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QUANTIFYING ANTI-CYTOMEGALOVIRUS (CMV) IMMUNITY WITH CMV ANTIGEN SPECIFIC T CELLS (CASTS) AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT (ALLOHCT)

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Reconstitution of CASTs after alloHCT has previously been quantified using CMV tetramers. We developed an assay for quantifying CASTs using CMV specific MHC multimers (Dextramers, Immudex, Denmark). CMV Dextramers consist of dextran polymers conjugated to optimized numbers of MHC-CMV peptide and fluorochrome molecules allowing higher avidity binding to CMV specific T cells. Dextramers were restricted to HLA-A01, -A02, -A03, -A24 -B07, -B08, and -B35, and were informative in 93% of our predominantly White (94%) population. Patient blood samples were prospectively collected pre-alloHCT and day +30, 100, and 365, and analyzed by flow cytometry for CASTs with CMV Dextramers. More than 300 CMV Dextramer measurements from 89 consecutive alloHCT patients treated from 2005 - 2011 with > 1 post-alloHCT CAST measurement were analyzed. CMV reactivation was defined as the presence of CMV antigen on > 2/50,000 cells, > 1/100,000 cells twice consecutively, or > 1/100,000 cells followed by preemptive CMV therapy. Absence of CMV reactivation was defined as 0 cells or 1/100,000 cells positive for CMV no more than once with no anti-CMV treatment. 30/89 (34%) patients reactivated CMV a median of 40 days (range 23-341) post-alloHCT. None of 37 recipients with undetectable CASTs before allo-HCT reactivated CMV by day +30 vs. 10/52 (19%) of recipients with detectable CASTs ($p = 0.005$), recapitulating CMV reactivation patterns seen in recipients with positive CMV serologies. We correlated the number of CASTs with recipient and donor CMV serology as a surrogate for anti-CMV immunity. In samples with CD3+ T cells ≥ 400/μL, CASTs correlated with recipient CMV serology with 67% sensitivity and 90% specificity (see Table). Sensitivity may have been lower than expected due to decreased anti-CMV IgG production secondary to chemotherapy and/or increased granularity of flow cytometry measurements for CASTs. In another analysis, CASTs at day +30 corresponded to positive CMV donor, but not recipient, serology, and not with CMV reactivation up to day +30, suggesting that the adoptive transfer of CASTs occurs with alloHCT. Preliminary analyses confirm prior reports of decreased relapse of underlying cancers after CMV reactivation. Our study suggests that Dextramers may be used to monitor reconstitution of CMV immunity post-alloHCT, that they may be a more sensitive test than CMV serology, and that adoptive transfer of anti-CMV immunity can be quantified.

Table 1. Correlation of CASTs and CMV Serology

	Pre-alloHCT Recipient CMV Serology		
Pre-alloHCT CASTs	Negative (N = 32)	Positive (N = 31)	N (row)
Undetectable	18 (56%)	2 (6%)	20
Detectable	14 (44%)	29 (94%)	43

*All samples with ≥400 CD3+ T cells

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SAFETY AND PERSISTENCE OF INFUSED CD19-CAR-MODIFIED MULTIVIRUS SPECIFIC CTLS IN B CELL MALIGNANCIES POST ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Allogeneic hematopoietic stem cell transplant (HSCT) may increase disease-free survival in patients with high-risk B-cell malignancies, but delayed immune reconstitution is associated with viral infections and disease relapse. We reasoned that a single T-cell platform mediating antiviral and antileukemic activity may benefit these patients. We prepared CTLs with native specificities directed towards EBV/CMV/adv, then engineered them to express chimeric antigen receptors (CAR) targeting CD19. We used donor-derived antigen presenting cells expressing adenovirus antigens & transgenic CMVpp65. After 3 stimulations, multivirus-specific CTL were transduced with a retroviral vector encoding CAR-CD19.

