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 500 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 alloHCT 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

<table>
<thead>
<tr>
<th>Pre-alloHCT Recipient CMV Serology</th>
<th>Pre-alloHCT CASTs</th>
<th>Negative (N = 32)</th>
<th>Positive (N = 31)</th>
<th>N (row)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undetectable</td>
<td>18 (56%)</td>
<td>2 (6%)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Detectable</td>
<td>14 (44%)</td>
<td>29 (94%)</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

*aAll samples with ≥400 CD3+ T cells

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 1.5-3x10^6 cells/m² without infusion related toxicity. Patient 2 developed fever, diarrhea and hypotension 4 weeks post T cell; 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.

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 allogeneic patients who had undergone allogeneic PBSCT after high dose conditioning. Patients had received either CD34-selected (CD34 group, n = 62) or unmanipulated PBSCT (PBSCT 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 60, 365, for CD34 and PBSCT groups respectively).

Results: Normal levels of NK (CD3- CD56+) cells and CD8+ (CD3+ CD8+) T cells were reached one month 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 60, 365, for CD34 and PBSCT groups respectively).

CD34- and PBSCT 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 60, 365, for CD34 and PBSCT 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 PBSCT patients. B cell counts attained 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 PBSCT patients. B cell counts attained 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 PBSCT patients. B cell counts attained 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 PBSCT patients. B cell counts attained 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 PBSCT patients. B cell counts attained