Published in final edited form as:
Exp Cell Res. 2014 December 10; 329(2): 220–226. doi:10.1016/j.yexcr.2014.07.030.

Getting more for your marrow: boosting hematopoietic stem cell numbers with PGE_2

Elliott J. Hagedorn, **Ellen M. Durand**, **Eva M. Fast**, and **Leonard I. Zon***
Stem Cell Program and Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute, Howard Hughes Medical Institute, Harvard Stem Cell Institute, and Harvard Medical School, Boston, MA 02115, USA

Abstract

Throughout the lifetime of an individual, hematopoietic stem cells (HSCs) self-renew and differentiate into lineages that include erythrocytes, platelets and all immune cells. HSC transplantation offers a potentially curative treatment for a number of hematopoietic and non-hematopoietic malignancies as well as immune and genetic disorders. Limited availability of immune-matched donors reduces the viable options for many patients in need of HSC transplantation, particularly those of diverse racial and ethnic backgrounds. Due to rapid availability and less stringent immune-matching requirements, umbilical cord blood (UCB) has emerged as a valuable source of transplantable HSCs. A single UCB unit contains a suboptimal number of HSCs for treating larger children or adults and there has thus been great clinical interest in expanding UCB HSCs *ex vivo* for use in transplantation. In this review we discuss the latest research and future avenues for the therapeutic use of small lipid mediator $dmPGE_2$ to expand HSC numbers for transplantation. Originally identified in a chemical screen in zebrafish, $dmPGE_2$ has now advanced to a phase II clinical trial as a therapy for patients with leukemia and lymphoma who are undergoing UCB transplantation.

Keywords

hematopoietic stem cells; *ex vivo* expansion; umbilical cord blood transplantation; self-renewal; engraftment; prostaglandin E_2

Introduction

Hematopoietic stem cells (HSCs) are a rare population of self-renewing progenitors that maintain life-long production of all blood and immune cell lineages[1]. HSCs are of

*Correspondence: zon@enders.tch.harvard.edu.

Conflicts of interest L.I.Z. is a founder and stockholder of FATE Therapeutics and Scholar Rock and a scientific advisor for Stemgent.

outstanding interest to clinical researchers for many reasons. The transplantation of these cells can be used to treat a variety of human diseases, including numerous cancers and a wide range of blood and immune disorders[2]. Transplantable HSCs can be obtained from three sources: bone marrow, peripheral blood and umbilical cord blood (UCB)[3]. Historically, bone marrow has been the standard source of HSCs for transplantation. Recently, it is becoming more common that HSCs are pharmacologically mobilized out of the marrow and then isolated from peripheral blood – an extraction procedure that is far less invasive.

The use of one's own stem cells, known as autologous transplantation, is the preferred method as it avoids immune differences that can lead to delayed engraftment, graft-versus-host disease and graft rejection[3]. This is often not possible, however, as it requires that healthy HSCs be obtained at an earlier date (e.g. pre-malignancy). The use of donor HSCs, known as allogenic transplantation, can work well if an HLA-matched donor is available. UCB serves as an attractive alternative source of HSCs for transplantation because the naïve state of immune cells within UCB reduces the stringency of immune-matching requirements[4]. UCB can be cryopreserved and stored in cord blood banks and is therefore more readily available. Although a rich source of HSCs, a single UCB unit contains an insufficient number of cells for treating larger children and adults. It is generally accepted that the number of transplanted HSCs directly correlates with the likelihood of successful engraftment and reduced time to immune recovery. It is therefore standard practice that two UCB units are administered, which puts a tremendous strain on cord blood bank resources. The use of two UCB units is also complicated by competition between the two cord bloods – one unit typically dominates by three months after the transplant[4]. The ability to expand the number of HSCs in a single UCB unit prior to transplantation would thus have great clinical significance.

Strategies to expand HSCs for transplantation

In recent years there have been a number of different strategies to expand UCB HSCs *ex vivo* for use in transplantation[5,6]. These efforts have in large part, however, seen limited success in terms of attaining clinically relevant numbers of cells. Expanding HSCs in culture requires maintaining the self-renewal properties of the cells while preventing their differentiation. These seemingly simple objectives have proven challenging to accomplish in a dish, when HSCs are outside of the *in vivo* microenvironment. Initial strategies to expand UCB HSCs *ex vivo* involved the addition of hematopoietic cytokines to the culture media, including factors such as SCF, Tpo, Flt-3L, IL-3, IL-6 and G-CSF[6]. Although the extent to which these factors enhance expansion is limited, it is worth noting that these so-called cytokine cocktails still serve as the basal media for many current HSC expansion protocols.