Safety: 4 patients (2 relapsed ALL, 2 B-CLL) were infused with 1.5-$3x10^7$ cells/m^2 without infusion related toxicity. Patient 2 developed fever, diarrhea and hypotension 4 weeks post T cells; findings were consistent with ileitis at a previous site of disease. Gut biopsy showed abnormal absence of normal & malignant B cells, but significant levels of CAR-CD19 T cells by qPCR.

Persistence: There was a predictable decline of T cells in peripheral blood following infusion (undetectable in 1-4 weeks). However, persistence up to 9 weeks after T cell administration is documented by their presence in disease sites like the gastrointestinal tract (patient 2, 4 weeks post CTL) and the bone marrow (patient 1 and 3, 9 & 4 weeks post CTL respectively).

Anti-tumor activity: Patient 1 (Ph+ ALL) had 4% blasts in the peripheral blood at time of CTL infusion #1 which cleared within 2 weeks. She received a 2nd infusion 2 months later. She became bcr-abl-negative but subsequently relapsed and died of progressive disease 7 months post CTL. Patient 2 with CLL had resolution of lymphadenopathy within 2 weeks but, following disappearance of CTLs from peripheral blood, showed evidence of disease progression 2 months post CTL and died weeks after. Patient 3 with CLL has had stable disease. Patient 4 (relapsed ALL) responded to the first CTL infusion with a decrease of blasts (0.5% to 0.3%) 4 days post CTL. He presented 2 weeks later with 4% blasts, requiring a 2nd dose of CTLs which decreased disease to 0.2% 2 days post-infusion.

Anti-viral activity: No patient developed viral infections post CTL.

These early results provide evidence of the safety, persistence, and effectiveness of monoculture CD19CAR trivirus T cells in the treatment of high risk B-cell malignancies post HSCT.

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LONGITUDINAL MONITORING OF IMMUNE RECONSTITUTION AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT): IMPACT OF T-CELL DEPLETION OF THE GRAFT

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Background: T cell depletion by positive selection of CD34+ cells of the graft is an effective procedure to reduce the incidence of graft-versus-host disease following allogeneic PBSCT. However, it has been suggested that it might impact immune reconstitution.

Patients and Methods: We retrospectively compared the kinetics of recovery of T, B and NK- cells compartments in 108 adult patients who had undergone allogeneic PBSCT after high dose conditioning. Patients had received either CD34-selected (CD34 group, n = 62) or unmanipulated PBSCT (PBSC group, n = 46). In the CD34 group, 43 of 62 patients had received pre-emptive CD8-depleted donor lymphocyte infusions (DLI) starting at days 40-60 after PBSCT in an effort to prevent disease relapse. Circulating cell phenotypes were studied by flow cytometry on days 28, 40, 60, 80, 100, 180 and 365 after PBSCT, and then yearly thereafter. Median follow-up was 6 months for both groups (ranging from 1 to 60 months). We also monitored T-cell repertoire reconstitution on days 100 and 365 post-PBSCT using third complementary region (CDR3) size spectratyping method (in 9 and 13 patients on days 100 and 2 and 7 patients on days 365, for CD34 and PBSC groups respectively).

Results: Normal levels of NK (CD3- CD56+) cells and CD8+ (CD3+ CD8+) T cells were reached one month after PBSCT and were similar in CD34 and PBSC patients. B cell counts attained lower limit of normal values six months after PBSCT and were similar in the CD34 and PBSC groups. CD4+ T-cell reconstitution was slow in the two groups of patients, with normal values reached only after 18 to 24 months post-PBSCT. The number of naive CD4+ T cells (CD4+ CD45RA+) was particularly low throughout the first

24 months after transplantation and was lower during the first 120 days in the CD34 group than in the PBSC group ($p<0.1$, with $p<0.01$ on day 40). This difference was more significant after excluding from the CD34 group patients who had received pre-emptive DLI ($p<0.05$, with $p<0.01$ on days 40 and 60). T-cell repertoire diversity was similar in both groups on days 100 and 365 post-PBSCT (with a median total number for the 24 BV families of 218 bands in both groups on day 100 post-PBSCT and 223.5 and 236 bands for the CD34 and PBSC groups respectively on day 365 ($p = 0.67$)).
Conclusion: This study suggests that the T-cell depletion of the graft induced a delayed immune reconstitution for naive CD4+ T cells after allogeneic PBSCT with high dose conditioning.

