Prostaglandin-Modulated Umbilical Cord Blood Hematopoietic Stem Cell Transplantation

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PGE2-modulated UCB Transplantation

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Key Points

- Molecular profiling was used to optimize an *ex vivo* modulation protocol with dmPGE₂ for UCB transplantation.
- Pulse treatment of UCB with dmPGE₂ is safe and may lead to accelerated UCB engraftment and preferential cord chimerism.
Abstract

Umbilical cord blood (UCB) is a valuable source of hematopoietic stem cells (HSCs) for use in allogeneic transplantation. Key advantages of UCB are rapid availability and less stringent requirements for HLA matching. However, UCB contains an inherently limited HSC count, which is associated with delayed time to engraftment, high graft failure rates and early mortality. 16,16 dimethyl prostaglandin E$_2$ (dmPGE$_2$) was previously identified to be a critical regulator of HSC homeostasis and we hypothesized that a brief ex vivo modulation with dmPGE$_2$ could improve patient outcomes by increasing the “effective dose” of HSCs. Molecular profiling approaches were used to determine the optimal ex vivo modulation conditions (temperature, time, concentration and media) for use in the clinical setting. A phase I trial was performed to evaluate the safety and therapeutic potential of ex vivo modulation of a single UCB unit using dmPGE$_2$ prior to reduced intensity, double UCB transplantation. Results from this study demonstrated clear safety with durable, multilineage engraftment of dmPGE$_2$ treated UCB units. We observed encouraging trends in efficacy, with accelerated neutrophil recovery (17.5 vs. 21 days, p=0.045), coupled with preferential, long-term engraftment of the dmPGE$_2$ treated UCB unit in 10 of 12 treated subjects.

This study is registered at www.clinicaltrials.gov, Identifier: NCT00890500.
Introduction

Umbilical cord blood (UCB) is a valuable source of hematopoietic stem cells (HSCs) for use in allogeneic transplantation when a suitable adult donor is unavailable.\textsuperscript{1,2} However, many of the publically accessible UCB units are currently unfit for use in adult transplantation due to a low HSC content, which is associated with delayed time to engraftment, high graft failure rates, and early mortality.\textsuperscript{3,4} Several approaches are being evaluated to address this limiting stem cell dose problem, including co-transplantation of multiple UCB units,\textsuperscript{5,6} co-transplantation with progenitor cells from an alternative source,\textsuperscript{7,8} direct intra-marrow injection of UCB units,\textsuperscript{9} or expanding HSCs in culture.\textsuperscript{10-12} While each strategy has its benefits, there are significant limitations associated with these approaches, including induction of differentiation of long-term HSCs during extended culture, high manufacturing cost and the risk of introducing infectious agents.

An alternative strategy to improve outcomes for patients undergoing UCB transplantation is to use a brief pulse treatment with a small molecule modulator to enhance the homing and engraftment potential of HSCs. One potential \textit{ex vivo} enhancing agent, the stable prostaglandin \textit{E}$_2$ (PGE$_2$) derivative 16,16-dimethyl prostaglandin E$_2$ (dmPGE$_2$), was previously identified in a chemical screen using zebrafish embryos to be a potent regulator of vertebrate hematopoietic stem cell homeostasis.\textsuperscript{13} dmPGE$_2$ modulates HSCs primarily through the G-protein coupled prostaglandin receptors PTGER2 (EP2) and PTGER4 (EP4), which use cyclic adenosine monophosphate (cAMP) as a secondary messenger to up-regulate the expression of genes involved in homing (\textit{e.g.}, CXCR4), proliferation (\textit{e.g.}, CyclinD1) and cell survival (\textit{e.g.}, Survivin).\textsuperscript{14,15} dmPGE$_2$ also increases Wnt signaling in HSCs, and enhances self-renewal, which is important for the long-term maintenance of HSCs.\textsuperscript{16} An extensive set of characterization studies in zebrafish, murine and non-human primate models demonstrated the ability of dmPGE$_2$ to enhance hematopoietic engraftment of HSCs.\textsuperscript{13-15,17}

Based on promising preclinical evidence and prior human safety data\textsuperscript{18,19}, a phase I clinical trial was initiated to evaluate the safety and efficacy of using an \textit{ex vivo} treatment with dmPGE$_2$ to improve HSC engraftment following reduced-intensity double UCB transplantation. Double UCB transplantation provides a unique platform to examine the potential of enhanced hematopoietic engraftment of dmPGE$_2$-modulated UCB in comparison with unmanipulated UCB by virtue of the
competitive engraftment between UCB units. In addition, the second unmanipulated UCB unit provides a safety mechanism in the event of damage to the treated UCB unit during the ex vivo manipulation. Herein we describe a point-of-care, ex vivo pulse treatment strategy designed to accelerate engraftment in UCB transplantation, describe the molecular studies that lead to the optimization of this ex vivo modulation process, and report outcomes treated on this phase I clinical trial.
Materials and Methods

