12 month median 2729 vs. 12062.5 unique TCR sequences, \( P = 0.22 \). Median lymphocyte counts did not increase between 3 and 12 months in the PD group as in the U+UD group, but the proportion of CD3⁺ cells within lymphocytes increased in both groups. PD T cell repertoire was less clonal than U+UD samples at 3 months (1-normalized entropy: 0.14 vs. 0.25, \( P = 0.09 \)) and 12 months (1-normalized entropy: 0.11 vs. 0.23, \( P = 0.05 \)), with more even frequency distribution of TCRs. PD T cell repertoire was more dynamic than the U+UD controls with a significantly decreased T cell clonal persistence between 3 and 12 months (TCR sequence overlap: 8.73% vs. 38.84%, \( P = 0.027 \)).

Conclusions: Ex vivo dmPGE₂ modulation with dmPGE₂ dominance resulted in delayed T cell and lymphocyte recovery, but less clonality despite a T cell repertoire restricted by low CD3⁺ counts. The reduced oligoclonality and rapid T cell turnover may indicate enhanced thymopoiesis. Correlation with TREC and clinical infectious outcomes is ongoing.

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**Monitoring CMV-Specific CD8⁺ T-Cell Responses After Allogeneic Stem Cell Transplantation: A New Way of Guiding Anti-Viral Therapy**

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CMV reactivation after allo-SCT is usually monitored by CMV-PCR. We present the results of a pilot study looking at the additional use of Multimers for monitoring CMV-specific CD8⁺ T-cells at different intervals after allo-SCT.

10 patients (4 males) that underwent allo-SCT from HLA-identical siblings (n = 4) or HLA-matched unrelated (n = 6) donors between May 2010 and May 2012 were enrolled. The diagnosis was acute leukemia (n = 6), lymphoproliferative disorders (n = 2) and myelodysplastic syndromes (n = 2). All patients and 8 donors were CMV seropositive. All patients were positive for the HLA-A*0201 allele. Pentamers (Proimmune, UK) and Streptamers (Gotttingen GmbH, Germany) were directed against the epitope NLVPMVATV of the pp65:495-503 protein of CMV in the context of HLA-A*0201. Samples were obtained from engraftment at 30-day intervals until day +180 and 2-monthly thereafter.

The median follow-up was 12.6 (4-27) months after SCT. Engraftment occurred in all patients with a median of 21.2 (15-27) days. Acute GVHD was diagnosed in 4 patients at a median of 63.3 (25-92) days. One patient developed acute GVHD following infusion of donor lymphocytes for the treatment of mixed chimerism on day +323.

Three patterns were observed. In 2 patients no CMV-specific CD8⁺ T-cells could be detected after SCT despite several episodes of CMV-PCR reactivation requiring prolonged antiviral treatment. In 5 patients CMV-PCR reactivation triggered a rapid increase of CMV-specific CD8⁺ T-cells (median 112.2 x 10⁶/L, range 1.3-279.7 x 10⁶/L). However, the CMV-PCR became immediately negative and antiviral treatment was stopped in all reactivations within 2 weeks. Subsequent CMV-PCR reactivations also lasted under 2 weeks in this group. Finally, 3 patients showed an early immune reconstitution with CMV-specific CD8⁺ T-cells detected (median 1.3 x 10⁶/L, range 0.3-2.3 x 10⁶/L) at a median of 21 (17-33) days post-SCT. No CMV-PCR reactivation was observed in this group.

We conclude that monitoring CMV-specific T-cell immunity after allo-SCT in combination with CMV-PCR may be able to distinguish patients at higher risk of CMV reactivation and in need of prolonged antiviral therapy. Patients with CMV-specific CD8⁺ T-cells detectable at the time of CMV-PCR reactivation may only need a short course of antiviral therapy, while those with early and persistent CMV-specific CD8⁺ T-cells may be at a very low risk of developing CMV disease in the long-term.

