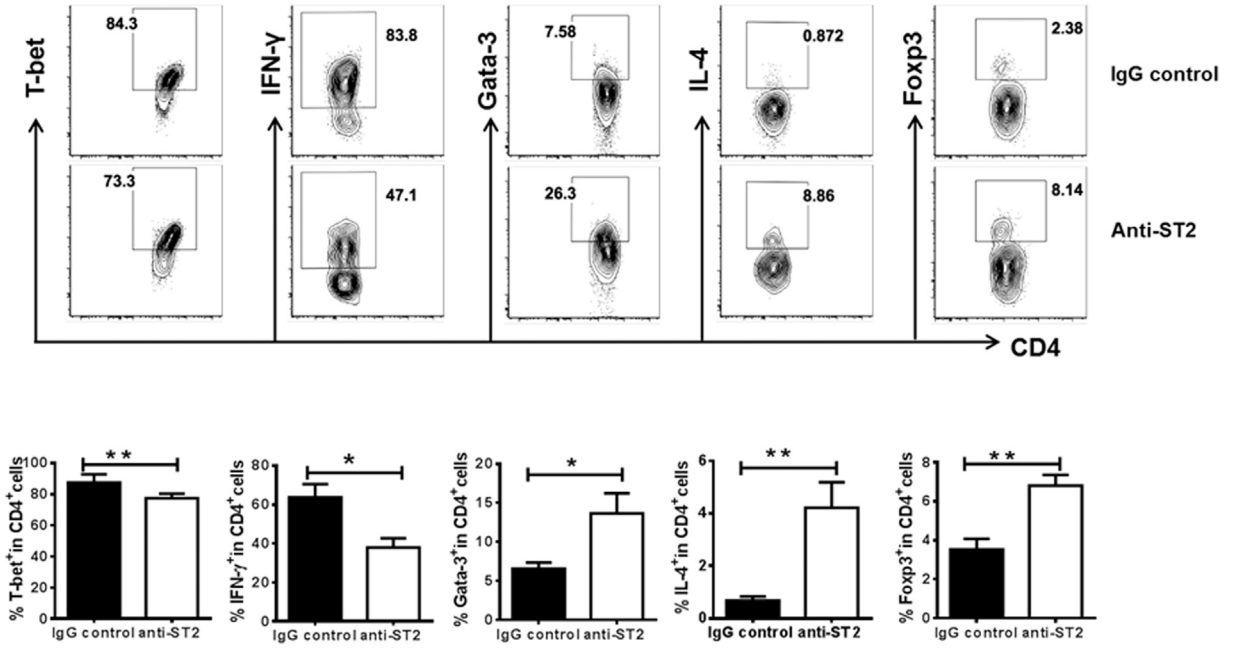

Figure 2. Blockade of ST2 shifts the Th1/Th2 balance toward Th2 phenotype and increases CD4+ regulatory T cells. Spleen T cells were collected for intracellular staining at day l0 post-transplantation. $^{*}p<0.05$, $^{**}p<0.01$.

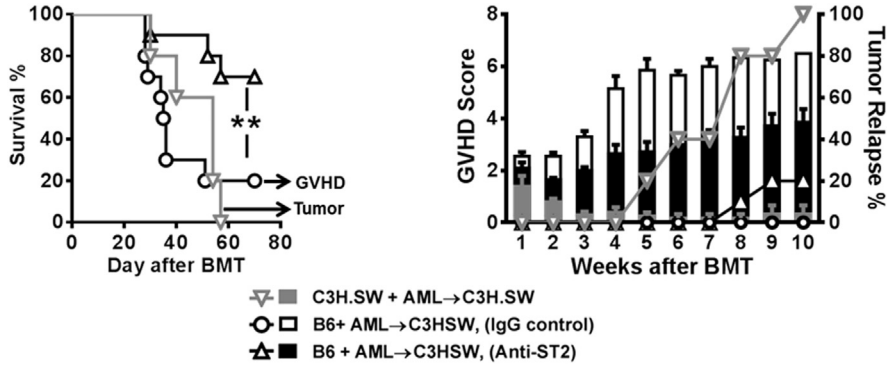

Figure 3. Anti-ST2 mAb treatment preserves substantial GVL activity. (A) Survival cure. (B) GVHD score (bars) and percent of tumor relapse (lines). $^{**}p<0.01$, IgG vs Anti-ST2