Patients and Treatment Program

The primary objective of this phase I trial was to evaluate the safety of dmPGE$_2$-treated UCB (dmPGE$_2$-UCB) co-transplantation with an unmanipulated UCB unit in patients with hematologic malignancies. Secondary objectives were to determine the kinetics of engraftment and the fractional chimerism of dmPGE$_2$-UCB after transplantation. Subjects with hematologic malignancies for whom no HLA-matched donor was available received conditioning with fludarabine (180 mg/m$^2$), melphalan (100 mg/m$^2$) and ATG (4 mg/kg) and received graft-vs.-host disease (GVHD) prophylaxis with sirolimus (target trough concentration 3-12 ng/ml) and tacrolimus (target trough concentration 5-10 ng/ml), as previously described. UCB units were required to be ≥ 4/6 HLA-allele matched with the recipient and each other. Each UCB unit was required to be ≥ 1.5 x 10$^7$ total nucleated cells (TNC)/kg pre-cryopreservation and the combined cell dose was required to be ≥ 3.7 x 10$^7$ TNC/kg. UCB units were hierarchically selected from international cord blood banks based on TNC count, HLA match and unit age. Units against which subjects had pre-formed anti-HLA antibodies were excluded.

On the day of transplantation, two cryopreserved UCB units were thawed and resuspended in a saline solution (0.9% NaCl) containing 5% human serum albumin (Baxter or Talecris) and 8% Dextran 40 (Hospira) (LMD/HSA). 2 cohorts of patients were enrolled. In cohort 1, one of the two UCB units was incubated with 10 µM dmPGE$_2$ (Fate Therapeutics) for 60 minutes at 4°C in LMD/HSA (9 patients). In the initial 6 patients, the smaller unit (by precryopreservation TNC) was treated with dmPGE$_2$, and in subsequent 3 patients, the larger unit was treated. Cohort 2 consisted of 12 patients where the larger of the two UCB units was incubated with 10 µM dmPGE$_2$ for 120 minutes at 37°C in LMD/HSA. Following incubation, excess dmPGE$_2$ was removed using a second centrifugation procedure and resuspended in LMD/HSA for infusion. All patients received both UCB units within 4 hours of each other, with the larger UCB (whether dmPGE$_2$-treated or not) always administered first. Standard post-transplantation care was delivered to all subjects. In accordance with the Declaration of Helsinki, all participants provided informed consent to participate, and this trial was prospectively registered at www.clinicaltrials.gov (NCT00890500). The study was approved by the Office for Protection of Research Subjects at the Dana-Farber/Harvard Cancer Center.
Patient baseline characteristics were reported descriptively. Neutrophil engraftment was defined as the first of three consecutive days with neutrophil recovery to at least $0.5 \times 10^9$ cells/L. Platelet engraftment was defined as the first day of a platelet count of at least $20 \times 10^9$ cells/L, without supporting transfusion in the prior 3 days. Donor chimerism was determined from peripheral blood mononuclear cells by analyses of informative short tandem repeat loci using the ABI Profiler-Plus Kit (Applied Biosystems Inc.) and the ABI 310 GeneticAnalyzer. Overall survival (OS) was defined as the time from transplant to death from any cause, while progression-free survival (PFS) was defined as the time from transplant to malignant disease progression or death from any cause. Surviving patients were censored at their date of last known follow-up. OS and PFS estimates were calculated using the method of Kaplan and Meier. A comparative control cohort of 53 subjects treated at our institution using the same UCB unit selection criteria, conditioning and GVHD prophylaxis regimen was used for comparison (Supplementary Table 1). All p-values are based on two-sided tests, and were computed using SAS v9.2 (SAS Institute, Cary, North Carolina).

**Primary Cells**

Cryopreserved human UCB CD34$^+$ primary cells were purchased from AllCells or Stem Cell Technologies.

**RNA preparation**

RNA was extracted using the PicoPure RNA Isolation kit (Life Technologies) using the manufacturer’s recommended protocol. Total RNA was quantified using the Nanodrop 2000 Spectrophotometer (Thermo Scientific). RNA integrity was confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies).