Recently, a number of small molecules have been shown to support HSC expansion. These include the ayrl hydrocarbon receptor agonist SR1[7], the copper chelator TEPA[8], the GSK-3β inhibitor BIO[9], NR-101[10] and prostaglandin E_2 (PGE_2)[11]. Interestingly, some of these compounds function as surrogates for the hematopoietic cytokines mentioned above. NR-101, for instance, binds and activates the Tpo receptor c-MPL and is even more effective at expanding HSCs than Tpo[10]. Compared to larger protein-based molecules, these

compounds offer several advantages as a method to modulate HSC numbers[12]. Small molecules are easier and cheaper to manufacture, and the window of treatment with small molecules can be tightly controlled. Chemicals can be removed, for example, before treated cells are introduced into patients – avoiding permanent modifications to the HSCs (e.g. retroviral gene insertion) that could potentially lead to dis-regulated proliferation and/or malignancy.

Other strategies the expand HSCs have been modeled more directly after the *in vivo* niche or the mechanisms that regulate HSC formation during development. Co-culturing HSCs with supportive cell types that are present in the bone marrow niche, such as mesenchymal stem cells, can enhance HSC expansion and improve the engraftment of UCB stem cells upon transplantation[13]. Modulation of certain developmental signaling pathways have also been shown to promote robust HSC expansion. Addition of soluble Wnt protein to the media increases HSC numbers and improves engraftment when the stem cells are transplanted into recipient animals[14]. Similarly, culturing UCB stem cells in the presence of an immobilized Notch ligand enhances HSC expansion and improves transplantability[15]. These latter approaches, targeting pathways that regulate HSC formation during normal development, have, to date, resulted in some of the best yields in *ex vivo* HSC expansion.

In this review we discuss the recent use of $dmPGE_2$ (a stable derivative of prostaglandin E_2) as a new strategy for HSC expansion that targets a developmental regulatory pathway while affording the therapeutic benefits of a small molecule. After just a brief *ex vivo* pulse of $dmPGE_2$, long-lasting effects on HSC function have been observed without over-proliferation side effects. $dmPGE_2$ was originally identified in a chemical screen in zebrafish and has now has now advanced to phase II clinical trials for UCB transplantation in patients with leukemia and lymphoma.

PGE_2 synthesis, reception and downstream intracellular signaling

Prostaglandins are members of the eiconsanoid family of lipid compounds – molecules that play major roles in inflammation and immune response, as well as regulate a number of physiological tissue responses such as blood pressure and stomach acid production[16]. Prostaglandins are synthesized by the cyclooxygenases Cox1 and Cox2, through oxidation of the derivative arachidonic acid to form PGG_2[17]. PGG_2 is then reduced to PGH_2, the molecule from which all three classes of prostaglandins are made. In general, Cox1 is more ubiquitously expressed, whereas Cox2 is silenced under normal physiology, but can be rapidly induced, for example, during times of stress.

Prostaglandins are not stored, but rather synthesized as needed and function locally as paracrine or autocrine lipid mediators, having a half-life of about one minute in circulation[16]. A number of different cell types produce PGE_2 and it can be found within most tissues throughout the body. PGE_2 can signal through four G-protein coupled E-prostanoid receptors PTGER1-4 (EP1-4), which, in contrast to PGE_2 itself, are expressed in more tissue-restricted patterns[18]. Activation of EP1, EP2 and EP4 result in positive signal transduction through either mobilization of intracellular Ca2+ (EP1) or stimulation of cAMP

production (EP2 and EP4). Binding to EP3, on the other hand, acts to negatively regulate cAMP.

A chemical screen in zebrafish identifies PGE_2 as a conserved regulator of vertebrate HSC homeostasis

In 2007 North *et al*. conducted a high-throughput chemical screen for small molecules that could modulate vertebrate HSC homeostasis[11]. Out of a library of nearly 2,500 biologically active compounds, the authors identified 82 chemicals that either increased or decreased the expression of *runx1* and *cmyb* (conserved HSC markers) in zebrafish embryos. Surprisingly, 10 of the identified compounds affected prostaglandin signaling. When embryos were treated with a long-acting derivative of PGE_2, 16,16-dimethyl-PGE_2 ($dmPGE_2$), a marked increase in HSCs numbers was observed in the aorta-gonad-mesonephros region (AGM) – the tissue from which definitive HSCs arise during development (Fig. 1A). Injection of morpholinos targeting Cox1 and Cox2 (the enzymes responsible for PGE_2 synthesis) or treatment with the Cox inhibitor indomethacin led to reduced *runx1/cmyb* staining in the AGM (Fig. 1A), indicating that endogenous prostaglandin signaling is necessary for HSC formation. Exposure to $dmPGE_2$ was also shown to enhance the marrow recovery of adult fish following a sub-lethal dose of irradiation. The authors went on to demonstrate that treatment with $dmPGE_2$ could amplify multi-potent progenitors in murine embryonic stem cell differentiation assays and enhance murine spleen colony forming units 12-days post-transplant. Limit dilution analysis in mice showed that treatment with $dmPGE_2$ also increased the frequency of long-term repopulating HSCs. Collectively, these studies demonstrated that PGE_2 was a conserved regulator of vertebrate HSC homeostasis.

