Prostaglandin E\textsubscript{2} enhances hematopoietic stem cell homing, survival, and proliferation

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**Running Title:** PGE\textsubscript{2} enhances HSC function

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ABSTRACT

Adult hematopoietic stem cells (HSC) are routinely used to reconstitute hematopoiesis after myeloablation; however, transplantation efficacy and multilineage reconstitution can be limited by inadequate HSC number, or poor homing, engraftment or self-renewal. Here we report that mouse and human HSC express PGE2 receptors, and that short-term ex vivo exposure of HSC to prostaglandin E2 (PGE2) enhances their homing, survival and proliferation, resulting in increased long-term repopulating cell (LTRC) and competitive repopulating unit (CRU) frequency. HSC pulsed with PGE2 are more competitive, as determined by head-to-head comparison in a competitive transplantation model. Enhanced HSC frequency and competitive advantage is stable and maintained upon serial transplantation, with full multi-lineage reconstitution. PGE2 increases HSC CXCR4 mRNA and surface expression and enhances their migration to SDF-1 in vitro and homing to bone marrow in vivo and stimulates HSC entry into and progression through cell cycle. In addition, PGE2 enhances HSC survival, associated with an increase in Survivin mRNA and protein expression and reduction in intracellular active caspase-3. Our results define novel mechanisms of action whereby PGE2 enhances HSC function and supports a strategy to use PGE2 to facilitate hematopoietic transplantation.
INTRODUCTION

Hematopoietic stem cell transplantation with bone marrow, mobilized peripheral blood, or umbilical cord blood (UCB), is a proven therapy for malignant and nonmalignant hematologic diseases and metabolic disorders. Repopulation of hematopoiesis is a multi-step process that can be adversely affected by the inability of HSC to migrate/home to appropriate marrow niches or poor engrafting efficiency and self-renewal. Insight into the intrinsic and extrinsic mechanisms regulating these critical functions can lead to new strategies to improve HSC transplantation efficacy.

Prostaglandin E2 (PGE2) is the most abundant eicosanoid and a mediator of numerous physiological systems. We and others have demonstrated regulatory roles for PGE2 in hematopoiesis. PGE2 dose-dependently inhibits growth of human and mouse granulocyte/macrophage colony forming cells (CFU-GM) in vitro and myelopoiesis in vivo but stimulates erythroid and multilineage progenitor cells. Short-term ex vivo treatment of marrow cells with PGE2 increases the proportion of mouse CFU-S and human CFU-GM in cell cycle. In addition, PGE2 can stimulate production of cycling human CFU-GM from a population of quiescent cells, possibly stem cells, which is critically dependent on timing, duration of exposure and concentration. Recently, it was shown that pulse exposure to PGE2 ex vivo increased HSC frequency of murine bone marrow cells and enhanced kidney marrow recovery in zebrafish. However, while it is clear that PGE2 can affect hematopoietic stem and progenitor cells, the mechanisms of action of PGE2 on stem cell function have yet to be determined.
In this report, we show that PGE$_2$ acts directly on murine HSC to enhance their frequency after transplantation and also provides a competitive advantage that is maintained during serial transplantation, with full multi-lineage reconstitution. Enhanced HSC engraftment induced by PGE$_2$ results from increased homing of HSC, mediated through up-regulation of the chemokine receptor CXCR4, implicated in HSC homing$^{11}$, and selective stimulation of primitive HSC survival and self-renewal associated with up-regulation of the inhibitor of apoptosis protein Survivin, required for HSC maintenance and entry into cell cycle$^{12,13}$. Our studies describe novel mechanisms for enhancement of HSC function by PGE$_2$ and support a translational strategy to facilitate HSC transplantation.
MATERIALS AND METHODS

Mice and Human Cord Blood

C57Bl/6 (CD45.2) mice were purchased from Jackson Laboratories (Bar Harbor, ME). B6.SJL-PtcrAPep3B/BoyJ (BOYJ) (CD45.1), C57Bl/6 X BOYJ-F1 (CD45.1/CD45.2) and NOD.Cg-Prkdcscid IL2rgtm1Wjl/Sz (NS2) mice were bred in-house. Mice used in transplant studies received Doxycycline feed for 30 days post-transplant. The Animal Care and Use Committee of IUSM approved all protocols. Human umbilical cord blood (UCB) was obtained from Wishard Hospital, Indianapolis, IN with IUSM IRB approval.

Flow Cytometry

All antibodies were purchased from BD Biosciences unless noted. For detection and sorting of KL and SKL cells, streptavidin conjugated with PE-Cy7 (to stain for biotinylated MACS lineage antibodies (Miltenyi Biotech, Auburn, CA)), c-kit-APC, Sca-1-PE or APC-Cy7, CD45.1-PE, CD45.2-FITC, and CD34-PE were used. For SLAM SKL, we utilized Sca-1-PE-Cy7, c-kit-FITC, CD150-APC (eBiosciences, San Diego, CA), CD48-biotin (eBiosciences) and streptavidin-PE. UCB CD34+ cells were detected using anti-human-CD34-APC. For multi-lineage analysis, APC-Cy7-Mac-1, PE-Cy7-B-220 and APC-CD3 were used. EP receptors were detected with rabbit anti-EP(1-4) antibodies (Cayman Chemicals, Ann Arbor, MI) and FITC-goat-anti-rabbit IgG (Southern Biotech, Birmingham, AL). CXCR4 expression was analyzed using streptavidin-PECy7, c-kit-APC, Sca-1-APC-Cy7, and CXCR4-PE. Apoptosis was measured with FITC-Annexin-V or FITC-anti-active caspase-3. For Survivin and active
caspase-3 detection, cells were permeabilized and fixed using the CytoFix/CytoPerm kit (BD) and stained with anti-active-caspase-3-FITC Flow Kit (BD) or Survivin-PE (R&D Systems, Minneapolis, MN). For cell cycle analysis, cells were stained with Hoechst-33342 (Molecular Probes, Eugene, OR) and Pyronin-Y (Sigma Aldrich, St. Louis, MO) or FITC-BrdU Flow Kit (BD). Analyses were performed on an LSRII and sorting on either a FACS Aria or FACS Vantage sorter (BD).

**dmPGE2 pulse-exposure**

16,16-dimethyl Prostaglandin E2 (dmPGE2) in methyl acetate (Cayman Chemicals) was evaporated on ice under N2, reconstituted in 100% ETOH at a final concentration of 0.1M and stored at -80 ºC. For pulse exposure, cells were incubated with dmPGE2 diluted in media, on ice, for 2 hours, with gentle vortexing every 30 minutes. After incubation, cells were washed twice in media before use. Vehicle-treated cells were processed in an identical manner, using the equivalent ETOH concentration.