**Genome-wide Microarray Analysis**

Biotinylated aRNA was prepared from 10-100 ng of total RNA involving the Message A mp II kit (Life Technologies) following the standard two-round amplification protocols and hybridized to Affymetrix U133-plus-2.0 GeneChips according to the manufacturer’s instructions. Arrays were processed in the GeneChip Fluidics Station 450 and scanned on the 3000 7G Scanner (Affymetrix). Probe intensities were normalized according to a log-scale robust multi-array
analysis (RMA) method (Affymetrix) and visualized in Spotfire for Genomics 4.5 (Tibco). Raw expression data files are available on Gene Expression Omnibus (GSE46569).

Parametric paired t-tests (Benjamini-Hochberg false discovery rate < 0.05, adjusted p-value/q-value < 0.05, and fold change > 3 fold) detected probes with significant changes due specifically to the dmPGE₂ treatment conditions. Biological pathway enrichment analysis of the up-regulated probes was performed against the Gene Ontology (GO) database (Singular Enrichment to GO Biological Process and FDR < 0.01).

**Microfluidic qPCR using the Fluidigm Platform**

The mRNA sequences of the dmPGE₂ signature genes were taken from the National Center for Biotechnology Information Gene database and amplification primers were designed using Primer3. (Supplementary Table 2) For real-time PCR transcript quantitation, we used the BioMark Dynamic Array microfluidics system and GE Dynamic Array 96.96 chips (Fluidigm Corporation) using the manufacturer's protocol. Amplification results were analyzed using BioMark Real-Time PCR Analysis software. Samples with Cycle Thresholds above 28 or amplified products with inappropriate melting curve properties were excluded from the calculations. Log₂ fold change results are displayed in Spotfire for Genomics 4.0 in heat-map format.

**Human CD34⁺ HSC Murine Homing**

Human CD34⁺ cells isolated from UCB were treated with 10 µM dmPGE₂ or vehicle (DMSO) for 2 hours at 37°C in StemSpan-SFEM (Stem Cell Technologies) or a minimal media containing 8% low molecular weight dextran with 5% human serum albumin (LMD/HSA). For the last 15 minutes of the incubation, Vybrant Dye Dil (Life Technologies) was added to label the cells. Post incubation, 1 x 10⁵ cells from each condition were washed and injected in sub-lethally irradiated NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice in groups of 5 per condition. Twenty hours following injections, hind limb long bones were harvested and the BM extracted. Flow cytometry was performed on the BM using a LSRII (Becton Dickinson) and gating criteria based on remaining Dil stained CD34⁺ cells and control mouse BM. This homing experiment was performed in duplicate using CD34⁺ cells from two separate UCB units. Data was plotted as the increase in homed events relative to the vehicle treated cells. Results were averaged for both donor groups in each of the conditions tested. All protocols using animals in this study were
approved by the Institutional Animal Care and Use Committee at Indiana University School of Medicine (see Supplemental Methods).

**Results**

**Cohort 1**

9 subjects with high risk hematological malignancies were treated using *ex vivo* modulation conditions based on pre-clinical studies (10 µM dmPGE₂, exposure for 60 min at 4°C in LMD/HSA media) between 5/2009 and 6/2010. Patient and UCB unit characteristics are described in Table 1. No safety concerns were identified in this cohort; however, neither an improvement in rates of neutrophil recovery nor preferential engraftment of the dmPGE₂-UCB over the unmanipulated UCB units was observed (Fig. 1a). The median times to neutrophil and platelet engraftment were 24 and 72.5 days, respectively. 2 of 7 engrafting patients demonstrated prolonged hematopoiesis from the dmPGE₂-UCB units, and 2 patients had primary or late graft failure (Fig. 1b & c).

**Optimization of the Ex Vivo Modulation**

Given the lack of accelerated engraftment in the initial cohort of patients, we sought to determine if the *ex vivo* incubation conditions were optimal in the clinical setting. To evaluate the effects of temperature, CD₃⁴⁺ cells were incubated at 4°C, 25°C or 37°C for 2 hours in the presence of dmPGE₂ or vehicle. Following the incubation, genome-wide expression arrays were used to identify genes upregulated by dmPGE₂. Only 2 probe sets across the entire human transcriptome were significantly upregulated (> 4-fold) when the CD₃⁴⁺ cells were incubated at 4°C (Figure 2a). Performing the *ex vivo* incubation at 25°C also resulted in a modest biological response, with 19 probe sets being significantly upregulated. In contrast, increasing the incubation temperature to 37°C resulted in a robust biological response with 192 probe sets being significantly upregulated, suggesting physiological temperatures are required for cryopreserved human CD₃⁴⁺ cells to effectively activate the prostaglandin signaling pathway. The gene ontology pathway analysis for the dmPGE₂ signature showing genes involved proliferation, migration and receptor signaling is shown in Figure 2b.