Although unexpected, this work was not the first time PGE_2 had been linked to hematopoiesis. As far back as the 1970s, it had been documented that unmodified PGE_2 had effects on colony forming unit-spleen (CFU-S) stem cells and on more lineage-restricted hematopoietic progenitors using *in vitro* colony-forming assays[19–21]. These earlier studies, however, produced varying and sometimes conflicting results and in the two decades that followed, few new insights had come about. These new findings in the fish not only revitalized interest in the area but also provided strong evidence that $dmPGE_2$ could provide an effective means to boost stem cell numbers in a transplantation setting.

$dmPGE_2$ signals through EP2/EP4 and an interaction with the Wnt pathway

In zebrafish, Ptger2 (EP2) and Ptger4 (EP4) are the PGE_2 receptors expressed on HSCs. Knockdown of *ptger2* or *ptger4* results in a decrease of *runx/cmyb* expression in the AGM that is not rescued with PGE_2 treatment[11]. This suggests that PGE_2 signals through Ptger2 and Ptger4 to regulate HSC formation in zebrafish embryos. Interestingly, it had been demonstrated in previous biochemical studies that binding of PGE_2 to EP2 or EP4 can lead to activation of the GSK-3/β-catenin signaling pathway via protein kinase A and phosphoinositide 3-kinase, respectively[22]. Additional work in the zebrafish by Goessling *et al*., demonstrated an *in vivo* PGE_2-Wnt interaction. PGE_2 enhanced the effect of Wnt signaling during embryogenesis by stabilizing β-catenin, and was required for Wnt-mediated regulation of HSC development (Fig. 1D)[23].

All four EP receptors are expressed on murine progenitor populations (LSK) as well as more purified stem cell populations (LSKCD48^{-}CD150^{+}). EP1–EP4 receptors are also found on human CD34^{+}CD38^{-} cells[24]. This data, combined with the observed genetic interaction between PGE_2 and Wnt, suggests the EP2 and EP4 receptors in particular play an important role in the $dmPGE_2$-mediated regulation of HSCs. Knockdown of EP4 in LSK cells results in decreased repopulation ability following transplantation, as well as a skewing towards T cell and myeloid lineages during differentiation[25]. In contrast, EP2 knockout mice exhibit normal hematopoiesis, reconstitution capabilities, and differentiation. In addition, treatment of LSK cells with an EP4 agonist, but not an EP2 agonist, lead to an increase in phosphorylation of GSK3β and β-catenin. Co-treatment of a PKA inhibitor with PGE_2 blocks the activation of GSK3β. Together, this data suggests that PGE_2 acts through EP4 to activate the cAMP/PKA pathway in HSCs.

$dmPGE_2$ improves engraftment by enhancing HSC homing, survival and proliferation

The aforementioned studies implicated particular EP receptors and an interaction with the Wnt pathway, but the transcriptional changes occurring downstream of $dmPGE_2$ treatment, through which HSC dynamics were being affected, remained incompletely understood. More recent studies have provided additional mechanistic insight into the stem-cell-enhancing activity of $dmPGE_2$. In 2009, a study by Hoggatt *et al*. reported that *ex vivo* treatment of murine whole bone marrow (WBM) with $dmPGE_2$ improved engraftment, likely through an enhancement in HSC homing (via up-regulation of CXCR4) and survival (via up-regulation of Survivin and down-regulation of caspase-3) (Fig. 1B and D)[24]. The improvement in HSC function was maintained in secondary transplantation, without additional $dmPGE_2$ treatment, suggesting there was an effect on long-term (LT)-HSCs.

These initial findings have since been confirmed by additional studies looking at global gene expression patterns in response to $dmPGE_2$ treatment[26,27]. PGE_2 seems to both activate general cell survival and proliferation pathways but also lead to a specific up-regulation in cytokine response. Interestingly, recent work has implicated HSCs as potent secretors of cytokines in response to infectious stimuli[28]. Treating HSCs with $dmPGE_2$ could therefore activate the endogenous hematopoietic regeneration program that is turned on during injury. Consistent with the $dmPGE_2$-induced improvement in HSC homing, it was recently shown that blocking prostaglandin signaling can lead to HSC mobilization[29]. The exact temporal requirement of different cell migration and survival pathways, as well as the interplay with the niche during UCB transplantation, remains to be determined for $dmPGE_2$ treatment.