**Limiting dilution competitive and non-competitive transplantation**

Whole bone marrow (WBM) cells (CD45.2) were treated on ice for 2 hours with 1 uM dmPGE2 (Cayman) or 1x10^-3 % ETOH per 1x10^6 cells in PBS. After incubation, cells were washed twice and mixed with 2x10^5 congenic CD45.1 competitor marrow cells at various ratios and transplanted intravenously into lethally-irradiated CD45.1 mice (1100-cGy split dose). Peripheral blood (PB) CD45.1 and CD45.2 cells were determined monthly by flow cytometry. For head-to-head competitive analysis, WBM from CD45.1 and CD45.2 mice were treated with vehicle or dmPGE2 and mixed with 2x10^5 competitor
marrow cells from CD45.1/CD45.2 mice at various ratios and transplanted into lethally-irradiated CD45.1/CD45.2 mice. The proportion of CD45.1, CD45.2, and CD45.1/CD45.2 cells in PB was determined monthly. HSC frequency was quantitated by Poisson statistics using L-CALC software (Stem Cell Technologies, Vancouver BC, Canada) with <5% contribution to chimerism considered negative. Competitive repopulating units (CRU) were calculated as described \(^{14}\). For secondary transplants, 2x10\(^6\) WBM from previously transplanted CD45.1/CD45.2 mice at a 1:1 ratio at 20 weeks post-transplant were injected into lethally-irradiated CD45.1/CD45.2 mice in non-competitive fashion.

**Analysis of HSPC homing**

WBM from CD45.2 mice was labeled with 5-(and -6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes) washed and treated with either 1 uM dmPGE\(_2\) or vehicle and 2x10\(^7\) cells transplanted into lethally-irradiated CD45.2 mice. After 16 hours, femurs and tibias were flushed, Lin\(^+\) cells depleted using MACS microbeads and Lin\(^\text{neg}\) cells stained for SKL and the total number of CFSE\(^+\) WBM, KL and SKL cells determined. For congenic homing studies, Lin\(^\text{neg}\) CD45.1 cells were treated with 1 uM dmPGE\(_2\), vehicle, or PBS and 2x10\(^6\) cells transplanted into lethally-irradiated CD45.2 mice. After 16 hours, CD45.1 SKL cells in recipient BM were quantitated. For competitive homing studies, Lin\(^\text{neg}\) cells from CD45.2 and CD45.1 mice were FACS sorted, treated with dmPGE\(_2\) or vehicle and 3x10\(^4\) CD45.1 (vehicle or dmPGE\(_2\) treated) plus 3x10\(^4\) CD45.2 (dmPGE\(_2\) or vehicle treated) SKL cells transplanted into lethally-irradiated CD45.1/CD45.2 mice. To evaluate the role of CXCR4 in homing,
Lin<sup>neg</sup> CD45.2 cells were treated with vehicle or 1 uM dmPGE2 plus 10 uM AMD3100 (AnorMed Inc., Vancouver, BC, Canada), 2x10<sup>6</sup> treated cells injected into lethally-irradiated CD45.1 mice and homed SKL cells analyzed 16 hours post-transplant. Homing of human UCB cells was evaluated in NS2 mice. UCB mononuclear cells were isolated on Ficoll-Paque<sup>TM</sup> Plus (Amersham Biosciences, Piscataway, NJ), treated with either dmPGE2 or vehicle, and 4x10<sup>7</sup> cells transplanted into each of 5 sublethally-irradiated (250 cGy) mice. Homed CD34<sup>+</sup> cells were analyzed 16 hours post-transplant.

**Expression of EP receptors, CXCR4 and Survivin**

Lin<sup>neg</sup> marrow cells were stained for SKL, SLAM or CD34, each of the 4 EP receptors and surface receptor expression on KL, SKL and SLAM SKL and CD34<sup>neg</sup> SKL cells determined by FACS. For human EP receptors, UCB CD34<sup>+</sup> cells were positively selected with MACS microbeads<sup>12</sup>, stained for CD34 and CD38 and each of the EP receptors and surface receptor expression determined by FACS. To evaluate CXCR4, Survivin and active caspase-3, Lin<sup>neg</sup> cells or CD34<sup>+</sup> UCB were treated on ice with either 1 uM dmPGE2 or vehicle control for 2 hours, washed, and then cultured in RPMI-1640/10% HI-FBS at 37ºC for 24 hours, stained for SKL (murine cells) and CXCR4, Survivin, and/or active caspase-3 as described earlier, and analyzed by FACS.

**Migration Assays**

Chemotaxis to SDF-1 was determined using a two-chamber Costar Transwell (Cambridge, MA), 6.5 mm diameter, 5 um pore) as previously described<sup>15</sup>. Briefly, dmPGE2 and vehicle-treated Lin<sup>neg</sup> bone marrow cells were cultured in RPMI/10% HI-
FBS overnight to allow for up-regulation of CXCR4, washed, resuspended at 2x10⁶ cells per ml in RPMI/0.5% BSA and 0.1 ml added to the top chamber of the transwells, with or without rmSDF-1α (R&D Systems) in the bottom and/or top chamber, and incubated for 4 hours at 37°C. Cells completely migrating to the bottom chamber were enumerated by flow cytometry. Percent migration was calculated by dividing total cells migrated to the lower well by the cell input multiplied by 100. SKL cell migration was determined by comparison of the proportion of SKL cells in input and migrated populations. For UCB migration, CD34⁺ cells were MACS selected as described and migration assays performed as described for mouse, using rhSDF-1α (R&D Systems).

**Cell Cycle Analysis**

For *in vitro* cell cycle analysis, Lin⁻ cells were treated with either 1 uM dmPGE₂ or vehicle and cultured in Stem Cell Pro Media (Stem Cell Technologies) with rmSCF (50 ng/ml) (R&D Systems), rhFlt-3 and rhTPO (100 ng/ml each) (Immunex, Seattle, WA). After 20 hours, cells were stained for SLAM SKL, fixed, permeabilized and stained with Hoechst-33342 followed by Pyronin-Y. The proportion of SLAM SKL cells in G₀, G₁, S and G₂/M phase was determined by FACS quantitation of DNA and RNA. For *in vivo* cell cycle analysis, CD45.2 mice were lethally-irradiated and transplanted with 5x10⁶ dmPGE₂ or vehicle-treated Lin⁻ CD45.1 cells. Recipient mice received 1 mg/mL BrdU (Sigma) in drinking water and 1 mg/mouse BrdU I.P. After 16 hours, recipient marrow was isolated, lineage depleted, stained for CD45.1, SKL and BrdU and the proportion of homed (CD45.1⁺) SKL cells that were BrdU⁺ determined by FACS.
Apoptosis Assays

Lin<sup>neg</sup> cells were treated with 1 uM dmPGE2 or vehicle, and incubated in RPMI/2% HI-FCS at 37 ºC without growth factors. After 24 hours, the cells were stained for SLAM SKL and Annexin-V or active caspase-3 and the proportion of apoptotic cells determined by FACS. For dose ranging studies, cells were cultured as described using a dose range of 0.1 nM to 1 uM dmPGE<sub>2</sub> or vehicle control.