To determine the optimal incubation time, CD₃⁴⁺ cells were treated with 10 µM dmPGE₂ or vehicle (DMSO) for increasing amounts of time at 37°C. Following the incubation, dmPGE₂
induced gene expression changes were measured using a high-throughput microfluidic qPCR-based system. Amplification primers were designed for 6 reference genes and 90 of the top dmPGE<sub>2</sub> up-regulated genes, selected based on the genome-wide expression analysis and biological significance for homing, proliferation and survival (Supplementary Table 1). Figure 3a shows that 2 hour incubation at 37°C is required to induce the maximal activation of the prostaglandin pathway. We also demonstrated that the prostaglandin must be present during the entire 2 hour incubation to achieve a full biological response (Supplementary Fig. 1). This eliminates the possibility of using a brief pulse treatment (< 60 minutes) to initiate a signaling event which could mature once the enhanced HSCs are infused into the patient.

To determine the optimal concentration of dmPGE<sub>2</sub> for ex vivo modulation, cryopreserved human CD34<sup>+</sup> cells were treated with increasing concentrations of dmPGE<sub>2</sub> (0.1 µM, 1 µM, 10 µM, 50 µM and 100 µM) for 2 hours at 37°C. Supraphysiologic concentrations of dmPGE<sub>2</sub> are permissible because the molecule is removed with the supernatant during washing prior to infusion. Expression changes in the prostaglandin signature genes were monitored using a microfluidic qRT-PCR platform. 10 µM was able to induce a maximal pathway response (Figure 3b). Higher concentrations of dmPGE<sub>2</sub> were not able to increase pathway activation at 4°C (Supplementary Fig. 2).

The majority of dmPGE<sub>2</sub> preclinical homing, engraftment and optimization studies were performed in “complete” media such as StemSpan-SFEM (Stem Cell Technologies, Vancouver) which contains glucose, amino acids and other nutrients designed to support long term culturing of HSCs. Unfortunately, this type of complete media cannot be used in a clinical setting because differentiated cell types (e.g., granulocytes) that do not survive the cryopreservation process lyse upon thawing and incubation in this media, which causes a significant reduction in the total nucleated cell counts (Supplementary Fig. 3). Typically, the media used in a clinical setting for thawing and washing cryopreserved UCB contains 8% low molecular weight Dextran with 5% Human Serum Albumin (LMD/HSA) to minimizes cell loss. We sought to determine whether the ex vivo modulation could be performed in this clinically compatible, nutrient-free media. To characterize the effects of media on the level of pathway activation, CD34<sup>+</sup> cells were treated with 10 µM dmPGE<sub>2</sub> for 2 hours at 37°C in either StemSpan-SFEM or LMD/HSA. Following the incubations, prostaglandin induced expression changes were analyzed using genome-wide
expression arrays. Figure 3c demonstrates that both media formulations were able to support activation of the prostaglandin pathway by dmPGE$_2$, however three-fold more probe sets were upregulated in StemSpan-SFEM (297 vs. 99 probesets). We used a mouse homing model to determine whether the incubation media impacts the functional properties of the enhanced HSCs. The homing properties of human CD34$^+$ cells were significantly improved in both types of media with a 2.2-fold increase in the number of human cells that had migrated to the bone marrow of mice when the CD34$^+$ cells were pulse treated in StemSpan-SFEM (p<0.001) compared to a 1.6 fold increase with LMD/HSA (p=0.002) (Figure 3d). The difference in homing properties between the two types of media (1.6-fold vs. 2.2-fold) is also statistically significant (p=0.03). A subtle decrease in viable cell recovery (7-AAD and CFU-C) was observed in the LMD/HSA relative to nutrient-rich media. Based on these results, we decided to move forward with the clinically proven LMD/HSA media for the initial clinical studies.

To characterize the engraftment properties of HSCs treated using the optimized conditions, lethally irradiated mice were injected with decreasing numbers of mouse bone marrow cells (200K, 100K, 50K or 10K) treated with 10 µM dmPGE$_2$ or vehicle for 2 hours at 37°C. Results from this experiment demonstrate that ex vivo treatment with dmPGE$_2$ significantly increased the survival rates of mice transplanted with limiting numbers of HSCs (Supplementary Fig. 4) We also observed accelerated recovery of neutrophils and platelets using this murine transplantation model (Supplementary Fig. 5a & b).