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**Elevated Gamma Delta T Cell Recovery Following Hematopoietic Stem Cell Transplantation Associated with Improved Long Term Overall Survival in Pediatric Patients with Acute Leukemia**

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Recent studies show that accelerated γδ T cell reconstitution after hematopoietic stem cell transplantation (HSCT) is associated with improved overall survival (OS) though the mechanisms have not been elucidated. We evaluated 102 consecutive pediatric patients with acute leukemia undergoing HSCT at St. Jude Children's Research Hospital from 1996-2011. The median age of the patients was 10.5 ± 3.9 yrs. (range 0.6-25.2) and median follow up was 2.7 ± 1.8 yrs. (range 0.2-6.0). There were 57% males, 43% females and 59% with ALL and 41% with AML. There were 14 patients with elevated γδ T cells (>1.75 x 10⁶ cells/ml) and 88 with low/normal γδ T cells (<1.75 x 10⁵ cells/ml). There were no significant differences between the two groups with respect to age, sex, disease or donor source, \( p = 0.7, 0.5, 1 \) and 0.07 respectively. Fours years after HSCT, Overall Survival (OS) was significantly higher for patients in the elevated group compared to the patients in the low/normal group, 93% and 60%, respectively, \( p = 0.0173 \). Survival without relapse or graft failure, Event Free Survival (EFS), was significantly higher in the elevated group compared to the low/normal group, 85.7% and 58.0%, respectively. Since T cell reconstitution following HSCT is age dependent, we determined if γδ T cell recovery correlated with age and/or CD3⁺ cells. Multivariate analysis showed no correlation between the number of CD3⁺ and γδ T cells. In fact, 13 of 14 patients that recovered with increased number of γδ T cells had normal or low numbers of CD3⁺ cells. Thus, γδ T cell recovery is not a simple correlate of T cell reconstitution. Because γδ T cells play a central role in maintaining intestinal epithelium integrity, we evaluated the incidence of gut GVHD. We found a significant lower rate of gut GVHD in the elevated group compared to the low/normal group, \( 0% \) and 17% respectively. Furthermore, the number of γδ T cells in patients with cGVHD (2.3 x 10⁵ cells/ml) was significantly lower compared to patients without cGVHD (6.2 x 10⁵ cells/ml), \( p = 0.01 \). This suggests that γδ T cell may protect against gut and cGVHD. Since accumulating evidence suggests that γδ T cells contribute to both innate and adaptive immune responses during infections, we evaluated the rate and types of infection between the two groups. We found a significant lower incidence of infections reported in the elevated group compared to the low/normal group, 21% and 54% respectively \( p = 0.02 \). Furthermore, the elevated group had only viral infections while the low/normal group had viral, bacterial and fungal infections. In summary, this is the first reported study of γδ T cell recovery after HSCT in pediatric patients and adds new insights into the role γδ T
cells by evaluating the relationship of the most common complications such as relapse, GVHD and infections.

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Vaccine Responses Following Unrelated Double Cord Blood Transplantation (CBT)
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Background: CBT recipients may respond to post-transplant vaccines differently than other transplant groups due to the lack of transfer of donor immunity.

Methods: We analyzed vaccine responses in 48 CBT recipients transplanted at our center from 2005-2010 for the treatment of hematologic malignancies with myeloablative or non-myeloablative conditioning. GHVD ppx included a calcineurin-inhibitor and MMF. All patients received double unit CB grafts and no pt received ATG. Vaccination criteria included CD4+ cell count > 200 cells/ul and IgG level > 500 mg/dl for at least 6 weeks following the last IVIG dose.

