Ex Vivo Programming of Donor Cells Prior to Allo-HCT Reduces GvHD without Compromising GvL

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ABSTRACT

While allogeneic hematopoietic cell transplantation (allo-HCT) is a potentially curative option for many hematologic malignancies, complications such as graft-versus-host disease (GvHD) result in significant morbidity and mortality. Conventional approaches to manage GvHD, such as prophylaxis with immunosuppressive agents or T-cell depletion strategies, are limited by increases in graft failure, viral-associated lymphoproliferative disorders, and disease relapse. Here we present a novel strategy to reduce the rates of GvHD by programming mobilized peripheral blood ex vivo with a cocktail of small molecules prior to allo-HCT.

An established xenogeneic mouse model was used to examine the potential of this cell programming strategy to reduce rates of GvHD. Sub-lethally irradiated NOD-scid IL2Rγc−/− (NSG) mice were transplanted with human peripheral blood mononuclear cells (PBMC) pulsed treated ex vivo with either vehicle or a cocktail of two small molecules (FT1050 + FT4145). Recipients of pharmacologically programmed PBMCs had significantly lower GvHD scores, decreased levels of circulating IFN-γ and enhanced survival relative to recipients of vehicle PBMCs (p < 0.0001, Mantel-Cox log rank).

In addition to xenograft-GvHD studies, we explored the impact of this cell programming strategy in a murine model of GvL. Lethally irradiated BALB/c recipient mice were transplanted with either control or FT1050 + FT4145 programmed C57Bl/6 (H-2Kd) CD34+ T cells and T-cell-depleted bone marrow. Prior to allo-HCT, recipients were injected with 2x107 luciferase-expressing A20 lymphoma cells (A20-luc). Bioluminescence imaging was used to monitor the tumor burden over a 28 day period post-HCT. Donor cells programmed with this small molecule cocktail significantly improved survival (p < 0.001) while retaining GvL effects against the A20 lymphoma cells. Combined, these studies demonstrate that pharmacologic programming of hematopoietic cells with FT1050 + FT4145 prior to allo-HCT may offer an innovative therapeutic approach to reduce rates of GvHD without compromising GvL activity.

RESULTS

Programmed mPB CD34+ Cells

Figure 1. Ex Vivo Programming of Human mPB CD34+ Upreregulates CXCR4 and Enhances Long-Term Engraftment in NSG Mice. A. Human CD34+ treated with two modulators exhibited significant upregulation of CXCR4 mRNA (40-fold increase, p < 0.0001, paired t-test). B. Increased CXCR4 surface protein levels (*N=3 mice/group; n=3 donors). C. Following ex vivo programming, mPB CD34+ cells (n=4 donors) demonstrated enhanced migration towards an SDF-1α gradient in a standard transwell assay. D. Sub-lethally irradiated (95 Gy) NSG mice were transplanted with 300,000 human PB CD34+ cells (control) or programmed with 10μM FT1050 + 10μM FT4145 (n=5 mice/group; 2 donors). E. The chimerism level of human cells in the peripheral blood was determined using flow cytometry at 8 weeks post-transplant. F. Multi-lineage engraftment (Myeloid, B, T-cells) was assessed in bone marrow upon termination (n=120 days post-transplant by flow cytometry).

Figure 2. Ex vivo programmed CD4+ and CD8+ T cells demonstrate reduced alloimmune responses and demonstrated a less inflammatory state following Type 1,2 differentiation. GCSF-mobilized peripheral blood units were obtained via leukapheresis from a total of 7 donors over a 3 month period. mPB MNC from these collections were programmed with either vehicle or FT1050 + FT4145. After the 4 hour programming, the cells were washed to remove the modulators and then assayed to assess T cell function over a 5 day period. A. Mixed lymphocyte reaction (MLR) to monitor allogeneic responsiveness of CD4+ and CD8+ T cells. Both cell populations were enumerated at the end of a five day co-culture with T cell depleted mismatched PBMC, costimulatory proteins (CD28, ICOS) were monitored by flow cytometry and reported as mean fluorescence intensity (MFI) compared to vehicle (CD4+ T cells). B. T cells were also assessed in T cell differentiation assays. After a 5 day incubation, cytokine release was monitored by intracellular cytokine staining using flow cytometry. Interferon-γ, Interleukin-4, Interleukin-10 B.

Figure 3. Xenogeneic acute GvHD model

Figure 4. Ex Vivo Programming of donor cells attenuates acute GvHD in a murine xenogeneic model. A. Murine acute GvHD model. Lethally irradiated (BG/Se) BALB/c recipients received ex vivo programmed (vehicle or FT1050 and FT4145) B6 donor cells (T-cell depleted BM, SP) B. Survival. C. GvHD score; D. Serum levels of IFN-γ in recipient mice was measured on day 7 by cytokometric bead array; E. Frequency of Th1 in peripheral blood day 7; F. spleen day 14. T regulatory cells were identified by flow cytometry as CD4+CD25hiCD127loGFp+ (p < 0.05). Results are representative of eight independent studies (n=10 recipients/group).

CONCLUSIONS

- Ex vivo programmed (FT1050 and FT4145) human CD34+ cells:
  - Enhanced CXCR4 expression and transwll migration
  - Enhanced homing and engraftment of CD34+ cells

- Ex vivo programmed (FT1050 and FT4145) T cells:
  - Reduced alloimmune responses (MLR)
  - Reduced surface expression of ICOS and 4-1BB (activating)
  - Reduced Th1 polarization and IFN-γ production
  - Increased Th2 polarization and IL-4 production
  - Increased IL-10 production

- Xenogeneic acute GvHD model demonstrated that ex vivo programming with FT1050 and FT4145 leads to significantly lower GvHD scores and enhanced survival

- Xenogene GvL studies demonstrated that ex vivo programmed T cells retain GvL response
- Ex vivo programmed human NK cells retain killing activity (not shown)
- Preclinical findings demonstrate the potential for using ex vivo modulation with FT1050 and FT4145 to enhance CD34+ engraftment and dampen alloreactive T cell responses while retaining the desirable anti-leukemic response.