Cohort 2
12 additional subjects were accrued between 8/2010 and 8/2011 and treated according to the optimized ex vivo dmPGE$_2$ modulation protocol(10 µM dmPGE$_2$ 2 hour exposure at 37°C in LMD/HSA media). Clinical and UCB unit characteristics are described in Table 1. The subject median age was 57.5 years (range 19 – 66) and the median weight was 78.7 kg (range 48.7 – 149.6 kg). The majority of UCB units were 4/6 matched to each other and the recipient, and the post-thaw cell doses between units were very similar. Ex vivo incubation with dmPGE$_2$ did not result in significant cell loss, with a mean viable CD34$^+$ cell recovery of 90%. Adverse events attributed to dmPGE$_2$-UCB infusion included grade 1-2 infusion-related events in 4 subjects, consisting of chills, flushing, abdominal pain, and cough. One additional subject with known
coronary artery disease experienced transient grade 4 ST-elevation following infusion and evidence of myocardial ischemia by cardiac troponin assay.

The median time to neutrophil engraftment was 17.5 days (range 14 - 31 days), which was shorter than a historical control regimen of similarly treated patients at our institution (21 days, n=53, p=0.045, Fig 4b), as well as shorter than the time to engraftment of patients in cohort 1 (p=0.09). No patient experienced primary graft failure. The median time to platelet engraftment was 43 days (range 26 - 60 days), and 11 of 12 patients had engrafted platelets by day 60 (Fig 4c). Chimerism assessment demonstrated that 10 of 12 patients had early and sustained engraftment of the dmPGE2-UCB unit, and that this unit contributed 100% to hematopoiesis (Fig. 4a). Using a binomial distribution and historical engraftment rates of the first administered cord, the probability of this occurring is only 2.4% (p = 0.03). Sustained dmPGE2-UCB hematopoiesis has been demonstrated for up to 27 months from transplantation. Chimerism in CD33+ myeloid and CD3+ lymphoid subsets mirrored total chimerism assessments.

Three subjects developed Grade I GVHD and 2 subjects developed skin-limited Grade II GVHD. One patient developed skin-limited chronic GVHD. Relapse occurred in 3 subjects, and 8 subjects have expired. Causes of death include relapse (3), treatment-related complications (4) and suicide (1). With a median follow-up among survivors of 24.6 months (range 21.9 – 27.4 months), the 1 and 2 year PFS were 61.7% and 31.3% respectively. The corresponding 1 and 2 year OS were 75% and 38.9%.
Discussion

The development of HSCs during embryogenesis is regulated by pathways that regulate HSC homing and engraftment after transplantation in adulthood. dmPGE$_2$ was originally identified as a small molecule that could increase HSC formation in the developing aorta of the zebrafish embryo, and enhanced HSC engraftment in murine HSC transplantation after brief ex vivo exposure. Further studies established that dmPGE$_2$ improved human UCB engraftment in immunodeficient mice, and demonstrated safety in long-term primate transplantation studies. Given that the limiting number of HSCs in UCB units results in delayed hematopoietic engraftment, graft failure and incomplete immunologic reconstitution, we sought to determine if modulation of UCB with dmPGE$_2$ prior to transplantation could improve these outcomes. Of note, this is the first study where the function a small molecule discovered in the zebrafish system has reached a human clinical trial. UCB units from the initial cohort of 9 subjects were treated under ex vivo modulation conditions designed to maximize viability of the HSCs. After demonstrating safety, but lack of clear efficacy, we enrolled a second cohort of patients using ex vivo modulation conditions designed to increase the prostaglandin pathway stimulation. Results from this second group of patients demonstrated a correlation between the enhanced biologic activity, accelerated engraftment and preferential hematopoiesis from the modulated unit in a double UCB transplantation model.