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KINETICS OF IL-7 AND IL-15 LEVELS AFTER ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (ALLO-PBSCT) FOLLOWING HIGH-DOSE OR NONMYELOABLATIVE CONDITIONING

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Background: Successful allo-PBSCT depends on the recovery of an adequate immune system, allowing not only to prevent lethal infections but also to induce immune-mediated graft-versus-tumor effects. Previous studies have demonstrated that immune recovery depends mainly on homeostatic peripheral expansion (HPE) of donor T cells contained in the graft the first 3-6 mo. following allo-PBSCT. IL-7 and IL-15 are the main driving forces of HPE, and are secreted in response to lymphodepletion. While high-dose conditioning regimens induce generally a profound lymphodepletion, progressive replacement of host-derived by donor-derived T cells is the rule after nonmyeloablative conditioning.
Patients and Methods: Data from 106 pts given allo-PBSCT after high-dose ($n = 36$) or low-dose TBI based nonmyeloablative ($n = 70$) conditioning were analyzed. Among patients given high-dose conditioning, 26 patients were given unmanipulated PBSC with ($n = 24$) or without ($n = 2$) added ATG and 10 patients received CD34-selected PBSC. No patient in the nonmyeloablative group received ATG or CD-34 selected PBSC. IL-7 plasma and IL-15 serum levels were measured with a commercial ELISA (R&D Systems, Minneapolis, MN, USA). Lymphocyte subset counts were analyzed by flow cytometry.
Results: CD4+ T cell and naive CD4+ T cell counts were lower ($P<0.001$) on days 28, 40, 60, 80, 100 and 180 in pts given grafts after high-dose than in those given nonmyeloablative conditioning. IL-7 levels were higher in pts given high-dose than in those given nonmyeloablative conditioning on days 7, 14, 28, 40, 60, 80 and 100 after transplantation ($p<0.01$). Day 28 IL-7 levels were highly correlated ($r<-0.52$, $P<0.0001$) with day 28 counts of T cells, CD4+ T cells, CD8+ T cells, and naive CD4+ T cells but not with day 28 counts of NK cells. IL-15 levels were higher in patients given high-dose than in those given non-myeloablative conditioning on days 7 ($p<0.01$), 14 ($p<0.01$), and 28 ($p = 0.055$) after transplantation. There was a correlation between IL-15 levels and day 28 counts of T cells ($r = -0.30$, $P = 0.002$) and NK cells ($r = -0.38$, $P = 0.001$).
Conclusions: Our data suggest that the physiology of immune recovery and particularly of HPE is significantly different in pts given high-dose than in those given nonmyeloablative conditioning and encourage further studies comparing immune recovery and immune recovery mechanisms in pts given high-dose or nonmyeloablative conditioning.

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EVIDENCE FOR QUANTITATIVE AND FUNCTIONAL IMMUNE DEVIATION IN PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE

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While HSCT is a curative therapy for sickle cell disease (SCD), SCD patients are at increased risk of graft rejection, especially after non-myeloablative transplant. To determine whether immune mechanisms might influence their increased risk of rejection, we evaluated quantitative and functional immunologic differences between 23 pediatric SCD patients and 18 age- and ethnicity-matched controls, using a prospective cross-sectional study design. All patients and controls were African American and were matched for gender and age. All patients were at their clinical baseline during evaluation: no patient was experiencing an acute complication of SCD. Following informed consent, each subject underwent quantitative flow cytometric immune analysis including enumeration of total WBC, ANC, ALC, total T cells, B cells and NK cells as well as CD4+ and CD8+ naive and memory T cell subpopulations. Functional immune assessment was also performed, using multiplexed analysis of 25 serum cytokines.

