initial pADLI#1, 3 have received a second infusion. 5 pts did not receive pADLI#2 because of relapse (n = 3), GvHD (n = 1), and transient uveitis in the absence of GvHD (n = 1). Overall incidence of acute GvHD (aGvHD) has been low; 1 pt developed aGvHD prior to pADLI#1, and 1 pt developed aGvHD after pADLI #1. No patient has developed chronic GvHD.

Conclusion: Preliminary results of this trial demonstrates that RIC with PBSCT followed by ex vivo costimulated pADLI for poor prognosis hematologic malignancies is safe and feasible with potential for enhancing GvT without increasing GvHD.

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CLINICAL EXPANSION OF CORD BLOOD DERIVED T CELLS FOR USE AS DONOR LYMPHOCYTE INFUSION AFTER CORD BLOOD TRANSPLANTATION

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Background: When no HLA-identical donor is available, cord blood transplantation (CBT) is an attractive option due to the rapid availability of the graft and its biological properties. One of the disadvantages associated with CBT is lack of possibility for donor lymphocyte infusion (DLI) after CBT. Here we report expansion and characterization of CD3 positive lymphocytes from CB grafts in connection to CBT.

Material and Methods: Lymphocytes from 13 CB grafts were expanded with CD3/CD28 beads and 200 IU/ml rIL2. Expanded cells were cryopreserved in ready-to-use DLI doses. Immunophenotyping and cytokine production assay were performed on expanded