balance by flow cytometry. Administration of anti-ST2 shifted Th1/Th2 balance toward a Th2 phenotype (Fig. 2). In addition, ST2 blockade also induced expansion of regulatory T cells (Tregs) (Fig. 2).

Given that anti-ST2 administration also up-regulated expression of genes, such as Granzyme A, that can mediate graft vs. leukemia (GVL) responses (Fig. 1E), we postulated that ST2 blockade would not affect therapeutic GVL activity. To confirm this, we developed a clinically relevant model of leukemia, in which C3H.SW recipients were challenged with syngeneic GFP+ MLL-AF9 induced acute myeloid leukemia cells. Our results indicated that administration of anti-ST2 preserved substantial GVL activity and resulted in significantly improved leukemia-free survival (Fig. 3), suggesting that anti-ST2 ameliorated GVHD and maintained GVL response.

In summary, we found that prophylaxis with anti-ST2 antibody could alleviate GVHD severity and mortality while preserving GVL effect. ST2 blockade increased the level of plasma IL-33, skewed the Th1/Th2 balance toward a Th2 phenotype, and induced Foxp3+Tregs (with preserved expression of membrane ST2 [not shown]). Our findings suggest that ST2 is a novel therapeutic target to ameliorate GVHD.

GRAFT PROCESSING

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Ex Vivo Expanded Multi-Specific Cytotoxic T Lymphocytes Derived from HIV+ Patients and HIV Negative Donors Using GMP Compliant Methodologies Recognize Multiple HIV Antigens and Suppress HIV Replication

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The therapeutic use of T cells has long been studied to boost HIV-specific T-cell immunity in HIV+ individuals. However, clinical efficacy has been relatively modest. Infusion of single-epitope specific CD8 T cells showed safety but could not provide lasting viral control, most likely due to their restricted specificity. Recently, the development of HIV entry resistant cells by other groups (e.g. CCR5 deletion) has allowed T cell therapy to be even more feasible for HIV. We hypothesized that broadly HIV-specific T cells could be expanded from patients on antiretrovirals (ARVs) as well as HIV negative individuals to effectively target HIV infection using a non-HLA restricted approach for patients receiving an autologous or allogeneic HSCT for HIV-associated hematologic diseases. PBMCs from healthy donors or HIV+ patients were stimulated with gag, pol, and nef peptide libraries (pepmixes) in the presence of co-stimulatory and growth factors. T cells expanded to clinically relevant numbers (Mean=1.62e8 cells, Range=3.72e7-2.87e8 cells, n=7) even in the presence of ARVs. 5 of 7 patient sample lines showed specific activity to all 3 HIV antigens in IFNY ELISPOT assays. The T cells were broadly specific to gag (99.33 SFC/10e5 cells), pol (131.11 SFC) and nef (337.26 SFC). HIV-specific T cells were also expanded from 9 healthy (HIV negative) donors. Expanded T cells released IFNγ in response to gag (163.79 SFC, n=9) and nef (291.25 SFC, n=6) but not an irrelevant antigen (7.0 SFC). Importantly, T cells expanded from both HIV+ and HIVneg were cytotoxic, as expanded T cells lysed HIV antigen loaded autologous PHA blasts (mean=67.55% specific lysis at 10:1 effector:target ratio) but not PHA blasts alone (0.46% specific lysis at 10:1 effector target ratio). Expanded T cells from HIV+ patients also showed a greater ability to suppress HIV outgrowth in vitro compared to unexpanded CD8+ T cells when co-cultured with autologous, reactivated resting CD4+ T cells, the authentic latent reservoirs. In 5 patients, a lower recovery of virus from resting CD4+ cells was seen in the presence of CTLs as compared to no effectors ($p<0.006$ by Mann Whitney), while the non-specific CD8+ T-cells showed only a modest trend towards decreased recovery ($p>0.9$). Similarly, HIV-specific T cells derived from HIVneg donors were able to suppress HIV replication more than non-specific CD8+ T-cells when co-cultured with autologous CD4+ T cells infected with HIV_{SF162} (HIV only p24=681.95 pg/mL, nonspecific CD8+ T-cells=448.80 pg/mL, expanded CTL=145.82 pg/mL). In summary, we have developed robust GMP-compliant methodology for expanding functional HIV-specific T cells from both HIV+ and HIVneg donors for use after autologous and allogeneic HSCT, respectively. We now plan to translate our approach to the clinical setting where we will test HIV-polyspecific T cell products as a part of a strategy to fully eradicate HIV infection after HSCT.