Quantitative-RT-PCR

Total RNA was obtained using the absolute RNA purification kit (Stratagene, La Jolla, CA). A constant amount of RNA was reverse transcribed with random primers (Promega, Madison, WI) and MMLV-reverse transcriptase (Promega) as described. DNase and RNase free water (Ambion, Austin, TX) was added to obtain a final concentration equivalent of 10 ng RNA/ul and 5 ul used for QRT-PCR. Primers for SYBR Green QRT-PCR were designed to produce an amplicon size of 75-150 bp. Sequences of primers are listed in Supplementary Table 1. QRT-PCR was performed using Platinum SYBR Green qPCR supermix UDG with ROX (Invitrogen, Carlsbad, CA) in an ABI-7000 (Applied Biosystems, Carlsbad, CA) or MxPro-3000 (Agilent, LaJolla, CA). Dissociation curves were determined on each analysis to confirm that only one product was obtained.

Statistical Analysis

All pooled values are expressed as Mean±SEM. Statistical differences were determined using the paired or unpaired two-tailed t-test function in Microsoft Excel as appropriate.
RESULTS

PGE₂ increases LTR-HSC frequency and engraftment

We previously showed that PGE₂ stimulates proliferation, cycling and differentiation of quiescent bone marrow cells into colony forming cells, suggesting that PGE₂ enhances HSC function ⁹. However, hematopoietic repopulation in myeloablated hosts is the only true measure of HSC function ¹⁴. A recent report by North et. al. ¹⁰ showed that pulse exposure to 16, 16 dimethyl PGE₂ (dmPGE₂) enhanced HSC frequency when transplanted into irradiated mice. We have confirmed enhancement of HSC frequency by PGE₂. In addition, using a limiting-dilution, competitive head-to-head transplant model of CD45.2 and CD45.1 congenic grafts in CD45.1/CD45.2 hybrid mice that permits quantitative comparison of engraftment and competitiveness of HSC from control and dmPGE₂ treatment groups, as well as endogenous repopulation of host cells within the same animal, we now show that short-term dmPGE₂ exposure produces stable long-term enhancement of HSC frequency and engraftment upon serial transplantation and that short-term exposure to dmPGE₂ increases the number of CRU and stably enhances HSC competitiveness (Figure 1A). At 12 weeks post-transplant, analysis of PB showed significantly increased chimerism of dmPGE₂-treated cells compared to vehicle-treated cells, with ~4-fold increase in HSC frequency and CRU, quantitative measures of long-term-repopulating capacity (Figure 1B). Throughout 20 weeks post-transplant, an ~4-fold increase in HSC frequency was maintained, indicating that the effect of dmPGE₂ pulse-exposure was stable (Figure 1C). At 32 weeks post-transplant, reconstitution was seen for peripheral B- and T-lymphoid and myeloid lineages, with no discernible
differences in lineage contribution between untreated competitor cells, dmPGE2 or vehicle-treated cells (Figure 1D).

Marrow was harvested from primary transplanted animals at 20 weeks post-transplant and transplanted into secondary recipients (Figure 1D) to validate expansion and self-renewal of LTRC previously exposed to dmPGE2 and vehicle. Analysis of PB 12 and 24 weeks after secondary transplant showed multi-lineage reconstitution by cells from all transplanted mice, clearly demonstrating the self-renewal of primary transplanted LTRC. Unlike the primary transplant, multi-lineage reconstitution by dmPGE2-treated cells showed an elevated myeloid lineage reconstitution. The increase in chimerism resulting from dmPGE2 exposure seen in primary donors was also observed in secondary transplants without any additional treatments.

**Murine and human HSPC express PGE2 receptors**

PGE2 interacts with 4 specific, highly conserved G-protein coupled receptors; EP1-EP4, with EP receptor repertoire accounting for multiple, sometimes opposing responses attributed to PGE2. While EP receptor expression has been observed in dendritic cells, monocytes, and early zebrafish hematopoietic tissue, EP receptor expression on hematopoietic stem and progenitor cell populations is not known. Analysis of EP receptors on c-kit+ Linneg (KL) cells, enriched for hematopoietic progenitor cells, Sca-1+ c-kit+ Linneg (SKL) cells enriched for multipotent progenitor cells as well as HSC, and SLAM (CD150+, CD48+) SKL and CD34+ SKL cells, highly enriched for primitive repopulating HSC, showed that all four EP receptors are expressed on these hematopoietic cell populations (Figure 2A). Analogous to murine cells, all four
receptors are expressed on human CD34⁺ UCB cells enriched for HSC and CD34⁺, CD38⁻ cells that contain the most primitive human HSC (Figure 2B). Quantitative RT-PCR showed that mRNA for all four EP receptors is detected in the whole bone marrow cell population and in FACS sorted KL, SKL and primitive CD34⁻ SKL cells (Figure 3A) and on common lymphoid (CLP) (Lin⁻, c-kitlow, Sca-1low, IL7R⁺) and myeloid (CMP) (Linneg, c-kit⁺, Sca-1⁻, CD34⁺) progenitor cells (not shown). Similarly, QRT-PCR analysis detected mRNA for all four EP receptors in purified CD34⁺ and CD34⁺, CD38⁻ UCB cells (Figure 3B).

**PGE₂ increases HSC homing efficiency**

Enhanced HSC engraftment by PGE₂ could result from increased HSC number and/or cell cycle status, effects on facilitating cells or effects on HSC homing or proliferation in the host marrow. In order to evaluate the mechanism of action of PGE₂ on HSC engraftment, we first utilized CFSE-labeled dmPGE₂ or vehicle-treated WBM cells transplanted into lethally-irradiated hosts to assess HSC homing. Total CFSE⁺ cells homing to bone marrow as well as the number of homed events within the KL and SKL cell populations were quantitated (Figure 4A). No difference in the percentage of CFSE⁺ cells homing to marrow was observed between dmPGE₂ and vehicle-treated cells when total WBM cells were evaluated; however, significantly more SKL cells homed to the marrow compared to control. In a congenic model, where homed cells are detected based upon CD45 cell surface variants, a significantly greater percentage of dmPGE₂-treated SKL cells homed to marrow (Figure 4B) compared to vehicle-treated or to non-
manipulated control cells. No difference in homing efficiency was seen between untreated and vehicle-treated cells.