The cellular context, culture conditions and dose of $dmPGE_2$ are important

A common theme to emerge from recent studies is that variations in the dose of $dmPGE_2$ or the duration of exposure can have significantly different effects on HSC dynamics. This is likely, in part, why early studies with PGE_2 often gave varying and sometimes conflicting results. A short 2-hour *ex vivo* pulse on ice was shown to be sufficient to increase HSC numbers and enhance engraftment of treated cells upon transplantation into mice[11,24]. As part of a recent phase I clinical study, Cutler *et al*. used expression profiling to conduct an

extensive analysis of various $dmPGE_2$ treatment conditions[26]. It was concluded that a 2-hour pulse of 10 μM $dmPGE_2$ at 37°C in nutrient-free media were the optimal conditions for treating UCB cells *ex vivo* in a clinical transplantation setting.

Work by Frisch *et al*. provided the first data on the effects of *in vivo* $dmPGE_2$ treatment in mice[30]. The authors adopted a PGE_2 treatment protocol previously used in rats. $dmPGE_2$ was delivered by intraperitoneal injection (6 mg/kg), twice daily for 16 days (Fig. 1C). Similar to the *in vitro* data, a significant increase in LSK cell numbers was observed without adversely affecting hematopoietic differentiation. SLAM receptors were used to differentiate long-term HSCs (LT-HSCs) from multi-potent progenitors (MPPs) and short-term HSCs (ST-HSCs) and it was found that *in vivo* $dmPGE_2$ treatment expanded the ST-HSC/MPP subpopulation without changing the frequency of LT-HSCs. Cells transplanted from these *in vivo*-treated mice displayed increased engraftment and hematopoietic reconstitution in recipient animals; however, the competitive advantage was eventually lost at six weeks post-transplantation and in secondary transplants no significant difference was observed. These experiments suggested that long-term *in vivo* exposure to $dmPGE_2$ was having a different effect on HSCs compared to the short *ex vivo* pulse treatments.

Continuous exposure to $dmPGE_2$ may induce multiple rounds of excessive proliferation from which cells cannot recover; it may also lead to receptor down-regulation, effectively changing the responsiveness of the cells to $dmPGE_2$. Supporting this notion, data from cell culture experiments has shown that long-term treatment with $dmPGE_2$ results in down-regulation of EP2 and EP4 receptors[31] and patients with mutations in the prostaglandin transporter gene, *SLCO2A1*, have elevated levels of circulating PGE_2 and experience pancytopenia with bone marrow hypocellularity[32].

In zebrafish, mouse and humans, components of the prostaglandin pathway and PGE_2 receptors are present on other cells types in addition to HSCs, such as endothelial cells, stromal cells and leukocytes[16,33]. It is likely that *in vivo* $dmPGE_2$ treatments also affect these other cells that then indirectly influence HSCs. Consistent with this, studies from the Calvi Lab have documented changes in trabecular bone microarchitecture and macrophage numbers following *in vivo* treatment with $dmPGE_2$[30,34]. In addition, the Lapidot Lab demonstrated that *in vivo* and *in vitro* treatment with $dmPGE_2$ increased expression of CXCL12 on a population of $nestin^+$ stromal cells that are adjacent to HSPCs and play a role in their preservation[35]. As many of the HSC sources used in *ex vivo* studies are heterogeneous cell populations (e.g. WBM, CD34+ and LSK cells), it is possible that similar non-cell autonomous effects could occur in these contexts. Interestingly, it was recently published that $dmPGE_2$ promotes the survival of naïve UCB T cells through the Wnt/β-catenin pathway[36]. This protective effect could enhance recipient T-cell chimerism following transplant, which would have significant clinical implications given that delayed immunological reconstitution is a huge risk factor for transplant patients.

Completion of a Phase I clinical trial for UCB transplantation

In 2010 preclinical studies were performed to evaluate the therapeutic potential of $dmPGE_2$ in UCB transplantation[27]. In this work, human and non-human primate HSCs were treated

with $dmPGE_2$. Treatment with $dmPGE_2$ improved engraftment of human HSCs upon xenotransplantation into NOD/SCID mice. $dmPGE_2$ treatment was also evaluated in autologous transplantation of HSCs from mobilized peripheral blood in non-human primates and stable engraftment was observed up to one year later. Together these results indicated that PGE_2 has evolutionarily conserved functions in regulating HSC numbers and provided sufficient preclinical data to move forward with an FDA-approved phase I clinical trial.