Results: Forty-eight of 69 (70%) eligible pts alive & disease-free @ 12 months post-HCT were vaccinated. Vaccinated patients engrafted with 6/6 (n = 2), 5/6 (n = 26), or 4/6 (n = 20) HLA-matched units. Twelve pts received rituximab (median 4 doses) as planned peri-transplant therapy for B-cell malignancies (n = 6), EBV viremia/lymphoma (n = 3), autoimmune hemolysis (n = 1), pure red cell aplasia (n = 1), or recurrent disease (n = 1). Prior to immunization, 13 patients had no acute or chronic GVHD whereas 35 had prior grade II-IV acute and/or chronic GVHD. Overall, the median time to vaccination was 16.9 months post-CBT; 18.9 months in pts who received rituximab; 15.23 months in those who did not (P = .056). Pre-vaccination titers obtained at a median of 1 yr demonstrated that > 85% of patients lacked protection against pneumococcus, H. flu, and pertussis and > 50% lacked immunity against tetanus, measles, & mumps. Seroconversion or > 3-fold rise in titer was observed in > 60% of patients in response to tetanus, diphtheria, H. flu, polio and pneumococcal (PCV7 or PCV13) vaccines. Following 3 doses of HBV vaccine, 52% seroconverted. Only 2/35 recipients of a single Tdap developed protective pertussis titers; 0/8 pts responded to a single protein-conjugated meningococcal vaccine. To date, 20 pts including 9 adults have received an MMR at a median of 2.3 years post-CBT. 8 pts received the live attenuated varicella vaccine. To date, seroconversion following measles, mumps, & rubella vaccine occurred in 56%, 41% & 93% of pts, respectively and 5 evaluable pts seroconverted after 1 (n = 3) or 2 doses of Varivax (n = 2). Survival in vaccinated patients is 100%. No serious reactions to any vaccine occurred.

Conclusion: CBT recipients, including adults & those with prior GVHD or rituximab therapy, are capable of responding to tetanus, diphtheria, H. flu, polio & PCV7 or PCV13 similar to other transplant groups. The sub-optimal response to pathogens associated with outbreaks in the community (Hepatitis B, Pertussis, meningococcus, measles, mumps, varicella) highlight the need to obtain pre- & post-vaccine titers to document response, and the need to define the optimal vaccination regimen in this population.

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Isolation, Expansion & Function of Cord Blood Natural Killer Cells
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The rate of immune reconstitution (IR) is directly correlated with the number of hematopoietic stem cells (HSC) infused and is particularly delayed in patients undergoing cord blood transplantation (CBT) secondary to the limited numbers of HSC. Thus, methods to increase the number of cord blood (CB) progenitors have the potential to accelerate IR after CBT. Natural killer (NK) cells play a crucial role in early IR after HCT because they are the first lymphocyte subset to recover after transplant. CB NK cells have been reported to have incomplete maturation and require activation for effective function. Here, we report a clinically relevant method for ex vivo expansion of NK cells isolated from CB without the use of a stromal layer. Our group has demonstrated that CB NK cells cultured in the presence of IL-2 and IL-15 results in a multi-log increase in the number of precursors that have a significant increase in cytotoxicity against several target cancer cell lines. After 21 days in culture, there is a 1.84 ± 0.341 log fold increase in the number of CD3-CD56+ NK cells (range, 0.420 to 3.108) (P < .001, N = 9). After culture, we also found a significant 2.612 ± 0.310 log fold increase (range, 0.979 to 3.622) (P < .001, N = 9) in the number of CD3-CD56+ NK cells. Evaluation of cytotoxicity against K562 cells, a chronic myelogenous leukemia, showed there was also a significant increase in cytolytic function at days 14 (31.52 ± 8.317% target cell lysis, P < .01, N=8) and 21 (44.22 ± 9.866% target cell lysis, P < .01, N=8) in culture when compared to day of isolation; similar results were seen using Jurkat cells, an acute T-cell lymphoblastic leukemia (T-ALL). Evaluation of NK cell resistant cell lines was also tested. While we found an increase in cytotoxicity toward the myeloid lymphoid leukemia cell line (MLL), MV411, after culture, the RS411 (MLL/ALL) and HDLM2 (Hodgkin’s lymphoma) cell lines remained resistant to cytolytic killing (N=3). Currently, we are investigating the NK cell population(s) responsible for the cytotoxic killing and the corresponding killer cell Ig-like receptor (KIR) ligand/adhesion molecule(s) that may be responsible for function. We hypothesize that using methods to increase the number of CB NK cells have the potential to prevent early relapse, infections and graft versus host disease, as well as facilitate engraftment when administered following CBT.

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In Vivo Generation of Thymus-Independent T Cells in a Tissue-Engineered T Cell Development Supporting Microenvironment
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Tissue engineering approaches based on implantation of biomimetic three-dimensional (3D) tissue constructs have been used for more than a decade for in vivo regeneration of