In translating this academic discovery into the clinic, one of the challenges that we faced was determining the optimal incubation conditions to use in the clinical setting. The incubation conditions used for the preclinical studies varied significantly with respect to the temperature, duration, dose, and media tested. Molecular profiling approaches were used to identify a set of ex vivo modulation conditions, which enable rapid and robust activation of the prostaglandin pathway in cryopreserved human cord blood HSCs. The key changes to the incubation protocol were increasing the temperature from 4°C to 37°C and extending the incubation from 1 hour to 2 hours. In making these changes, we had to consider the potential impacts of the optimized conditions on cell viability, especially in the clinically established nutrient-free media. Prior to human use, we confirmed that these optimized conditions were not only safe, but resulted in enhanced engraftment and hematopoiesis in murine models.
There are limitations to the conclusions that can be made from this phase 1 clinical trial. In the second cohort of patients, it was always the larger unit pre-cryopreservation that was modulated with dmPGE₂ and it was this unit that dominated hematopoiesis in 10 of 12 treated patients. While firm conclusions cannot be drawn based on the small sample sizes tested, two important factors should be noted. First, in the latter patients enrolled in Cohort 1, the same protocol of treating and infusing the larger unit first was employed as in Cohort 2, and no signal hinting at early engraftment or chimerism dominance was noted, and this was the factor that lead to the early closure of the trial and the re-design of the ex vivo expansion process. In addition, in Cohort 2, while the larger unit (based on pre-cryopreservation TNC) was to be infused first, the UCB units in fact were extraordinarily comparable with respect to TNC (1.8 vs. 1.7 x 10⁷ TNC/kg, p=0.43), CD34⁺ cells (0.74 vs. 0.56 x 10⁵ CD34⁺/kg, p=0.71) and CFU growth (4.5 vs. 6.9, p = 0.77) (Table 1). In dual UCB transplantation, one unit dominates in the vast majority of patients based on incompletely understood biological factors. Factors such as total nucleated cell dose, CD34⁺ cell dose, CD3⁺ T cell dose, HLA match, CXCR4 expression, and CFU assay have been suggested as relevant contributors to this phenomenon. To determine if order of infusion or cell size was relevant in the outcomes we report in this manuscript, we compared the UCB dominance patterns with those in the historical control cohort, and noted that UCB dominance was nearly random in the control group, with approximately 50% of subjects engrafting with each of the transplanted units, using varying thresholds for the determination of UCB dominance. The dominance seen with the dmPGE₂ treated patients in the active cohort suggests that enhanced homing may be relevant to this process. We have previously demonstrated that CXCR4 expression is enhanced on human UCB cells treated with dmPGE₂. As a chemokine receptor to SDF1, increased CXCR4 expression may enhance engraftment. We also demonstrated that dmPGE₂ functions as a modifier pathway for Wnt signaling via cAMP, and this could lead to enhanced self-renewal of the HSCs. Rather than increasing the number of HSCs, this pharmacologic ex vivo modulation increases the “effective stem cell dose” for use in transplantation. Many additional cell types (e.g., regulatory T cells, CD4⁺ T cells, CD8⁺ T cells, monocytes, dendritic and NK cells) in UCB units express the EP2/4 receptors and have been shown to be effected by dmPGE₂. It is likely that effects on differentiated cells types in cord blood are involved in stimulating engraftment and driving hematopoiesis. We are currently in the process of characterizing the molecular responses
across these cell types and the potential mechanism(s) by which a pulse treatment with dmPGE2 may impact UCB unit dominance, GVHD, rates of infections and rates of viral reactivation.

Other groups have attempted to expand HSCs to improve engraftment and clinical outcomes after UCB transplantation. A recent trial established that the notch ligand, Delta1\textsuperscript{ext-1G} could be used to expand UCB in culture for 14 days, and these cells could be infused with a second UCB unit.\textsuperscript{11} While the expanded UCB unit did engraft early, these cells did not contribute to long-term hematopoiesis, and likely acquired a committed myeloid progenitor phenotype during \textit{ex vivo} culture. Similarly, a mesenchymal stem cell co-culture UCB expansion trial demonstrated a 40-fold expansion of CD34\textsuperscript{+} cells; however transplantation of these cells with a second unexpanded UCB unit similarly resulted in the loss of the expanded cells over time.\textsuperscript{12} We have demonstrated sustained multilineage dmPGE2-UCB derived hematopoiesis for 27 months after transplantation, but further optimization of these and other \textit{ex vivo} protocols may prove that each approach has utility in the transplantation setting.