Our results provide evidence for significant quantitative and functional immune deviation in SCD patients compared to controls. In addition to the well-documented increases in total WBC and ANC (1.9-fold and 1.7-fold compared to controls, respectively, $p<0.05$), SCD patients also demonstrated significantly higher total lymphocytes, monocytes and both cytotoxic and cytokine-secreting NK cells (2.9, 2.4, and 2.2-fold compared to controls, $p<0.01$ for all). Circulating B cells were significantly higher in SCD patients (3.3 fold, $p = 0.0005$). Circulating T cells were also increased in SCD patients, and this effect was specific to CD4+ T cells (1.7-fold, $p = 0.002$). There was no difference in CD8+ T cells ($p = 0.4$). The increased CD4+ T cell count predominantly involved memory CD4+ T subpopulations, with both central and effector memory CD4+ T cells significantly increased compared to controls (1.9-fold and 2.5-fold, respectively, $p< 0.01$). SCD patients also showed evidence of functional immune activation at steady state; plasma cytokine analysis revealed higher circulating CXCL10, CCL4 and IL-15 (2.4, 2.1, and >10 fold compared to controls, $p<0.05$ for all).

Our results provide strong cellular and molecular evidence for quantitative and functional immune deviation in SCD patients. These observations suggest that strategies targeting NK cell, B cell, and CD4 T cell function may be required for successful engraftment of SCD patients during non-myeloablative HSCT.

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SAFETY OF LIPOSOMAL CYTARABINE PROPHYLAXIS IN PEDIATRIC BONE MARROW TRANSPLANT (BMT) RECIPIENTS WITH ACUTE LEUKEMIA (AL) AND NON HODGKIN LYMPHOMA (NHL) AT HIGH RISK OF CNS RELAPSE

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Liposomal Cytarabine (LC) is a derivative of cytarabine designed for intrathecal treatment of leukemia/lymphomatous meningitis (LLM). LC allows for sustained release with prolonged drug exposure of 8 to 14 days in children and adults, respectively. Adult trials have shown 50mg of LC to be both safe and effective in the treatment of LLM (Glantz et al, JCO 1999). Bomgaars et al. investigated the use of LC in children in a phase I dose escalation trial and demonstrated the maximal tolerated dose to be 35mg and that it was safe, well tolerated and effective (Bomgaars et al., JCO 2004). However, there remains a paucity of data on the use of LC prophylaxis to prevent LLM in pediatric allogeneic stem cell (AlloSCT) recipients.
Objective: We report on the safety and tolerability of liposomal cytarabine prior to and following stem cell transplant in pediatric BMT recipients with AL and NHL.
Methods: Pediatric stem cell recipients with a history of AL or NHL and high risk of CNS disease received 35mg of LC (<21yr) or 50mg (>21yr) via lumbar puncture or omaya reservoir pre conditioning and every 3months for 1-2yrs post stem cell transplant. Patients received dexamethasone (0.15mg/kg/dose BID) concomitantly x 5days. There were 8 matched unrelated donor transplants, 9 matched related donor transplants and 3 autologous transplants. Twelve patients received a TBI (1200 cGy) containing conditioning regimen. Seven patients received an additional CNS boost of up to 1200 cGy.
Results: To date there have been 21 patients: (12 ALL, 2 AML, 3 Burkitt, 3 DLBCL and 1 T-LL). At the time of transplant, 7 patients were CR1/PR1, 10 patients CR2 and 4 patients CR3. Patients have received a total of 46 doses (median = 1, range 1-12). Median age: 12yr (range 6-22). Mean follow up: 517 days (median = 294days). Eight of 21 patients had a history of CNS AL/NHL involvement