Ex Vivo Modulation of Donor Cells Results in Enhanced Survival and Reduced GvHD Mortality
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ABSTRACT
Allogeneic hematopoietic stem cell transplant (HSCT) represents a potential curative treatment for a number of life threatening blood malignancies. Despite improvements, the utility of this treatment regimen is limited by a number of side-effects including graft versus host disease. Acute graft versus host disease (GvHD) is a serious complication of allogeneic HSCT and occurs in approximately half of all transplant patients. Treatment for patients with acute GvHD has remained unchanged for several decades and consists of high doses of steroids which are only effective approximately 35 percent of the time and also have a number of side effects. Therefore, identification of therapeutic approaches for reducing GvHD represents a large unmet medical need. Here we present a fundamentally novel strategy for potentially reducing GvHD by ex vivo programming donor mobilized peripheral blood cells with small molecules prior to HSCT.

To this end, we applied our screening platform to identify a combination of small molecule modulators (FT1050, FT4145) that promote the activation of genes implicated in cell cycle, immune tolerance and anti-viral properties of T-cells, as well as in the survival, proliferation and engraftment potential of CD34+ cells. Genome-wide expression analysis of the T-cell compartment of mobilized peripheral blood following treatment with FT1050+FT4145 revealed the induction of genes involved in cell cycle (e.g., CCND1, CCNE1), immune tolerance (e.g., ALDH4, AREG) and anti-viral properties (e.g., EFNBB2). A number of T-cell assays to assess T-cell phenotype and function were conducted on mononuclear cells cultures after donor mobilized peripheral blood units after treatment with FT1050 and FT4145 either alone or in combination. Overall, ex vivo programming of mobilized peripheral blood resulted in reduced allogeneic T-cell responses and was accompanied by reduced capacity of modulated T-cells to produce interferon-gamma (IFN-γ). Concomitantly, there was enhancement in the ability to make interleukin 4 (IL-4), as well as the survival, proliferation and engraftment potential of CD34+ cells.

RESULTS

Programmed mPB CD34+ Cells

A. CXCR4 mRNA 
B. CXCR4 Surface Expression
C. Directional Migration

Programmed mPB CD3+ T Cells

A. CD4+ T-cell proliferation in MLR
B. CD8+ T-cell proliferation in MLR

EX Vivo Programming of Human CD34+ Cells Upregulates CXCR4 and Enhances Long-Term Engraftment in NSG Mice. A. Human CD34+ treated with two modulators exhibited significant upregulation of CXCR4 mRNA (*4-fold increase, p=0.001; paired t-test). B. Increased CXCR4 surface protein levels (*4-fold increase, p=0.005; paired t-test) measured by flow cytometry. 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 (5Gy) NSG mice were transplanted with 200,000 human mPB CD34+ cells, unmodulated or modulated with 10µm FT1050 + 10µm FT4145 on untreated cells (n = 5 mice/group per donor; 3 donors). The chimerism level of human cells in the peripheral blood was determined using flow cytometry at 8 weeks post-transplant.

Figure 1. Ex Vivo Programming of Human CD34+ Cells Upregulates CXCR4 and Enhances Long-Term Engraftment in NSG Mice.

EX Vivo Programming of Donor Cells attenuates acute GvHD in a murine allogeneic model. A. Murine acute GvHD model: lethally irradiated (5Gy) Balb/c recipients received ex vivo modulated (vehicle or FT1050 and FT4145) B6 donor cells (T-cell depleted BM). B. Survival. C. Serum levels of IFN-gamma in recipient mice was measured on day 7 by cytokometric bead array; D. GVHD score at day 12. Results are representative of seven independent studies (n=10 recipients/group). E. GVHD score using B6-FOXP3(+) donors. F. Peripheral blood day 7. B. Splenocytes day 34. Frequency of Tregs was monitored by flow cytometry. T regulatory cells were identified by flow cytometry as CD4+ CD25hi CD127low GFP+ *p<0.05 (A-D, B6-GFP-FOXP3)(**p<0.001)

Figure 4. Ex Vivo Programming of donor cells attenuates acute GvHD in a murine allogeneic model.

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Figure 5. Ex Vivo Programming of donor cells attenuates acute GvHD in a xenogeneic model. A. Xenogeneic acute GvHD model: Sub-lethally irradiated (25Gy) NGS recipients received unsorted or ex vivo modulated (vehicle or FT1050 and FT4145) human PBMC. B. Serum levels of IFN-gamma in recipient mice was measured on day 7 by cytokometric bead array. C. Mice were observed three weekly for clinical GvHD signs and symptoms including diarrhea, inactivity, hunched posture, ruffled fur, weight loss, GVHD score and skin integrity. D. Survival Results are representative of three independent studies (n=8 recipients/group).

CONCLUSIONS
- The combination of FT1050 and FT4145 on Human CD34+ cells:
  - Enhanced CXCR4 expression and transwell migration
  - Enhanced homing and engraftment of CD34+ cells
- The combination of FT1050 and FT4145 in the T cell compartment:
  - Reduced allogeneic responses (MLR)
  - Reduced surface expression of ICOS and 4-1BB (activating)
  - Reduced Th1 polarization and IFN-gamma production
  - Increased Th2 polarization and IL-4 production
  - Increased IL-10 production
- Murine allogeneic and xenogeneic acute GvHD models demonstrated that ex vivo programming with FT1050 and FT4145 leads to significantly lower GvHD scores and enhanced survival.

Future studies to characterize potential impacts on T cells (Tregs), NK cells (killing host APC and DCs (tolerogenic induction) and the graft-versus-leukemia response

Therapies are likely to be more effective if they have synergistic effects on multiple cell types

Preclinical findings demonstrate the potential for using ex vivo modulation with FT1050 and FT4145 to enhance CD34+ engraftment and dampen alloreactive T-cell responses.