The fact that PGE_2 was already being used for various purposes in the clinic facilitated the transition to phase I clinical studies. A two-year trial was undertaken at Dana Farber Cancer Center and Massachusetts General Hospital to examine the safety of *ex vivo* $dmPGE_2$ treatment to expand UCB HSC for transplantation in human patients[26]. The trial enrolled 12 patients that were undergoing treatment for leukemia or lymphoma. Each patient received two UCB units that were infused in succession – the first being treated with $dmPGE_2$ and the second being untreated (Fig. 2). Neutrophil engraftment was observed in 100% of patients in the trial, compared to 90% of historic control and the median time to engraftment was 17.5 days versus 21 days, respectively. $dmPGE_2$-treated UCB displayed enhanced engraftment by outcompeting the untreated UCB unit in 10 out of 12 patients. These initial results demonstrate that this $dmPGE_2$ treatment regimen is clinically well tolerated and on the basis of these results patients are now being enrolled in a randomized phase II clinical trial to evaluate the efficacy of $dmPGE_2$ treatment. These studies will involve 40–80 patients and will be conducted at multiple centers. In addition, new phase I studies have been initiated to examine the use of $dmPGE_2$ in single UCB transplantation and in autologous peripheral blood HSC transplantation.

Directions for future studies with $dmPGE_2$

Significant advances have been made towards understanding how $dmPGE_2$ affects HSCs and initial studies suggest it may be possible in the not-too-distant future to sufficiently expand the number of HSCs in a single UCB unit for transplantation. At the same time there remain many important questions for future research. Fully elucidating the downstream transcriptional changes that occur following $dmPGE_2$ treatment will be crucial for better understanding how this compound exerts its effects on HSCs. Genomic approaches like the one used by Cutler *et al*. could be performed on individual HSCs or on a subpopulation basis (e.g. on cycling versus quiescent cells or on sorted MPPs, ST-HSCs and LT-HSCs).

It was recently shown that short 2-hour *ex vivo* pulse treatment of murine HSCs with $dmPGE_2$ can have effects that remarkably last for as long as five serial transplantations without malignant transformation (Fig. 1B)[37]. On a cell-by-cell basis, however, the HSCs were not inherently more competitive, suggesting the observed effects may be due to an increase in HSC numbers through proliferation or a conversion in HSC fate (e.g. a change in ST-HSC fate to a more LT-HSC phenotype). Such changes could be due, in part, to alterations in the epigenetic state of the cells. PGE_2 has been shown to affect DNA methylation patterns in the intestine and increase global DNA methylation via induction of DNMT3a in fibroblasts[38]. It will be interesting in future studies to explore potential roles for epigenetic modifiers and to examine DNA methylation patterns before, during and after treatment with $dmPGE_2$. As a clearer picture of the downstream cellular mechanisms

emerges, so will new therapeutic opportunities to enhance the positive effects of $dmPGE_2$ on HSCs or to alleviate medical conditions in which PGE_2 levels are aberrant.

Exploring additional UCB stem cell expansion protocols

In a recent clinical trial it was shown that co-culturing UCB cells with mesenchymal stem cells leads to a 40-fold increase in transplantable CD34+ cells[13]. A similar trial demonstrated that culturing UCB cells for 14 days in the presence of the Notch ligand Delta could expand stem cells numbers and that these cells could then be infused with a second UCB unit[15]. In both of these cases, however, the expanded stem cells were unable to contribute to long-term hematopoiesis and were eventually lost. Also being evaluated in a clinical trial is the small molecule StemRegenin1 (SR1), which was shown to have remarkable effects on the expansion of CD34+ cells[7]. Similar to $dmPGE_2$, treatment with SR1 prior to transplantation increased the frequency of short-term repopulating units without affecting long-term engraftment or multi-lineage reconstitution ability. It has become clear that crosstalk between signaling pathways regulates HSC formation and homeostasis. Treatment with the GSK-3β inhibitor BIO (a Wnt signaling activator), for example, leads to activation of Notch in UCB cells and may promote Notch signaling during *ex vivo* expansion[9]. Given that PGE_2 and Wnt signaling pathways interact to regulate HSC function[23], it seems likely that targeting additional pathways and/or adapting a co-culture-type expansion protocol, in combination with $dmPGE_2$ treatment, will be more effective. Interestingly, both SR1 and $dmPGE_2$ were used in a recent study to better preserve HSCs such that the cells could be cultured long enough for targeted genome editing[39]. Future approaches involving the integration of multiple different strategies are likely provide the most effective means to enhance the *ex vivo* expansion of UCB stem cells for clinical transplantation.

Acknowledgments

This work was supported by NIH grants R01 HL04880, 5P30 DK49216, R24 DK092760 and 5U01 HL10001-05. In addition, L.I.Z. is a Howard Hughes Medical Institute Investigator. E.J.H. is a Howard Hughes Medical Institute Fellow of The Helen Hay Whitney Foundation.