To determine whether the enhancing effect of dmPGE2 on HSC homing was direct or indirect, we compared homing of highly purified SKL cells from both CD45.2 and CD45.1 mice in a head-to-head model. FACS sorted SKL cells were treated with dmPGE2 or vehicle and transplanted into CD45.1/CD45.2 mice. An additional cohort was transplanted with congenic strain and treatment groups switched to test for strain bias. Similar to studies using WBM, dmPGE2 pulse-exposure of purified SKL cells increased their homing efficiency by 2-fold (Figure 4C), strongly suggesting a direct effect on HSC. Although SKL cells are not a homogenous HSC population, they are highly enriched for LTRC25.

Immunodeficient mice offer the ability to evaluate human HSC function in an in vivo setting26 and are a validated model for human HSC homing27. To verify that the enhancing effect of dmPGE2 on mouse HSC homing is also seen on human HSC, UCB mononuclear cells were pulsed with dmPGE2 or vehicle and HSC homing evaluated in sublethally-irradiated NS2 mice (Figure 4D). Similar to mouse HSC, dmPGE2 pulse-exposure significantly enhanced the homing efficiency of UCB CD34+ cells.

**PGE2 increases HSPC CXCR4 and chemotaxis to SDF-1α**

The stromal-cell-derived factor-1α (SDF-1α)/CXCR4 axis is believed to play a major role in HSPC trafficking and chemoattraction/homing to the bone marrow microenvironment11. In addition, up-regulation of CXCR4 on human CD34+ cells28 and endothelial cells29 by PGE2 has been reported, and PGE2 can increase monocyte
chemotaxis to SDF-1$\alpha$ \(^{19}\). We therefore evaluated whether the mechanism of improved homing of dmPGE$_2$-treated HSPC resulted from up-regulation of SDF-1$\alpha$/CXCR4 signaling. Pulse-exposure to dmPGE$_2$ increased CXCR4 expression on KL and SKL cells (Figure 5A). Similarly, dmPGE$_2$ increased CXCR4 expression on CD34$^+$ UCB cells. QRT-PCR demonstrated elevated CXCR4 mRNA levels in dmPGE$_2$-treated SKL cells compared to vehicle (2.65-fold).

In vitro, HSPC selectively migrate to a gradient of SDF-1$\alpha$ \(^{30}\), a process that is believed to reflect their marrow homing capacity. We evaluated the effect of dmPGE$_2$-treatment on HSC chemotaxis to a gradient of SDF-1 in in vitro transwell migration assays to determine if PGE$_2$-mediated CXCR4 up-regulation enhanced chemotaxis. Both vehicle and dmPGE$_2$-treated SKL cells demonstrated significant migration to 1-1000 ng/ml SDF-1, however, chemotaxis was significantly higher in cells treated with dmPGE$_2$ (Figure 5B). Analysis of positive and negative gradients indicated that the dmPGE$_2$-enhancing effect on SKL cell chemotaxis did not result from a nonspecific increase in chemokinesis (Figure 5B-top inset). Enhanced migration to SDF-1 by dmPGE$_2$ was also observed using FACS-sorted SKL cells, suggesting a direct effect on HSC (Figure 5B-bottom inset). Chemotaxis of UCB CD34$^+$ cells to SDF-1 was also significantly enhanced by pulse-exposure to dmPGE$_2$ and migration was blocked by the selective CXCR4 antagonist AMD3100 \(^{31}\), indicating a specific effect mediated through the CXCR4 receptor (Figure 5C).

To specifically determine if up-regulated CXCR4 played a role in the enhanced homing observed after dmPGE$_2$ treatment, cells were treated with AMD3100 prior to evaluation of in vivo homing. PGE$_2$ pulse-exposure increased homing of SKL cells as
described earlier, and incubation of vehicle or dmPGE2-pulsed cells with AMD3100 significantly reduced SKL cell homing (Figure 5D) and abrogated the improved homing efficiency of dmPGE2-pulsed cells. Pulse-exposure to dmPGE2 enhanced SKL cell homing efficiency by 2.6±0.3 fold (P<0.05), which was reduced to 1.3±0.2 fold (P=NS) in the presence of AMD3100. AMD3100 reduced overall homing by 42±5% (range 31-64), consistent with previous reports 32;33.

**PGE2 decreases HSC apoptosis and increases Survivin**

PGE2 treatment produces a ~4-fold increase in HSC and CRU frequency (Figure 1), but only a ~2-fold enhancement in homing (Figure 4), which suggests that other mechanisms are involved in enhanced engraftment. Apoptosis is an important regulatory process in normal and malignant hematopoiesis 34 and PGE2 has been implicated in anti-apoptotic signaling 35;36. Moreover, activation of cAMP, a downstream signaling molecule of EP receptors, inhibits apoptosis in CD34+ cells 37. We hypothesized that dmPGE2-treatment affects survival and/or proliferation of HSC, contributing to enhanced engraftment.

Under conditions of reduced serum concentration, dmPGE2 pulse-exposure significantly reduced intracellular active caspase-3 in SLAM SKL cells (Figure 6A). Dose ranging studies using Annexin-V as an additional marker of apoptosis indicated that dmPGE2 inhibited apoptosis in a dose-dependent fashion, reaching ~65% inhibition at 1 uM (Figure 6A-Inset).

We previously showed that the inhibitor of apoptosis protein Survivin regulates apoptosis and proliferation of HSC 12;38 and PGE2 has been reported to alter Survivin levels in cancer cells 39;40. We therefore evaluated if PGE2 affected Survivin expression in
HSPC. At 24 hours post-dmPGE2-pulse, intracellular Survivin levels were significantly higher in murine SKL cells and human CD34+ UCB cells (1.7 and 2.4 fold, respectively) compared to control (Figure 6B). QRT-PCR analysis of treated SKL cells similarly indicated elevated Survivin mRNA compared to control (2.94-fold). In a kinetic analysis, decreased active caspase-3 coincident with an increase in Survivin was seen at 24, 48, and 72 hours post-exposure of SKL cells to dmPGE2 compared to control (Figure 6C), consistent with the caspase-3 inhibiting activity of Survivin.

