Ex Vivo Modulation of Donor Cells Results in Enhanced Survival and Reduced GvHD Mortality

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ABSTRACT

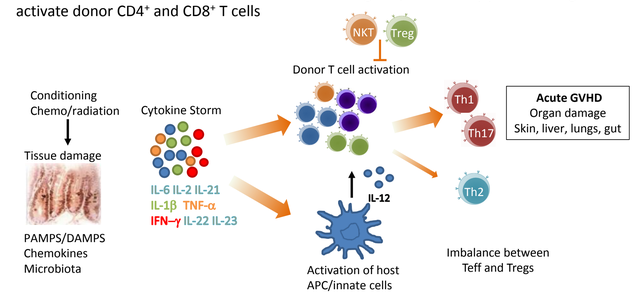
Allogeneic hematopoietic stem cell transplant (HSCT) represents a potential curative treatment for a number of life threatening blood malignancies. Despite improvements, the utility of this treatment regimen is limited by a number of side-effects including graft versus host disease. Acute graft-versus-host disease (aGVHD) 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 affective 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.,* ALDH, AREG) and anti-viral properties (*e.g.,* EFNB2). A number of T cell assays to assess T cell phenotype and function were conducted on mononuclear cell 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-ɣ). Concomitantly, there was enhancement in the ability to make Interleukin 4 (IL-4) and 10 (IL-10) suggesting a polarization of these cells towards a less inflammatory functional state. This was further evidenced by reduced expression of the activation markers 41BB and ICOS.

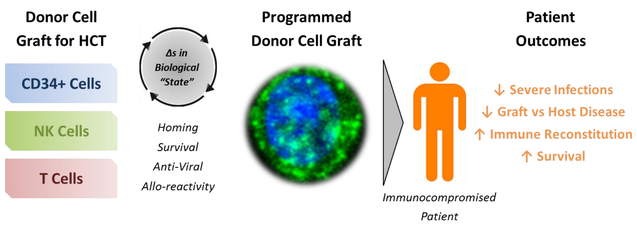
We next examined the potential beneficial role of ex vivo programming with FT1050+FT4145 in a major histocompatibility complex (MHC) mismatched HSCT mouse model. Briefly, lethally irradiated BALB/c mice received bone marrow and splenocytes from C57BL/6 donor mice pulse treated with vehicle or FT1050+FT4145. Significantly less GvHD, as determined by survival, weight loss, GVHD score (diarrhea, inactivity, hunched posture, ruffled fur, eye lesion, snout swelling/skin integrity), cytokine production and histopathology of GvHD target organs was observed in FT1050+FT4145 treated compared to vehicle control recipients. In addition, we observed increased levels of donor T regulatory cells (Tregs) in secondary lymphoid organs concomitant with decreased levels of circulating IFN-ɣ. Based on the attenuation of alloreactive T cell responses in both human MLR and mouse GVHD studies following pulse treatment with FT1050+FT4145, we believe these findings provide a compelling scientific basis to support the clinical evaluation of *ex vivo* programmed mobilized peripheral blood in patients undergoing hematopoietic stem cell transplantation for the treatment of hematologic malignancies.

Acute GvHD occurs rapidly after HSCT

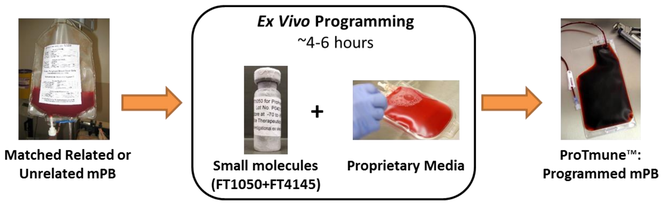
- Following conditioning, a state of immune reactivity is induced by damaged host tissues, the "cytokine storm", and lymphopenia
- Host DCs become activated and migrate into draining LNs, where they present antigens and activate donor CD4⁺ and CD8⁺ T cells



Cell-Based Immunotherapeutic Strategy



ProTmune™: Programmed Mobilized Peripheral Blood



RESULTS

Programmed mPB CD34⁺ Cells

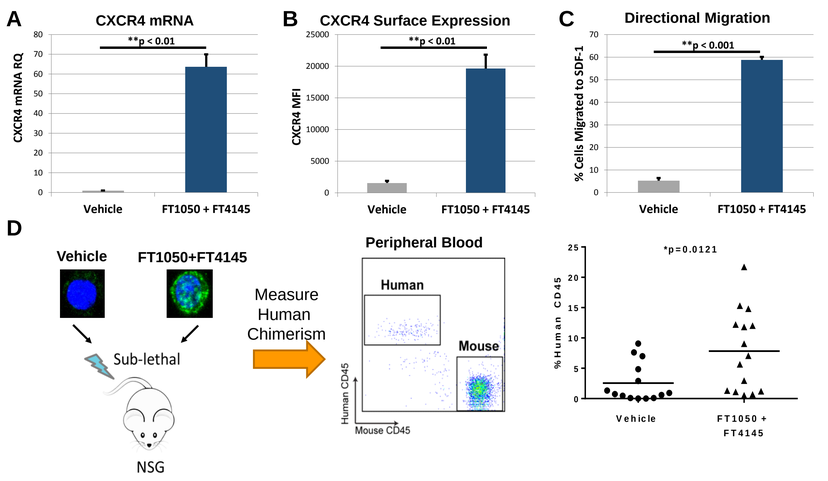


Figure 1. Ex Vivo Programming of Human mPB CD34+ Upregulates CXCR4 and Enhances Long-Term Engraftment in NSG Mice. A. Human CD34+ treated with two modulators exhibited significant upregulation of CXCR4 mRNA (~60-fold increase, p=0.0001; paired t-test) B. Increased CXCR4 surface protein levels (~4-fold increase, p=0.0005; 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 (3Gy) NSG mice were transplanted with 200,000 human mPB CD34⁺ cells unmodulated or modulated with 10μM FT1050 + 10μM FT4145 or 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.

Programmed mPB CD3⁺ T Cells

Allogeneic response
-one-way MLR
-flow readout integrates activation, cytokine, and degranulation markers

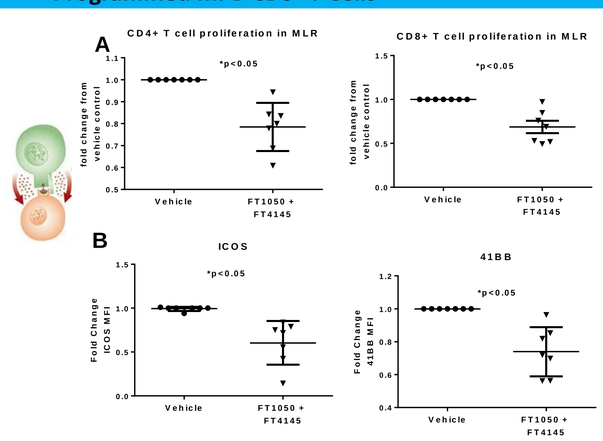


Figure 2. Ex vivo programmed CD4+ and CD8+ T cells demonstrate reduced allogeneic responses and had decreased levels of costimulatory receptors. Mobilized peripheral blood units were obtained via leukapheresis from a total of 7 donors over a 3 month period. mPB MNC from these collections were incubated with either vehicle or the combination of FT1050 + FT4145. After the ex vivo 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 Assay (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; B. Costimulatory proteins were monitored by flow cytometry and reported as mean fluorescence intensity (MFI) compared to vehicle (CD4+ T cells).

T cell differentiation
- 4 conditions
-10 color panel
-cytokine expression in CD4 and CD8

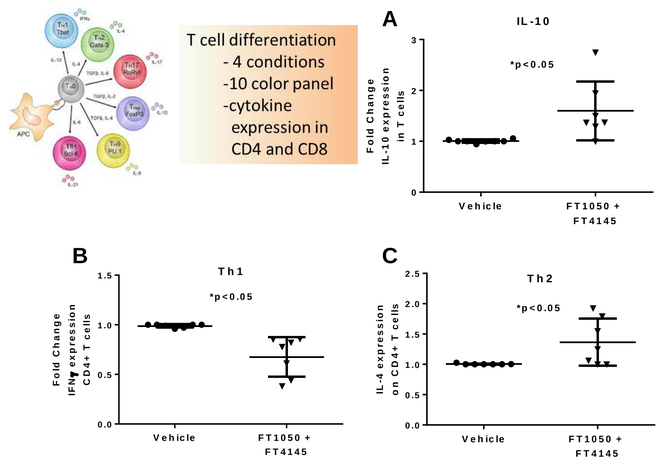


Figure 3. Figure 2. Ex vivo programmed T cells demonstrated a less inflammatory state following Type 1,2 differentiation. Mobilized peripheral blood units were obtained via leukapheresis from a total of 7 donors over a 3 month period. mPB MNC from these collections were incubated with either vehicle or the combination of FT1050 + FT4145. After the ex vivo programming, the cells were washed to remove the modulators and then assayed to assess T cell differentiation. After a 5 day incubation, cytokine release was monitored by intracellular cytokine staining using flow cytometry. A. Interleukin-10 B. Interferon-gamma; C. Interleukin-4.

Murine Allogeneic acute GVHD model

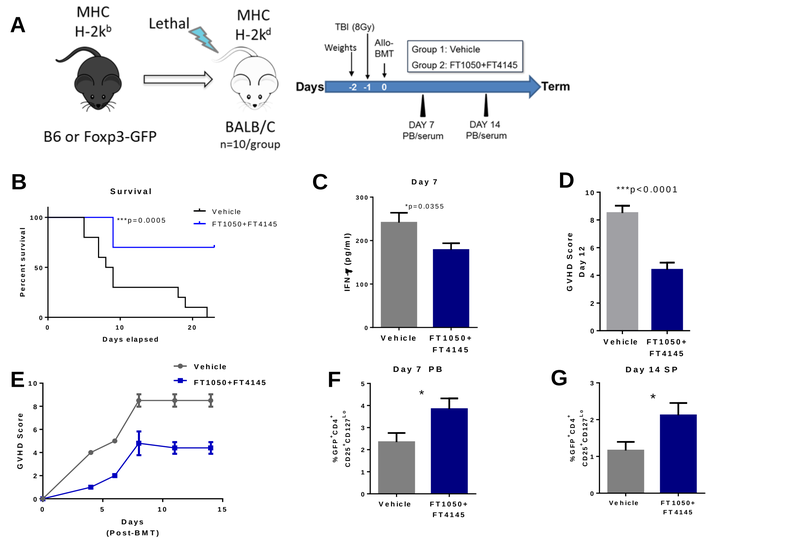


Figure 4. Ex Vivo Programming of donor cells attenuates acute GvHD in a murine allogeneic model. A. Murine acute GVHD model: Lethally irradiated (8Gy) BALB/c recipients received ex vivo modulated (vehicle or FT1050 and FT4145) B6 donor cells (T-cell depleted BM, SP) B. Survival; C. Serum levels of IFN-gamma in recipient mice was measured on day 7 by cytometric 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^{EGFP} donors; F. Peripheral blood day 7; B. Splenocytes day 14; Frequency of Tregs was monitored by flow cytometry. T regulatory cells were identified by flow cytometry as CD4+ CD25hi CD127lo GFP+ *P< 0.05 (A-D= B6 donors, E-G= B6-Foxp3^{EGFP})

Xenogeneic acute GVHD model

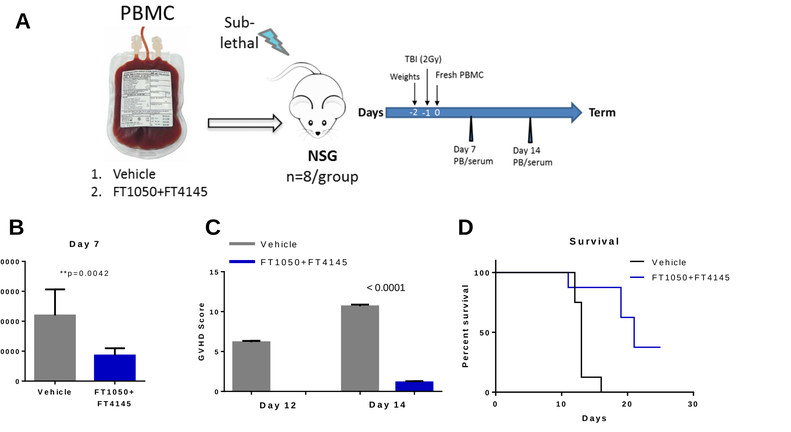


Figure 5. Ex Vivo Programming of donor cells attenuates acute GvHD in a xenogeneic model. A. Xenogeneic acute GVHD model: Sub-lethally irradiated (2Gy) NSG recipients received 15x10^6 ex vivo modulated (vehicle or FT1050 and FT4145) human PBMC; B. Serum levels of IFN-gamma in recipient mice was measured on day 7 by cytometric bead array; C. Mice were observed three times weekly for clinical GvHD signs and symptoms including: diarrhea, inactivity, hunched posture, ruffled fur, eye lesion, snout swelling/skin integrity. Weight was monitored as an independent parameter; 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