References

1. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. Cell. 2008; 132:631–644. doi:10.1016/j.cell.2008.01.025. [PubMed: 18295580]
2. Shizuru JA, Negrin RS, Weissman IL. Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. Annual review of medicine. 2005; 56:509–538. doi:10.1146/annurev.med54.101601.152334.
3. Ballen KK, et al. The national marrow donor program 20 years of unrelated donor hematopoietic cell transplantation. Biology of blood and marrow transplantation: journal of the American Society for Blood and Marrow Transplantation. 2008; 14:2–7. doi:10.1016/j.bbmt.2008.05.017.
4. Cutler C, Ballen KK. Improving outcomes in umbilical cord blood transplantation: state of the art. Blood reviews. 2012; 26:241–246. doi:10.1016/j.blre.2012.08.001. [PubMed: 22958984]
5. Walasek MA, van Os R, de Haan G. Hematopoietic stem cell expansion: challenges and opportunities. Annals of the New York Academy of Sciences. 2012; 1266:138–150. doi:10.1111/j.1749-6632.2012.06549.x. [PubMed: 22901265]
6. Kelly SS, Sola CB, de Lima M, Shpall E. Ex vivo expansion of cord blood. Bone marrow transplantation. 2009; 44:673–681. doi:10.1038/bmt.2009.284. [PubMed: 19802023]

7. Boitano AE, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. Science. 2010; 329:1345–1348. doi:10.1126/science.1191536. [PubMed: 20688981]
8. Peled T, et al. Chelatable cellular copper modulates differentiation and self-renewal of cord blood-derived hematopoietic progenitor cells. Experimental hematology. 2005; 33:1092–1100. doi:10.1016/j.exphem.2005.06.015. [PubMed: 16219531]
9. Ko KH, et al. GSK-3beta inhibition promotes engraftment of ex vivo-expanded hematopoietic stem cells and modulates gene expression. Stem cells. 2011; 29:108–118. doi:10.1002/stem.551. [PubMed: 20960517]
10. Nishino T, et al. Ex vivo expansion of human hematopoietic stem cells by a small-molecule agonist of c-MPL. Experimental hematology. 2009; 37:1364–1377. e1364. doi:10.1016/j.exphem.2009.09.001. [PubMed: 19744539]
11. North TE, et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. Nature. 2007; 447:1007–1011. doi:10.1038/nature05883. [PubMed: 17581586]
12. Tavassoli A, Hamilton AD, Spring DR. Small molecules in biology. Chemical Society reviews. 2011; 40:4269–4270. doi:10.1039/c1cs90028e. [PubMed: 21720613]
13. de Lima M, et al. Cord-blood engraftment with ex vivo mesenchymal-cell coculture. The New England journal of medicine. 2012; 367:2305–2315. doi:10.1056/NEJMoa1207285. [PubMed: 23234514]
14. Austin TW, Solar GP, Ziegler FC, Liem L, Matthews W. A role for the Wnt gene family in hematopoiesis: expansion of multilineage progenitor cells. Blood. 1997; 89:3624–3635. [PubMed: 9160667]
15. Delaney C, et al. Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. Nature medicine. 2010; 16:232–236. doi:10.1038/nm.2080.
16. Smyth EM, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. Journal of lipid research. 2009; 50(Suppl):S423–428. doi:10.1194/jlr.R800094-JLR200. [PubMed: 19095631]
17. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annual review of biochemistry. 2000; 69:145–182. doi:10.1146/annurev.biochem.69.1.145.
18. Regan JW. EP2 and EP4 prostanoid receptor signaling. Life sciences. 2003; 74:143–153. [PubMed: 14607241]
19. Feher I, Gidali J. Prostaglandin E2 as stimulator of haemopoietic stem cell proliferation. Nature. 1974; 247:550–551. [PubMed: 4150455]
20. Lu L, et al. Enhancement of the proliferation of human marrow erythroid [BFU-E] progenitor cells by prostaglandin E requires the participation of OKT8-positive T lymphocytes and is associated with the density expression of major histocompatibility complex class II antigens on BFU-E. Blood. 1986; 68:126–133. [PubMed: 3487351]
21. Pelus LM, Broxmeyer HE, Kurland JI, Moore MA. Regulation of macrophage and granulocyte proliferation. Specificities of prostaglandin E and lactoferrin. The Journal of experimental medicine. 1979; 150:277–292. [PubMed: 313430]
22. Fujino H, West KA, Regan JW. Phosphorylation of glycogen synthase kinase-3 and stimulation of T-cell factor signaling following activation of EP2 and EP4 prostanoid receptors by prostaglandin E2. The Journal of biological chemistry. 2002; 277:2614–2619. doi:10.1074/jbc.M109440200. [PubMed: 11706038]
23. Goessling W, et al. Genetic interaction of PGE2 and Wnt signaling regulates developmental specification of stem cells and regeneration. Cell. 2009; 136:1136–1147. doi:10.1016/j.cell2009.01.015. [PubMed: 19303855]
24. Hoggatt J, Singh P, Sampath J, Pelus LM. Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. Blood. 2009; 113:5444–5455. doi:10.1182/blood-2009-01-201335. [PubMed: 19324903]
25. Ikushima YM, et al. Prostaglandin E(2) regulates murine hematopoietic stem/progenitor cells directly via EP4 receptor and indirectly through mesenchymal progenitor cells. Blood. 2013; 121:1995–2007. doi:10.1182/blood-2012-06-437889. [PubMed: 23315170]