**PGE2 increases HSC proliferation**

In previous reports, we showed that Survivin regulates HSC entry into and progression through cell cycle. Furthermore, β-catenin, implicated in HSC proliferation and self-renewal, lies downstream of EP receptor pathways. The ability of PGE2 to modulate these cell cycle regulators suggests that an increase in HSC self-renewal and proliferation might contribute to the enhanced engraftment of dmPGE2-pulsed cells. To test this hypothesis, we analyzed the cell cycle status of dmPGE2 or vehicle-pulsed SKL cells *in vitro*. Pulse-exposure to dmPGE2 increased SKL cell cycling (Figure 7A), with 60% more SKL cells in G1+S/G2M phase of the cell cycle after dmPGE2 treatment compared to controls. To evaluate the effect of PGE2 exposure on primitive, quiescent HSC, we performed additional *in vitro* studies using SLAM SKL cells. Similar to SKL cells, *in vitro* dmPGE2 pulse-exposure significantly increased the proportion of SLAM SKL cells in cell cycle (G1+S/G2M) by 24% (Table 1). No significant effect on cell cycle rate of KL or Lin<sup>neg</sup> cells was seen (not shown); suggesting that dmPGE2 selectively increases HSC cycling state.
To confirm the effect of dmPGE2 on enhancement of HSC cell cycle observed *in vitro*, bone marrow cells were pulsed with dmPGE2 and injected into congenic mice treated with BrdU post-transplant, and the proportion of donor BrdU+ SKL cells determined 16 hours later (Figure 7B). A ~2-fold increase in the proportion of homed SKL cells in S+G2/M phase was observed for cells pulsed with dmPGE2 prior to transplant, confirming that short-term exposure of HSC to dmPGE2 stimulates HSC to enter and progress through cell cycle *in vivo*. 
DISCUSSION

It is well documented that PGE\textsubscript{2} participates in regulation of hematopoiesis, both inhibiting myelopoiesis \textit{in vitro} \textsuperscript{2} and \textit{in vivo} \textsuperscript{4} and promoting erythroid and multi-lineage colony formation \textsuperscript{5,6} and enhancing proliferation of CFU-S \textsuperscript{7} and CFU-GM \textsuperscript{8}. In addition, PGE\textsubscript{2} stimulates production of cycling HPC from the quiescent bone marrow compartment \textsuperscript{9}, suggesting that PGE\textsubscript{2} has biphasic effects on hematopoiesis. These studies implicated PGE\textsubscript{2} in stem cell function, but did not directly evaluate HSC. Moreover, one cannot rule out that inhibition of colony formation by PGE\textsubscript{2} resulted from modulation of HSC commitment to self-renewal versus differentiation, thus reducing colony formation. Recently, \textit{ex vivo} exposure of bone marrow cells to PGE\textsubscript{2} was shown to facilitate murine hematopoietic cell engraftment \textsuperscript{10}, validating previous studies that PGE\textsubscript{2} enhances HPC production and extending the role of PGE\textsubscript{2} to stimulation of HSC function. However, the mechanism by which PGE\textsubscript{2} produced this effect was not defined.

We now demonstrate, for the first time, that PGE\textsubscript{2} has direct and stable effects on long-term repopulating HSC, as determined by serial transplantation, and facilitates HSC engraftment by increasing CXCR4, enhancing migration to SDF-1 and homing to bone marrow, up-regulating Survivin expression that blocks HSC apoptosis, and increasing the proportion of LTR-HSC entering into and progressing through cell cycle.

Direct comparison in competitive transplant models showed that short-term exposure of HSC to PGE\textsubscript{2} produces a ~4-fold competitive advantage, consistent with published results \textsuperscript{10}. However, previous studies showed a maximal effect on HSC frequency at 12 weeks post-transplant with reduced HSC frequency at 24 weeks,
suggesting a greater effect on short-term rather than long-term repopulating cells. Our studies show that PGE2-induced enhancement of HSC frequency was stable throughout a >20 week period and was maintained in secondary transplants through 24 weeks, clearly indicating a sustained effect on LTRC. The reasons for this difference in repopulating stability are not clear, but may relate to more precise head-to-head quantitation of HSC competition in our model.

Full hematopoietic reconstitution was observed in serially transplanted recipients using either control or PGE2-treated cells, indicating no adverse impact of PGE2 on HSC self-renewal. In fact, a trend towards increased LTRC activity was seen, indicating that the enhancing effect of short-term PGE2 exposure on HSC observed in primary transplants was long lasting, since no additional treatment was performed on cells or animals before secondary transplants. While it is commonly assumed that a single HSC compartment gives rise to all hematopoietic lineages, recent studies have demonstrated the presence of normal HSC biased towards lymphoid or myeloid differentiation 44. In secondary transplants, we observed a myeloid bias in mice transplanted with PGE2-treated HSC, suggesting a possible selective effect of PGE2 on myeloid-biased HSC. However, white blood cell counts in secondary transplanted mice have remained within normal ranges. Continued analysis of transplanted mice and re-transplantation studies will validate these findings and determine their significance, if any.

While it was suggested that PGE2 does not affect HSC homing, earlier studies evaluated WBM 10 and did not specifically assess HSPC. When evaluating total transplanted cells we also observed no difference in homing efficiency between control and PGE2-treated cells; however, enhanced homing of SKL cells by PGE2 was clearly
evident. Furthermore, enhanced homing efficiency of PGE$_2$-treated, sorted SKL cells was observed, suggesting a direct effect on HSC. PGE$_2$ also enhanced homing of human CD34$^+$ UCB in immunodeficient NS2 mice, strongly indicating translation of HSC enhancement to human stem cell products. Although more primitive populations of HSC than defined by SKL can be identified, (e.g. CD34$^-$ SKL and SLAM SKL), the small number of homed events that can ultimately be detected using these markers precludes the ability to define effects of PGE$_2$ on these extremely rare cells in vivo in individual mice as we performed. The fact that we see similar activities of PGE$_2$ on LTRC and on SKL and SLAM SKL cells in a number of assays of HSC function without significant effects on the progenitor cell-enriched KL cell population, indicates that the SKL cell fraction is a valid indicator of the effects of PGE$_2$ on HSC homing.