HISTOCOMPATIBILITY/ALTERNATIVE STEM CELL SOURCES

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Impact of High-Resolution Typing for HLA-A, -B, -C and -DRB1 on Single-Unit Cord Blood Transplantation in Pediatric Patients

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Background: Current practice in choosing potential donors for umbilical cord blood transplantation (UCBT) involves matching at the antigen level for HLA-A and HLA-B by low-resolution (LR) typing and at the allele level for HLA-DRB1 by high-resolution (HR) typing.
Objectives: To determine the significance of HR HLA matching on UCBT outcome in 62 eligible pediatric patients <=21 years-of-age. We further explored the presence of anti-HLA antibodies (AHA) in our patient population.
Design/Method: Through IRB approved retrospective chart review, we identified 62 UCBT recipients (>=2.0X10^7TNC/kg) for malignant (53%) and non-malignant (47%) disorders. We compared original HLA typing (LR HLA-A-B; HR HLA-DRB1) to HR HLA typing (HR HLA-A-B-C-DRB1). We determined incidence of engraftment, relapse, acute and chronic graft-versus-host disease (GVHD), early transplant-related infections and mortality (from any cause and non-relapse/non-disease progression mortality) and analyzed each of these variables against subgroups of HR and original HLA matching using the Fisher exact test. Lastly, we determined the presence of pre-UCBT AHA.
Results: Retrospective determination of HR HLA matching showed 16% of pairs were matched at all HR loci; 6% were mismatched at 1, 14% at 2, 29% at 3, 27% at 4, 3% at 5, and 2% at both 6 and 7 alleles. Ten of 13 (77%) transplants originally matched at LR HLA-A-B and HR HLA-DRB1 remained matched at the allelic level (HR HLA-A-B-C-DRB1).

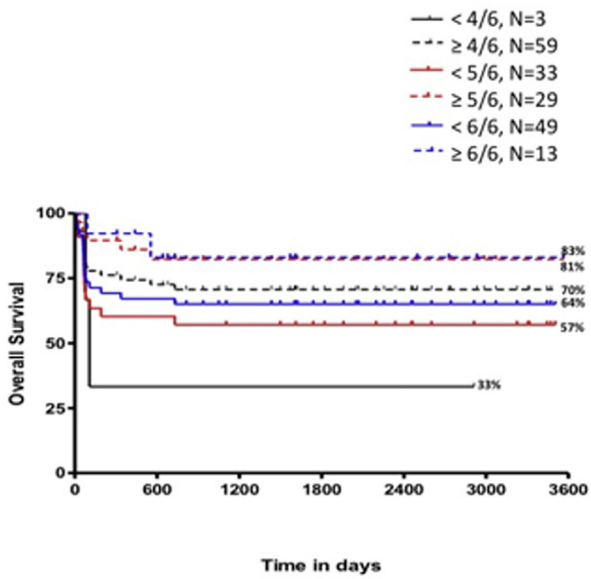

Figure 1. Survival by Original HLA Match.