26. Cutler C, et al. Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. Blood. 2013; 122:3074–3081. doi:10.1182/blood-2013-05-503177. [PubMed: 23996087]
27. Goessling W, et al. Prostaglandin E2 enhances human cord blood stem cell xenotransplants and shows long-term safety in preclinical nonhuman primate transplant models. Cell stem cell. 2011; 8:445–458. doi:10.1016/j.stem.2011.02.003. [PubMed: 21474107]
28. Zhao JL, et al. Conversion of danger signals into cytokine signals by hematopoietic stem and progenitor cells for regulation of stress-induced hematopoiesis. Cell stem cell. 2014; 14:445–459. doi:10.1016/j.stem.2014.01.007. [PubMed: 24561084]
29. Hoggatt J, et al. Differential stem- and progenitor-cell trafficking by prostaglandin E2. Nature. 2013; 495:365–369. doi:10.1038/nature11929. [PubMed: 23485965]
30. Frisch BJ, et al. In vivo prostaglandin E2 treatment alters the bone marrow microenvironment and preferentially expands short-term hematopoietic stem cells. Blood. 2009; 114:4054–4063. doi: 10.1182/blood-2009-03-205823. [PubMed: 19726721]
31. Desai S, April H, Nwaneshiudu C, Ashby B. Comparison of agonist-induced internalization of the human EP2 and EP4 prostaglandin receptors: role of the carboxyl terminus in EP4 receptor sequestration. Molecular pharmacology. 2000; 58:1279–1286. [PubMed: 11093764]
32. Diggle CP, et al. Prostaglandin transporter mutations cause pachydermoperiostosis with myelofibrosis. Human mutation. 2012; 33:1175–1181. doi:10.1002/humu.22111. [PubMed: 22553128]
33. Feng Y, Renshaw S, Martin P. Live imaging of tumor initiation in zebrafish larvae reveals a trophic role for leukocyte-derived PGE(2). Current biology: CB. 2012; 22:1253–1259. doi: 10.1016/j.cub.2012.05.010. [PubMed: 22658594]
34. Porter RL, et al. Prostaglandin E2 increases hematopoietic stem cell survival and accelerates hematopoietic recovery after radiation injury. Stem cells. 2013; 31:372–383. doi:10.1002/stem.l286. [PubMed: 23169593]
35. Ludin A, et al. Monocytes-macrophages that express alpha-smooth muscle actin preserve primitive hematopoietic cells in the bone marrow. Nature immunology. 2012; 13:1072–1082. doi: 10.1038/ni.2408. [PubMed: 22983360]
36. Li L, et al. Prostaglandin E2 promotes survival of naive UCB T cells via the Wnt/beta-catenin pathway and alters immune reconstitution after UCBT. Blood cancer journal. 2014; 4:e178. doi: 10.1038/bcj.2013.75. [PubMed: 24442207]
37. Hoggatt J, Mohammad KS, Singh P, Pelus LM. Prostaglandin E2 enhances long-term repopulation but does not permanently alter inherent stem cell competitiveness. Blood. 2013; 122:2997–3000. doi:10.1182/blood-2013-07-515288. [PubMed: 24047650]
38. Huang SK, et al. Prostaglandin E(2) increases fibroblast gene-specific and global DNA methylation via increased DNA methyltransferase expression. FASEB journal: official publication of the Federation of American Societies for Experimental Biology. 2012; 26:3703–3714. doi: 10.1096/fj.11-203323. [PubMed: 22645246]
39. Genovese P, et al. Targeted genome editing in human repopulating haematopoietic stem cells. Nature. 2014; 510:235–240. doi:10.1038/nature13420. [PubMed: 24870228]