PGE$_2$ treatment increased SKL CXCR4 mRNA and surface expression, consistent with effects of PGE$_2$ on CXCR4 in CD34$^+$ cells$^{28}$. This increase in CXCR4 corresponds directly with a functional increase in chemotaxis to SDF-1, and chemotaxis was blocked using AMD3100. In addition, AMD3100 significantly reduced the enhancing effect of PGE$_2$ on homing in vivo; suggesting that increased CXCR4 expression and chemotaxis to marrow SDF-1 are largely responsible for the enhanced homing effect, although additional effects on adhesion molecule expression or function cannot be excluded. We also found elevated mRNA and protein levels of Survivin, with concomitant reduced active caspase-3 in PGE$_2$-treated SLAM SKL cells. It is likely that enhanced HSC survival, mediated through Survivin, also contributes to enhanced engraftment.
Pulse-exposure to PGE2 increased the proportion of HSC in cell cycle by ~2-fold, with increased frequency of HSC, CRU and homed BrdU+ SKL cells and maintenance of enhanced HSC frequency in primary and secondary transplants, suggesting that PGE2 pulse-exposure initiated at least a single round of HSC self-renewal. EP2 and EP4 receptor activation can result in phosphorylation of glycogen synthase kinase-3 (GSK-3) and increased β-catenin signaling, which is downstream of the Wnt pathway that has been implicated in HSPC survival and self-renewal. Signaling by PGE2 through EP4 can directly increase β-catenin and synergistic cross-talk between COX-2 and Wnt pathways has been suggested. Further exploration of specific signaling pathways and EP receptors involved in mediating the effects of PGE2 may refine our understanding of the role of PGE2 on HSC function. While it has been suggested that cycling cells have reduced marrow homing, which may be the result of triggered apoptosis, it is clear that PGE2-treated cells have both enhanced homing and enhanced migration, despite their enhanced cycling. This may be explained by the increase in CXCR4 migratory response overcoming deficits in cycling-cell homing and/or increased homing occurring before an increase in cycling. Additionally, PGE2 may protect homed cycling HSC from apoptosis, thus allowing for simultaneous enhanced homing, survival and proliferation in these cells.

We previously reported that Survivin is required for HSC to enter and progress through cell cycle and deletion in conditional knockout mice indicates Survivin is required for HSC maintenance (Fukuda et. al, Blood 108: 258a, 2007). Survivin also facilitates HSPC cell cycle through p21WAF1/CDKN1, known to be involved in HSC function, and blocks caspase-3 activity, recently implicated in HSC self-renewal.
Our findings that PGE$_2$ up-regulates Survivin, which is consistent with previous reports in cancer$^{40}$ and dendritic cells$^{39}$, and decreases intracellular levels of active caspase-3 in primitive HSC, suggest that the Survivin pathway may also be involved in the effects of PGE$_2$ on self-renewal. It is interesting to note that Survivin$^{51}$ and CXCR4$^{52}$ transcription are both up-regulated by the transcription factor hypoxia-inducible factor-1$\alpha$ (HIF-1$\alpha$), which can be stabilized by PGE$_2$$^{53}$, potentially linking these PGE$_2$ responsive pathways.

In summary, we have defined specific mechanisms of action and new pathways for enhancement and regulation of HSC function by PGE$_2$. The 4-fold increase in HSC frequency and engraftment produced by exposure to PGE$_2$ results from the cumulative effect of a 2-fold increase in HSC homing and a 2-fold increase in HSC cell cycle activity. Although the precise signaling pathways remain to be determined, enhanced engraftment involves up-regulation of CXCR4 and Survivin, with subsequent increased chemotactic response to SDF-1 and reduced apoptosis. The ability to easily improve homing and survival/proliferation of HSC by short-term PGE$_2$ exposure is exciting from a clinical perspective, especially in transplant settings where insufficient or low HSC numbers are found, e.g. UCB and some mobilized peripheral blood stem cell products. Our limiting dilution transplant studies show that equivalent engraftment is achieved with one-fourth the number of PGE$_2$-treated cells compared to controls, supporting a use for PGE$_2$ when HSC numbers are limiting. Homing and migration studies utilizing UCB CD34$^+$ cells clearly suggest potential translation to human hematopoietic grafts. Lastly, it will be interesting to determine if enhanced engraftment/recovery can be achieved by administering PGE$_2$ in vivo or if PGE$_2$ used in vivo can further facilitate engraftment of
HSC exposed to PGE₂ *ex vivo*. In COX2 knockout mice, recovery from 5-FU is delayed suggesting that COX2 activation and subsequent PGE₂ production may be critical for HSC expansion.
ACKNOWLEDGEMENTS

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AUTHORSHIP

Contribution: J.H. designed research, performed research, collected data, analyzed data, performed statistical analysis, and wrote the paper. P.S. designed research, performed research, collected data and analyzed data. J.S. participated in performance of research and collection and analysis of data. L.M.P. participated in designing research, analysis of the data, coordination and performance of the study, and wrote the paper.

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Table 1

Effects of short term *in vitro* exposure of SLAM SKL cells to dmPGE₂ on cell cycle

<table>
<thead>
<tr>
<th>In vitro treatment</th>
<th>G₀</th>
<th>G₁</th>
<th>S+G₂M</th>
<th>% cells in cycle b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>63.4±2.5</td>
<td>2.6±0.7</td>
<td>33.8±2.1</td>
<td>36.4±2.4</td>
</tr>
<tr>
<td>1 μM dmPGE₂</td>
<td>54.8±2.2*</td>
<td>6.8±1.9*</td>
<td>38.4±1.6*</td>
<td>45.2±2.2*</td>
</tr>
</tbody>
</table>

a. Lin⁰neg cells treated with either 1 μM dmPGE₂ or vehicle for 2 hours and cultured in the presence of growth factors (50 ng/ml rmSCF, 100 ng/ml each of rhFlt-3 and rhTPO) for 20 hours, were stained for SLAM SKL, Hoechst-33342 and Pyronin-Y and the proportion of SLAM SKL cells in G₀, G₁, S and G₂/M phase of the cell cycle determined by quantitation of DNA and RNA content by FACS. Data are Mean ± SEM for n=9 mice, each assayed individually.

b. Percentage of cells in G₁+S+G₂M; Combined data for n=9 mice.

* P<0.05 compared to vehicle control
FIGURE LEGENDS

Figure 1. PGE$_2$ enhances hematopoietic stem cell engraftment

(A) Test bone marrow from CD45.1 or CD45.2 mice were treated with vehicle or dmPGE$_2$ respectively. CD45.1/CD45.2 hybrid marrow cells were used as competitors. Limiting dilutions were transplanted into lethally irradiated (1100cGys, split dose) CD45.1/CD45.2 hybrid mice and chimerism in PB analyzed for 20 weeks. A representative flow plot detecting each cell population is shown.

(B) Frequency analysis (top) for vehicle (red) or dmPGE$_2$ (blue) pulsed cells, determined by Poisson statistics, at 12 weeks; $P_0=85,560$ (vehicle) and $P_0=23,911$ (dmPGE$_2$ treated). Chimerism in PB and CRU analysis is shown at 12 weeks (Mean ± SEM). Data represent 2 pooled experiments, n=5 mice/group/experiment, each assayed individually. * P<0.05 compared to vehicle control.

(C) HSC frequency analysis in recipients of vehicle or dmPGE$_2$-treated bone marrow over 20 weeks. Fold change indicates increase in frequency of engraftment of dmPGE$_2$-pulsed cells compared to vehicle.