Despite the limited conclusions that can be drawn from the clinical data, the potential implications of this prospective competitive engraftment clinical trial are broad. First, by generating more effective HSCs, the minimum nucleated cell dose required for adult UCB transplantation might be lowered, increasing the number of acceptable UCB units currently available in public UCB banks. Currently, fewer than 5\% of UCB units in the National Marrow Donor Program inventory are of adequate size for use in single UCB transplantation for average weight American adults. By increasing the effective stem cell dose by as little as 4-fold, 98\% of these units would be accessible for this use (Personal communication, M. Boo, National Marrow Donor Program). In addition, by increasing the number of accessible units, the likelihood of identifying better HLA-matched units would be increased, potentially leading to improved UCB transplantation outcomes.\textsuperscript{32,33} From a resource point of view, reducing the need for costly second UCB units, and reducing the length and complexity of hospital stays represents a substantial potential cost savings for health care payers. In addition, unlike the significant time requirements associated with HSC expansion approaches, this simple \textit{ex vivo} manipulation procedure with dmPGE2 is inherently exportable to all stem cell processing facilities.
In summary, in this preliminary human experience, we have demonstrated that ex vivo modulation of UCB stem cells using dmPGE₂ results in enhanced and more rapid engraftment in human UCB transplantation. Based on these positive results, we are now expanding the use of dmPGE₂ in randomized phase II trials in addition to initiating new phase I studies in single UCB transplantation and autologous peripheral blood stem cell transplantation.
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Conflict of Interest Statement

P. Multani, D. Robbins, T. Le, C. Desponts, B. Rezner, J. Mendlein, and D. Shoemaker are employees of, and own stock in, Fate Therapeutics. C. Cutler, H.T. Kim, J. Hoggatt and L. Pelus have received consulting fees from Fate Therapeutics. W. Goessling and T.E. North receive patent royalties and consulting fees from Fate Therapeutics. D. Scadden and L.I. Zon are founders of, own stock in, and serve as members of the Scientific Advisory Board for Fate Therapeutics.
Authorship Statement

1. Designed research
2. Cared for patients
3. Collected data
4. Performed statistical analyses
5. Performed pre-clinical laboratory studies
6. Performed clinical laboratory studies
7. Drafted manuscript
8. Approved manuscript

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Reference List


### Table 1 Patient and umbilical cord blood characteristics

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<tr>
<th>Patient Characteristics</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
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<tr>
<td>Sample Size</td>
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<td>12</td>
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<tr>
<td>Median Age (range)</td>
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<td>57.5 (19 - 66)</td>
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<td>Male Gender</td>
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<td>8 (66.7%)</td>
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<td>Median Weight (kg, range)</td>
<td>73.8 (44.7 - 126)</td>
<td>78.7 (48.7 - 149.6)</td>
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<tr>
<td>Primary Malignancy</td>
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<tr>
<td>AML</td>
<td>3 (33.3%)</td>
<td>5 (41.7%)</td>
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<tr>
<td>MDS</td>
<td>2 (22.2%)</td>
<td>4 (33.3%)</td>
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<tr>
<td>NHL/CLL</td>
<td>2 (22.2%)</td>
<td>3 (25.0%)</td>
</tr>
<tr>
<td>ALL</td>
<td>2 (22.2%)</td>
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</tr>
<tr>
<td>Prior Autologous Transplant</td>
<td>2 (22.2%)</td>
<td>2 (16.7%)</td>
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<tr>
<td>CMV Seropositive</td>
<td>7 (77.8%)</td>
<td>7 (58.3%)</td>
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<tr>
<th>Umbilical Cord Blood Unit Characteristics</th>
<th>dmPGE$_2$-UCB</th>
<th>Untreated UCB</th>
<th>p</th>
<th>dmPGE$_2$-UCB</th>
<th>Untreated UCB</th>
<th>p</th>
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<tr>
<td>HLA Match</td>
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<tr>
<td>4/6</td>
<td>8</td>
<td>8</td>
<td>NS</td>
<td>10</td>
<td>8</td>
<td>0.64</td>
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<tr>
<td>5/6</td>
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<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0.02</td>
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<td>Pre-Cryopreservation</td>
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<tr>
<td>TNC (x$10^7$/kg)</td>
<td>3.03</td>
<td>2.53</td>
<td>0.43</td>
<td>2.64</td>
<td>1.95</td>
<td>0.02</td>
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<tr>
<td>CD34$^+$ (x$10^5$/kg)</td>
<td>1.58</td>
<td>1.54</td>
<td>0.79</td>
<td>1.21</td>
<td>1.01</td>
<td>0.69</td>
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<tr>
<td>Post-Thaw</td>
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<tr>
<td>TNC (x$10^7$/kg)</td>
<td>2.17</td>
<td>1.8</td>
<td>0.45</td>
<td>1.8</td>
<td>1.7</td>
<td>0.43</td>
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<td>CD34$^+$ (x$10^5$/kg)</td>
<td>0.7</td>
<td>0.68</td>
<td>0.94</td>
<td>0.74</td>
<td>0.56</td>
<td>0.71</td>
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<tr>
<td>CFU-GM (x$10^3$/kg)</td>
<td>6.76</td>
<td>2.65</td>
<td>0.19</td>
<td>4.5</td>
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<td>First Infused Unit</td>
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<td>6</td>
<td>12</td>
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| dmPGE$_2$ Processing                     |       |       |   |       |       |   |
| CD34$^+$ cell count                     | -20.92% |       | -7.87% |
| TNC Viability (7-AAD)                   | -0.27%  | 1.81%  |
| CD34$^+$ Viability (7-AAD)              | -1.40%  | -1.28% |
Figure Legend