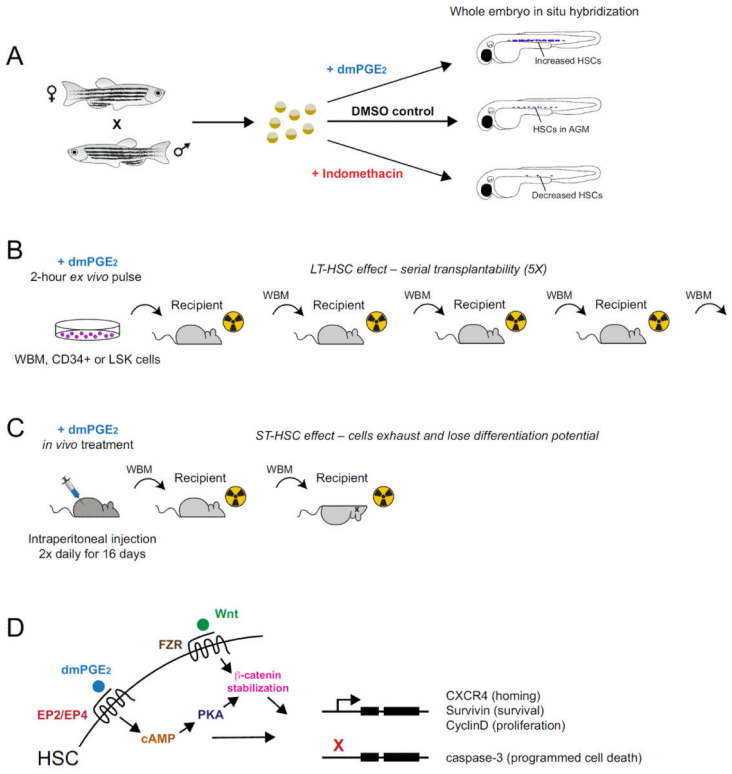

Figure 1. dmPGE$_2$ regulates vertebrate HSC function

(A) A schematic representation depicts the basic workflow for the zebrafish chemical screen that identified dmPGE$_2$ and an endogenous role for prostaglandin signaling in HSC formation. Adult zebrafish were in-crossed and collected embryos were treated with chemicals from the 3-somite stage to 36 hours post fertilization. Whole embryo in situ hybridization to *runx1/cmyb* (conserved HSC markers) was then used to examine HSC formation. Embryos treated with the stable derivative dmPGE$_2$ displayed increased *runx1/cmyb* staining in the AGM whereas treatment with the Cox inhibitor indomethacin led to reduced HSC formation. (B) 2-hour *ex vivo* pulse treatment with dmPGE$_2$ leads to increases in HSC function that last for as long as five serial transplantations. (C) Prolonged *in vivo* exposure to dmPGE$_2$ leads to enhanced engraftment; however, the treated cells eventually exhaust and lose differentiation potential. (D) dmPGE$_2$ signals through EP2 and EP4 receptors and an interaction with Wnt signaling. Downstream transcriptional changes lead to improved homing, survival and proliferation of the HSCs.

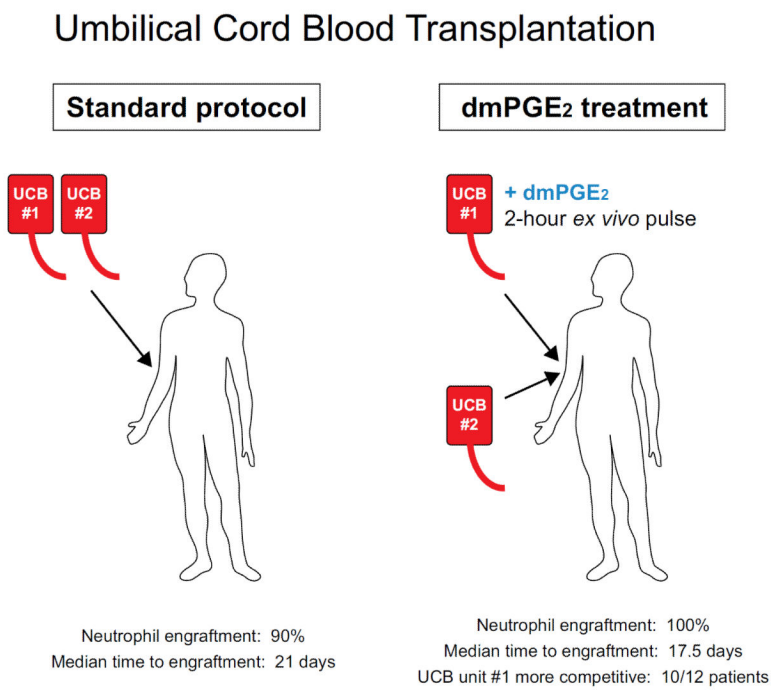

Figure 2. The clinical application of dmPGE$_2$ to improve UCB transplantation

The schematic on the left depicts the standard protocol in which two UCB units are administered to the patient. The clinical strategy for using dmPGE$_2$ to improve UCB transplantation is shown on the right. The first UCB unit is treated with a 2-hour *ex vivo* pulse of dmPGE$_2$ before infusion into the patient. A second untreated UCB is administered in succession. Clinical outcomes for each group are listed at the bottom of the figure.