(D) Representative FACS plots of multi-lineage reconstitution (myeloid (M), B (B) and T-lymphoid (T)). Multi-lineage analysis for primary transplant (32 weeks) and a cohort of 4 mice that received transplants from primary transplanted mice at 20 weeks, with analysis 12 weeks later. For primary transplanted mice at 32 weeks, vehicle-treated cells were (Mean ± SEM) 14.1±3.5% M, 70.8±1.1% B, and 17.8±1.4% T, vs. dmPGE$_2$-treated cells which were 15.7±2.5% M, 76.9±3.4% B, and 7.5±1.2% T. For secondary transplanted mice at 12 weeks, vehicle-treated cells were 15.7±5.3% M, 60.3±4.8% B, and 22.1±3.6% T, vs. dmPGE$_2$-treated cells which were 37.0±6.5% M, 52.3±5.4% B, and
9.0±1.4% T. * P<0.05 vs. vehicle control. Increased chimerism of dmPGE2-treated cells
vs. vehicle is shown for primary transplant at 20 weeks (time of secondary transplant)
and in a sub-cohort at 32 weeks (time of 12 week analysis of secondary transplant), for
secondary transplant at 12 weeks and 24 weeks. Data for 20 week primary transplant
were from 2 pooled experiments, n=5 mice/group/experiment, each assayed individually.
Data for secondary transplants were from n=5 mice/group, each assayed individually.

**Figure 2. PGE2 receptors are expressed on murine and human HSPC**

(A) Representative FACS gating of Lin^- murine bone marrow showing c-kit^+ and Sca-
1^+ gates and SLAM (CD150^+, CD48^-) and CD34 gating of SKL cells. EP1-EP4 surface
receptor expression on murine KL, SKL, SLAM SKL and CD34^- SKL cells is shown.
(B) Representative FACS gating of human CD34^+ and CD34^+, CD38^- UCB cells. EP
surface receptor expression on CD34^+ and CD34^+, CD38^- cells is shown.

**Figure 3. Amplification plots for mRNA for PGE2 receptors**

(A) Primers designed to specifically detect murine EP1, EP2, EP3 or EP4 were used for
QRT-PCR (with SYBR green) and plots with an activation step of 50 °C for 2 min,
denaturation at 95 °C for 2 min and amplification for 45 cycles at 95 °C-15 sec, 50 °C-30
sec, 72 °C-30 sec are shown. Plots corresponding to specific EP receptors are indicated in
each amplification plot, where the legend key on the right shows the relative order of
transcripts top to bottom. * Denotes the presence of at least 2 dissociation peaks
indicating the presence of splice variants.
(B) EP receptor amplification on human UCB CD34+ and CD34+ CD38- cells with the same QRT-PCR procedure as above.

**Figure 4: PGE₂ increases homing efficiency of HSPCs**

(A) Test murine bone marrow cells were labeled with CFSE and treated with vehicle (red) or dmPGE₂ (blue) and 2x10⁷ labeled and treated WBM cells were transplanted into lethally irradiated mice. Sixteen hours later, bone marrow was analyzed by FACS for homed events. Data are expressed as Mean ± SEM, n=6 mice/group, each assayed individually.

(B) Test bone marrow cells from CD45.1 mice were treated with PBS, vehicle or dmPGE₂, and 2x10⁷ treated WBM cells were transplanted into lethally irradiated CD45.2 mice. Sixteen hours later bone marrow was analyzed for homed SKL cells. The left panel shows representative data from 1 experiment, n=3 mice/group, each assayed individually. The right panel shows the combined increase in homing efficiency of SKL cells after dmPGE₂ treatment for 3 experiments (n=6 mice/group/expt, each assayed individually).

(C) SKL cells from CD45.1 and CD45.2 mice were isolated by FACS sorting and treated with either dmPGE₂ or vehicle. Five lethally irradiated CD45.1/CD45.2 hybrid mice received 3x10⁴ vehicle-treated CD45.1 sorted SKL plus 3x10⁴ dmPGE₂-treated CD45.2 SKL cells (top panel). Five mice received a similar transplant with treatment groups switched between strains (bottom panel). Representative flow gating of marrow 16 hours post-transplant and combined data for the homing efficiency of dmPGE₂ or vehicle-
treated, sorted SKL cells (Mean ± SEM, n=10 mice, each assayed individually) are shown.

(D) Low density UCB mononuclear cells (LDMC) were isolated and treated with either dmPGE₂ or vehicle. Five sublethally irradiated NS2 mice received dmPGE₂ treated LDMC and 5 received vehicle treated LDMC. Bone marrow was analyzed 16 hours later and the number of CD₃₄⁺ cells determined and homing efficiency calculated. Data are Mean ± SEM for n=5 mice, each assayed individually.

**Figure 5. CXCR4 receptor expression is increased on murine and human HSPC after dmPGE₂ treatment**

(A) CXCR4 expression (Mean ± SEM; n=3) on murine KL and SKL cells, and human CD₃₄⁺ UCB cells 24 hours after treatment with dmPGE₂. Data are expressed as % change in mean fluorescence intensity (MFI) of CXCR4 due to treatment with dmPGE₂ or vehicle.

(B) Freshly isolated Lin⁻ cells were pulsed with dmPGE₂ or vehicle for 2 hours, washed and resuspended in media with 10% HI-FCS and cultured at 37 °C for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA and allowed to migrate to rmSDF-1α for 4 hours. Total cell migration was quantitated by flow cytometry. Data are the Mean ± SEM percent migration for 3 experiments. † P<0.05 for dmPGE₂ treated cells compared to cells treated with vehicle. (Top Inset) Percent migration of gated SKL cells to positive (100 ng/ml SDF-1 in bottom chamber), negative (100 ng/ml SDF1 in upper chamber) or neutral (100 ng/ml SDF-1 in both upper and bottom chambers)
gradients. Data are the Mean ± SEM percentage migration for 3 experiments. † P<0.05 for dmPGE2 treated cells compared to cells treated with vehicle. (Bottom Inset) Percent migration of sorted SKL cells to 100 ng/ml SDF-1. Data are the Mean ± SEM percentage migration for 3 experiments. † P<0.05 for dmPGE2 treated cells compared to cells treated with vehicle.

(C) Freshly isolated UCB CD34+ cells were pulsed with dmPGE2 or vehicle for 2 hours, washed and resuspended in media with 10% HI-FCS and cultured at 37 °C for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA and migration to rhSDF-1 quantitated by flow cytometry. To block the CXCR4 receptor, replicate cells were incubated with 5 μg/ml AMD3100 for 30 minutes prior to the migration assay. Data are the Mean ± SEM percentage migration for 3 experiments. † P<0.05 for dmPGE2 treated cells compared to cells treated with vehicle.

(D) Homing efficiency of vehicle and dmPGE2 treated cells to bone marrow in the absence and presence of 10 μM AMD3100. Cells were incubated with AMD3100 for 30 minutes prior to the homing assay. Data are expressed as Mean ± SEM; n=3 mice/group, each assayed individually.

Figure 6. PGE2 decreases apoptosis, increases Survivin expression and decreases active caspase-3 in SKL cells

(A) Lin− bone marrow cells were treated with dmPGE2 or vehicle and cultured in media supplemented with 2% FBS without growth factors for 24 hours to induce apoptosis. Cultured cells were stained for SKL and SLAM and PE-anti-active caspase-3 and/or FITC-Annexin-V and the proportion of SKL or SLAM SKL cells undergoing apoptosis
determined by FACS. (Inset) dose response analysis of the effects of dmPGE$_2$ on SKL cell apoptosis.

(B) Fold increase in intracellular Survivin levels (mean fluorescence intensity (MFI)) in control and dmPGE$_2$-pulsed murine SKL and human CD34$^+$ cells 24 hours after treatment; data are Mean ± SEM from 3 experiments, n=3 mice/group; each assayed individually, or 3 separate cord blood samples.

(C) Evaluation of intracellular Survivin and active caspase-3 levels (MFI) in SKL cells 24, 48, and 72 hours post-treatment with dmPGE$_2$. Data expressed as Mean ± SEM for 3 experiments; n=3-6 mice/group, each assayed individually; *P<0.05.

**Figure 7. PGE$_2$ increases the proliferation of SKL cells in vitro and in vivo**

(A) Lin$^{neg}$ cells were treated with either vehicle or 1 uM dmPGE$_2$ for 2 hours, washed and cultured in media with rmSCF, rhFlt3 and rhTpo. After 20 hours cells were stained for SKL and Hoechst-33342 and Pyronin-Y. The proportion of SKL cells in cell cycle were quantitated by FACS. Representative flow plot showing cell cycle distribution of gated SKL cells and combined data for fold increase in cell cycle for dmPGE$_2$-treated cells compared to vehicle control from 3 experiments, Mean ± SEM, n=9 mice, each assayed individually.

(B) CD45.1 Lin$^{neg}$ bone marrow cells were treated with dmPGE$_2$ or vehicle and transplanted into lethally irradiated CD45.2 mice. Immediately after transplantation, BrdU was provided in drinking water and administered by IP injection. Bone marrow was analyzed 16 hours later and the proportion of CD45.1$^+$, SKL cells that were BrdU$^+$
was analyzed by FACS analysis. Data are Mean ± SEM, n=5 per mice/group, each assayed individually.
**Figure 1**

A. Schematic representation of the experimental setup:
- **Vehicle**
- **dmPGE\textsubscript{2}**
- Competitors

CD45.1 → CD45.1/2 → Transplant into irradiated recipient

B. Graphs showing:
- Repopulating cell frequency over weeks post-transplant.
- % Chimerism vs. competitor ratio.
- CRU per 100K Cells.

C. Table summarizing repopulating cell frequency:

<table>
<thead>
<tr>
<th>Weeks Post-transplant</th>
<th>Repopulating Cell Frequency</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1:69,466</td>
<td>4.18</td>
</tr>
<tr>
<td>8</td>
<td>1:85,560</td>
<td>3.48</td>
</tr>
<tr>
<td>12</td>
<td>1:85,560</td>
<td>3.58</td>
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<tr>
<td>16</td>
<td>1:85,560</td>
<td>3.58</td>
</tr>
<tr>
<td>20</td>
<td>1:89,586</td>
<td>4.12</td>
</tr>
</tbody>
</table>

D. Bar charts comparing primary and secondary transplants:
- % of Total WBC: T-Lymphoid, B-Lymphoid, Myeloid.
- % Chimerism at different time points:
  - 20 Weeks Primary: P<0.001
  - 32 Weeks Primary: P<0.01
  - 12 Weeks Secondary: P<0.02
  - 24 Weeks Secondary: P<0.01
Figure 2

A

Sca-1 PE

c-kit APC

EP Receptors

Counts

CD48

CD150

CD34

SKL

KL

B

SSC

CD34

Counts

EP Receptors

CD34

CD38

Counts

EP1

EP2

EP3

EP4

Iso

EP1

EP2

EP3

EP4

For personal use only.
Figure 4

A. Label and ex vivo treat CD45.2

Transplant CD45.2

Analyze homed events @ 16 hours

% CFSE +

WBM, KL, SKL

B. Ex vivo treatment CD45.1

Transplant CD45.2

Analyze homed events @ 16 hours

% Homing Efficiency SKL cells

Vehicle dmPGE2

% Homing Efficiency SKL cells

P<0.05

P<0.001

C. Vehicle dmPGE2

CD45.1

Transplant CD45.2

CD45.1/2

dmPGE2 Vehicle

CD45.1

Transplant CD45.2

CD45.1/2

% Homing Efficiency SKL cells

P<0.001

D. % Homing Efficiency CD34+ UCB cells

Vehicle dmPGE2

P<0.05
Figure 5

A. % ΔMFI for CXCR4

B. Migration of SKL (% of Input)

C. % CD34+ cell migration

D. % Homing Efficiency SKL Cells
Figure 6

A

% change over vehicle control

Time after exposure to dmPGE₂

B

% Annexin-V+

vehicle dmPGE₂

C

% change over vehicle control

Time after exposure to dmPGE₂

P<0.05

P<0.005

vehicle dmPGE₂
A

<table>
<thead>
<tr>
<th>Pyronin</th>
<th>Hoeschst</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>dmPGE₂</td>
</tr>
<tr>
<td>30.43%</td>
<td>38.61%</td>
</tr>
<tr>
<td>8.70%</td>
<td>25.04%</td>
</tr>
<tr>
<td>53.26%</td>
<td>28.36%</td>
</tr>
<tr>
<td>7.61%</td>
<td>7.99%</td>
</tr>
</tbody>
</table>

Fold Increase in S+G₂M

B

Treat Lin⁻
Marrow

CD45.2

BrdU i.p.

Transplant

Analyze homed SKL for BrdU @ 16 hours

Fold Change in SKL G₁+S/G₂M

P<0.01

vehicle dmPGE₂

P<0.05

vehicle dmPGE₂
Prostaglandin E\textsubscript{2} enhances hematopoietic stem cell homing, survival, and proliferation

Jonathan Hoggatt, Pratibha Singh, Janardhan Sampath and Louis M. Pelus