Figure 1 Cohort 1 clinical results
a) Donor chimerism of the dmpGE$_2$ treated umbilical cord blood unit over 360 days post transplantation (% of total) as determined from peripheral blood mononuclear cells by analyses of informative short tandem repeat (STR) loci.
b) Cumulative incidence of neutrophil engraftment of greater than 500 cells/mm$^3$, in cohort 1 (n=9, blue) compared with historical institutional controls (n=53, black).
c) Cumulative incidence of platelet engraftment in Cohort 1 (n=9, blue) compared with historical institutional controls (n=53, black).

Figure 2 Evaluation of ex vivo modulation temperature with cryopreserved human UCB CD34$^+$ cells with dmpGE$_2$

a) Genome-wide expression analysis on Affymetrix U133 plus 2.0 GeneChips of human UCB CD34$^+$ cells treated with 10 µM dmpGE$_2$ for 2 hours at 4°C, 25°C, and 37°C. RMA log$_2$ normalized expression levels for cells treated with dmpGE$_2$ (y-axis) in comparison to vehicle treated cells (x-axis). There were 2, 19, and 192 probe sets (red) with expression levels changing greater (or less) than 4 fold due to dmpGE$_2$ treatment at 4°C (left), 25°C (center), and 37°C (right) respectively.
b) Gene Ontology (GO) enrichment analysis showing biological processes enriched in the up-regulated probes induced by treatment with dmpGE$_2$ at 37°C. Genes up-regulated by dmpGE$_2$ treatment at 37°C in CD34$^+$ are listed for each GO category.

Figure 3 Optimization of incubation time, dmpGE$_2$ concentration, and modulation media in cryopreserved UCB CD34$^+$ cells
a) Microfluidic RT-PCR gene expression analysis of the top 90 signature genes after 10 µM dmpGE$_2$ treatment at 37°C incubated for 0 to 240 minutes. The heat map shows log$_2$ fold changes in expression levels relative to vehicle control treatments of the same time.
b) Microfluidic RT-PCR gene expression analysis of the top 90 signature genes after 120 minutes at 37°C with varying dmpGE$_2$ concentrations. The heat map shows log$_2$ fold changes in expression levels relative to vehicle control treatments.
c) Genome-wide expression analysis on Affymetrix U133 plus 2.0 GeneChips of human UCB CD34$^+$ cells treated with 10 µM dmpGE$_2$ for 2 hours at 37°C in 8%LMD/5%HSA or StemSpan-SFEM. RMA log$_2$ normalized expression levels of expression for cells treated with dmpGE$_2$ (y-axis) in comparison to vehicle treated cells (x-axis). There were 99 and 297 probe sets (red) with expression levels changing greater (or less) than 4-fold due to dmpGE$_2$ treatment in LMD/HSA and StemSpan-SFEM respectively.
d) Fold increase of homed CD34$^+$ cells over control (vehicle treated) after incubation with 10 µM dmpGE$_2$ for 2 hours at 37°C in LMD/HSA (Green) and StemSpan-SFEM (Red) (t-test, p=0.03).

Figure 4 Cohort 2 clinical results
a) Donor chimerism of the dmpGE$_2$ treated umbilical cord blood unit over 360 days post transplantation (% of total) as determined from peripheral blood mononuclear cells by analyses of informative short tandem repeat (STR) loci.
b) Cumulative incidence of neutrophil engraftment of greater than 500 cells/mm$^3$, in cohort 2 (n=12, red) compared with historical institutional controls (n=53, black).
c) Cumulative incidence of platelet engraftment in Cohort 2 (n=12, red) compared with historical institutional controls (n=53, black).
Figure 1 | Cohort 1 clinical results
Figure 2 | Evaluation of *ex vivo* modulation temperature with cryopreserved human UCB CD34⁺ cells with dmPGE₂
Figure 3 | Optimization of incubation time, dmPGE₂ concentration, and modulation media in cryopreserved UCB CD34⁺ cells
Figure 4 | Cohort 2 clinical results
Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation