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PHENOTYPIC AND FUNCTIONAL COMPARISON OF MOBILIZED PERIPHERAL BLOOD VERSUS UMBILICAL CORD BLOOD MEGAKARYOCYTE POPULATIONS

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Abstract

Background aims—Hematopoietic stem cell transplantation of mobilized peripheral blood progenitor cell (PBPC) products results in rapid platelet engraftment while use of cord blood (CB) shows significant delays. The difference in the quality and number of megakaryocyte (MK) progenitors that may be responsible for the delay in platelet engraftment has not been fully defined. The objective of this study was to quantitate the cells of the MK lineage in PBPC and CB products to determine if potential differences existed.

Methods—We examined PBPC or CB for differences in surface markers and subpopulations as well as polyploidization status within the MK lineage. Colony forming assays (CFU-MK) were used to determine if differences existed in the clonogenic MK progenitor cell. Finally, we transplanted PBPC and CB MNCs into NOD/SCID/IL2R $\gamma^{-/-}$ (NSG) mice to study platelet engraftment rates.

Results—Equivalent MK populations and polyploidization was observed in PBPCs and CB. MK progenitors were present only in CD34+ cells and had little difference in colony growth between PBPC and CB. Additionally, MK subpopulations were similar in either product with a slightly more progenitor-enriched phenotype in CB. Finally, when PBPC or CB was transplanted at similar doses, equivalent platelet engraftment rates were observed.

Conclusions—PBPC and CB contain similar frequencies of MK populations and when transplanted in comparable doses, CB is as effective as PBPCs in producing platelet engraftment *in vivo*. Understanding the differences in MK populations between PBPC and CB could help generate protocols to improve platelet engraftment after CB transplantation.

Keywords

megakaryocyte; platelet engraftment; cord blood; peripheral blood progenitor cell

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DISCLOSURE OF INTEREST

There are no conflicts of interest to declare.

INTRODUCTION

Allogeneic hematopoietic stem cell (HSC) transplantation is used to effectively treat a wide variety of hematologic malignancies. Bone marrow (BM) was first utilized as the source for hematopoietic support, becoming successful upon understanding the role the human leukocyte antigens (HLA) and proper matching of donor BM cells with the patients. Transplantation with unrelated BM following myeloablative therapy results in recovery from neutropenia in a median of 18 days while recovery from thrombocytopenia in a median of 32 days [1]. Comparison studies of autologous or allogeneic BM demonstrate similar times to neutrophil and platelet recovery [2, 3]. However, the invasiveness of BM harvest prompted investigators to find alternative cellular sources for transplantation. Circulating peripheral HSCs had limited success in early clinical transplantations but technological advances in apheresis and cryopreservation enabled investigators to concentrate numbers of peripheral blood (PB) HSCs and improvements in time to engraftment were observed [4, 5]. The use of G-CSF mobilized PB progenitor cells (PBPCs) promoted more rapid platelet recovery in a median of 15 days when compared to BM (median 39 days), while the time to neutrophil engraftment was 9 days with PBPC and 10 days with BM recovery [6–8].

Although the use of PBPC has improved the success of HSCT, a significant number of patients needing an allotransplant lack a suitable HLA-matched adult donor. The utilization of cord blood (CB) as a cellular source for transplantation has provided an alternative approach for such patients [9]. Compared to allogeneic BM, CB transplantation is associated with reduced graft-versus host disease (GVHD) and similar rates of survival, but results in delayed engraftment of neutrophils (26 days with CB, 18 days with BM) and platelets (44 days with CB, 24 days with BM)[10]. In clinical studies, double CB transplantation was established to increase the cell doses available for larger patients. With double CB transplantation, however, the time to engraftment remained delayed with 26 days to neutrophil and 53 days to platelet recovery [11]. It is unclear why such discrepancies exist between PBPC and CB transplantation with regard to platelet recovery. The differences between PBPC and CB transplantations might be due to the quality of the engrafting population responsible for platelets or simply to the lower cell dose in CB grafts. Based on clinical evidence, CB units might have lower doses or completely lack unique MK populations responsible for rapid platelet recovery that are contained within BM and PBPC grafts. Here, we compared the MK lineage-specific phenotypic surface expression including known MK subpopulations as well as the polyploidization status of the MKs to identify these potential differences. Additionally, we performed transplantation studies with increasing doses of CB and PBPCs in NOD/SCID/IL2R γ –/– (NSG) mice to effectively compare the two sources in promoting fast engraftment of total hematopoietic cells and platelets.

MATERIALS AND METHODS

Cord Blood (CB) and Mobilized Peripheral Blood Progenitor Cells (PBPC)

CB products and G-CSF mobilized PBPCs were obtained under MD Anderson IRB approved protocols with informed consent. Blood was layered over Histopaque (Sigma, St. Louis, MO) and MNCs were collected from the buffy coat.

Flow cytometry phenotypic analysis

Cell surface phenotyping of MKs was performed using anti-CD45 PerCp, anti-CD61 APC, anti-CD34 APC-e780, anti-CD41a V500 or anti-CD42b PE/Pecy5 monoclonal antibodies. Antibodies were obtained from either eBioscience (San Diego, CA) or BDBiosciences (San Jose, CA). Cells were acquired on a LSR Fortessa (BD Biosciences) and analyzed with FlowJo (Tree Star, Ashland, OR) software. A minimum of 50,000 events were acquired.

Megakaryocyte Polyploid Analysis

Cells were stained with cell surface markers (CD61, CD41a, CD42b, CD45, CD34) and subsequently washed. Cells were then fixed with Foxp3 Perm Buffer set (eBioscience) following manufacturer's instructions. After fixation, cells were incubated with 100 ng/test RNase for 15 minutes at RT in dark before staining with 400 ul Propidium Iodide (Sigma) (40 ug/ml). Cells were acquired on LSR Fortessa and analyzed using Flowjo software. A minimum of 500,000 events were acquired.

CD34+ Isolation

CD34+ cells were isolated using MACS (Miltenyi Biotech, Cologne, Germany) magnetic column separation. CB or PBPC MNCs were stained with CD34 Microbeads (Miltenyi Biotec) and selected following manufacturer's instructions. CD34- cells were also retained from the eluted fractions.

CFU-MK colony forming assay

Graded cell doses were plated into a collagen based Megacult-C megakaryocyte colony forming assay (Stem Cell Technologies, Vancouver, British Columbia, Canada). The assay was performed following manufacturer's instructions. The assay was cultured in a 150mm Petri dish with an open 35mm sterile water dish and placed in a 37C, 5% CO2 incubator for 14 days. Slides were fixed and stained with anti-CD41a antibody. Images of CFU-MK colonies on microscope slides were taken using an Olympus DP-10 camera (Olympus Imaging, Center Valley, PA).

Transplantation

NSG mice were sublethally irradiated with 300 rads, 24 hours before transplantation. PBPC and CB MNC samples were prepared in PBS and transplanted into mice by tail vein injection. Mice were bled twice weekly by retroorbital vein collection. Samples were split into two with half used to perform a CBC/DIFF count using an ADVIA120 Hematology Analyzer (Siemens Corporation, Munich, Bavaria, Germany). The other half was RBC lysed with RBC Lysis 1X (Biolegend, San Diego, CA) for 5 minutes and washed twice. Cells were first stained with anti-CD41a FITC and anti-CD45 PE murine monoclonal antibodies for 30 minutes and washed. Cells were subsequently stained for anti-CD61, anti-CD41a and anti-CD45 human monoclonal antibodies for 30 minutes and washed. Prior to acquiring cells on flow cytometer, 25 ul counting beads (Spherotech, Lake Forest, IL) were added to enable counting of events/ul. Cells were acquired on an LSR Fortessa and analyzed by FlowJo software. A minimum of 5,000 bead events was acquired.

Platelet Analysis

Platelets were analyzed by flow cytometry using the Forward Scatter (FSC) and Side Scatter (SSC) settings in log phase to observe the platelet fraction found to the left of the RBC/debris field. Platelets were analyzed by using FSC (x-axis) in logarithmic phase and anti-CD61 (y-axis) to identify the platelet population.

RESULTS

Phenotypic characterization of the megakaryocyte lineage

We isolated MNCs from fresh PBPC and CB and phenotyped expression of common megakaryocyte lineage surface markers, CD34, CD41a and CD61 (Figure 1/Table 1). Similar frequencies of CD34 expression were detected from both sources (Figure 1A). The frequency of CD41a and CD61 expression was increased non-significantly in PBPC (Figure 1B/C). Since MKs begin to express CD42b, or glycoprotein 1b, during late maturation we tested for surface expression on PBPC and CB and detected only minimal differences in expression similar to CD41a and CD61 (Figure 1D). When analyzed together, the traditional MK dual-markers of CD41a/CD61 exhibited elevated expression on PBPC compared to CB but not statistically significant (Figure 1E). The non-significant results observed attest to the variability and heterogeneity of cell populations within these blood sources.

The presence of CD41a on multipotent progenitors has led to the suggestion that CD34+CD41a+ cells may contain the MK progenitor population with CD42b expression as a late surface antigen of MK differentiation [12, 13]. As shown in Figure 1F/Table 1, though highly variable between samples, CB may have a higher frequency of the more immature MK/progenitor population (CD34+CD41a+CD42b-) compared to PBPC, though this is not significant. Levels of the early stage MK (CD34+CD41a+CD42b+) were similar while the late stage MK (CD34-CD41a+CD42b+) were elevated in PBPC compared to CB yet not significant ($p=0.06$). Whether expressed as a percentage of CD34+ cells (Figure 1F) or from total MNCs (Figure 1G), the trends in relative population frequencies were similar. Overall the comparison of PBPC to CB does not exhibit significant differences or absence of a particular population within the MK lineage. However, these results suggest that CB may have a more immature subpopulation phenotype within the MK lineage.

Ploidy analysis of CB and PB

MKs become polyploid as differentiation to platelet-shedding-MKs occurs. Several studies have demonstrated that the level of ploidy in MKs correlates to the level of platelet release [14]. We tested whether there were any major differences in ploidy between PBPC and CB relative to the MK lineage. The majority of PBPC and CB CD41a+ MNCs were in 2N stage (94.87 ± 2.67 and $92.62\pm2.07\%$ respectively) (Figure 2A). Similarly the CD61+ fraction also contained a majority of cells in 2N in both PBPC and CB (95.55 ± 2.45 and $92.95\pm2.96\%$ respectively) (Figure 2B). Interestingly, both the CD41a+ and CD61+ cell populations had a trend towards elevated high-ploidy stages in CB compared to PBPC, with CD41a >4N ploidy CB of $5.66\pm2.54\%$ compared to PBPC of $3.58\pm2.28\%$ and CD61 >4N ploidy of CB of $5.47\pm3.07\%$ compared to PBPC of $3.37\pm2.09\%$. However, none of the differences were statistically significant. The polyploidy analysis in CD34+ cells revealed 30% in 2N stage in

both products (CB, $33.48 \pm 18.97\%$; PBPC, $28.32 \pm 20.78\%$) with an additional 50% in 4N stage (CB, $53.25 \pm 14.06\%$; PBPC, $55.65 \pm 19.08\%$) demonstrating a population undergoing cell cycle and polyploidization of the nucleus (Figure 2C). However, again these were trends as there were no statistically significant differences. Again, slightly elevated high-ploidy populations ($>8N$) were observed in CB CD34+ cells ($9.6 \pm 4.4\%$) compared to PBPC CD34+ cells ($6.4 \pm 5.15\%$) but these were at lower numbers of cells and not significant. Of additional note was that CB buffy coat MNCs had an increased frequency of platelets compared to PBPC (61.6 ± 5.68 , 39.3 ± 16.1 $p=0.08$) but these results may be an artifact of the buffy coat collection and centrifugation steps (Figure 2D). These results further suggest that CB and PBPC have no major differences in MKs with respect to ploidy and the slight differences observed are possibly attributed to the lower cell frequencies at these high-ploidy stages.

Megakaryocytic colony forming assay

To test the level of progenitor content of the MK lineage between PBPC and CB we used the semi-solid methylcellulose colony forming unit megakaryocyte assay (CFU-MK). We isolated CD34+ and CD34- cells from both PBPC and CB and plated cells in the MK colony forming assays at various cell doses (Figure 3). The MK colony forming potential resided solely in the CD34+ fraction validating the hypothesis that the MK progenitor differentiates from this population. When comparing similar seeded cell doses between PBPC and CB we observed a greater frequency and size of colonies in CB. The increased frequency of MK colonies suggests that CB is enriched in MK progenitor populations or possibly enriched in multipotent progenitors favorable to the MK lineage.

Megakaryocyte Progenitor Populations

The exact surface marker denoting the development and lineage specificity of MKs is not clearly defined. Currently, researchers have used CD34+ for the “stemness” of the MK progenitor with CD41a and CD61 as co-expressing markers and CD42b as a late-stage marker. One study attempted to identify the specific marker with regards to the MK precursor and subsequent platelet recovery in patients, identifying CD34+CD61+ as having the best correlation to platelet recovery [15]. We used this marker subset as a baseline to phenotype the MK populations in PBPC and CB (Figure 4). Initial observations, which was confirmed in multiple analysis, identified 5 sub populations, CD34+CD61-, CD34+CD61+, CD34+CD61++, CD34-CD61++ and CD34-CD61+ (Figure 4A). As expected, CD41a expression increased linearly with CD61 expression (Figure 4B). Interestingly though, the CD42b expression profile was expressed in the CD34+CD61++ and CD34-CD61++ populations and minimally in the CD34+CD61+ and CD34-CD61+ populations (Figure 4B). These populations, CD34+CD61++CD41a++CD42b+ and CD34-CD61++CD41a++CD42b+ demonstrated the early-stage MK and late-stage MK, respectively. Figure 4C depicts the proposed Mk lineage development as phenotyped by this flow cytometry profile. As shown in Figure 4D, when compared as a percentage of MNCs with respect to the MK progenitor populations and subpopulations, CB had non-significant higher progenitor cell frequencies (CD34+CD61-/CD34+CD61+) and contained lower frequencies of the mature MK populations (CD34-CD61+/CD34-CD61++) with similar levels of the early-stage MK

(CD34+CD61++). The subpopulation Mk data is further tabulated in Table 2. This validates the previous results suggesting that CB has a more immature population in the MK lineage.

Clinically, the time to platelet recovery is faster with PBPC (17–30 days) than with CB (50–70+ days [11, 16]. This could be due to the lack of a specific MK population required for platelet production in CB products. However, our observations demonstrate that though small differences exist between blood sources, there is no significant difference or loss of any specific MK population. Within the clinic, a greater number of total cells are infused with PBPC ($\sim 2.0 \times 10^8$ cells) than with CB ($\sim 2.0 \times 10^7$ cells) (Figure 5A). Based on the frequencies we described above for the various MK subpopulations and using the standard total cell numbers typically transplanted in the clinic, we would expect a greater number of each population being transplanted with PBPC than with CB (Figure 5B–C). This discrepancy in total cell numbers and subsequent fewer MK populations could contribute to the decrease in platelet recovery observed in CB transplant patients.

To study the potential of platelet recovery and engraftment simultaneously, we isolated MNCs from PBPC and CB and transplanted them at equivalent numbers of MNCs into sub-lethally irradiated NSG mice. When comparing sources at similar levels, CB had a slightly faster time to CD45 engraftment that was dose-dependent and a more rapid platelet recovery in the peripheral blood of mice (Figure 5D/E). This result suggests that if transplanted at similar numbers, PBPC and CB were comparable and the difference clinically is due to the lower numbers transplanted in CB transplant patients.

DISCUSSION

Although stem cell transplantation has had a major impact on the treatment of many hematological diseases, there is need to optimize CB grafts which are associated with delays in neutrophil and in particular platelet recovery [16]. A comprehensive understanding of the MK precursors in the different hematopoietic sources, BM, PBPC and CB would allow us to potentially develop optimal stem cell products for platelet engraftment [17, 18].

Several groups have studied the differences between CB and PBPC *in vitro*, however the differences in *in vivo* potential for platelet engraftment by specific MK subpopulations remains to be defined [14, 19, 20]. We found that although expression of individual MK surface markers were similar between PBPC and CB, the subpopulations in the MK lineage revealed a potential greater immature MK frequency in CB with more mature MK populations present in the PBPC. Differences of MK differentiation in response to cytokines in *ex vivo* cultures have been demonstrated by multiple reports confirming the distinctiveness of BM, CB and PBPC [20, 21].

We detected slightly higher levels of ploidy in CB MNCs conflicting with studies that demonstrated that CB MKs are unable to generate high-ploidy cells; however the polyploidy analysis in this study was done after *in vitro* culture, which may alter the specific state of freshly isolated MKs [14]. In fact, reports have demonstrated that CB MKs do not complete maturation upon thrombopoietin-induced activation *in vitro*, which could be explained by the length of the culture and/or specific conditions to which MKs are exposed [22]. In

addition, though *in vivo* studies in patients have further demonstrated smaller MKs after CB transplantation this could be attributed to variances in the CB units and further studies are needed to better quantitate this hypothesis thus complicating an interpretation [23]. Another plausible explanation is that platelet-shedding MKs might simply exist in the BM and high-ploidy mature MKs are not detected until stimulated into the periphery.

The colony-forming potential of the MK progenitor cell (CFU-MK) resides solely in the CD34+ fraction of the MNCs, which has been also been observed in other studies [19, 24]. CB demonstrated higher frequencies of CFU-MK colonies with greater sizes when compared to PBPC. This suggests that CB does have a more immature profile within the MK lineage which may contribute to the differences in response to cytokines and subsequent development of more mature MKs.

Studies exploring MK precursors by cell surface marker expression have been limited and unique markers to identify MK subset development from the hematopoietic stem/progenitor population into the MK lineage have not been identified. However, one study correlating platelet recovery with infused MKs reported a minimally enhanced time to engraftment with the CD34++HLA-DR-CD61+ population [15]. We demonstrated that phenotyping MNCs with CD34 and differential levels of CD61 expression identified five unique subpopulations that further profiled different with regards to CD41a and CD42b expression (Figure 4). This method of profiling the MK lineage by using CD61 differential expression is unique and to our knowledge has not been previously reported. Surface marker expression of CD34, CD41a and CD42b have been used to study cultures comparing PBPC and CB but this was not applied to the MK lineage and specific MK lineage differentiation [24]. In an *in vivo* transplant model of MKs, a CD34-CD61+CD42b+ population only generated human platelets in the PB for about 4 days [25]. In our phenotypic analysis, this population represents about 45% of the PBPC and 37% of the CB CD34-CD61++ population or 98% (PBPC) and 96% (CB) of the total CD34-CD61+ population. Attempting to characterize MKs by CD41a/CD61 expression alone does not account for the heterogeneous MK populations that are expressed at different frequencies in blood products. This limitation may explain the discrepancies observed among different studies regarding effectiveness of MK expansion and platelet production. Better definition of the MK lineage with respect to differential levels of cell surface marker expression may provide insights into the development of MKs from stem cell to MK-progenitor cell to mature MK. Since cryopreserved CB is utilized in clinical settings, we briefly examined frozen CB for potential losses of CB populations and did not detect any differences preserving the potential of using CB for MK *ex vivo* expansion.

The lack of statistically significant differences in population frequencies or loss of an entire MK subpopulation suggests that the delay in platelet recovery might simply be attributed to lower cell numbers in CB. Clinical standards for PBPC transplantation is related to efficient engraftment and platelet/neutrophil recovery post-transplantation and lower CB cell numbers might require transplantation of more numbers of MK progenitors for faster engraftment. This was observed in our transplantation study where comparison of equivalent numbers of cells from PBPC to CB provided similar human CD45 engraftment and platelet recovery in the periphery (Figure 5). One caveat of the NSG model is the faster engraftment

capability of the SCID repopulating cell (SRC) with CB CD34+ cells, requiring only 500,000 for optimal engraftment versus 5.0 e6 from mobilized PBPC. As seen in Figure 5F/G, the comparison of 0.1 e6 CB TNC to 1.0 e6 PB TNC (10X difference) revealed almost identical patterns of engraftment within this model, providing some evidence that PBPC and CB at equivalent cell numbers provide similar activities.

Unfortunately, the low engraftment of human platelets in the NSG transplant model could be attributed to the lack of complete thrombocytopenia in the mouse where normal homeostatic mechanisms from unablated murine MKs would reestablish platelet levels. We have performed subsequent studies with increasing amounts of radiation and detected a longer duration of murine thrombocytopenia after higher levels of radiation. This improved model would allow us to better understand the effect of human MK transplantation and subsequent platelet engraftment when comparing PBPC to CB. Furthermore, these findings also highlight the potential differences of engraftment of human cells into a murine model. It is not known if the discrepancies in engraftment and homing between CB and PBPC in NSG mice are also present when transplanted into patients. Developing the NSG model into a more thrombocytopenic model mimicking typical clinical settings will enable more efficient studying of MK and platelet engraftment *in vivo* but translation into clinical medicine would be more problematic due to the sensitive nature of clinical studies. *Ex vivo* expansion of CB to equivalent cell numbers as PBPC would grant better insight into whether CB and PBPC have similar engraftment and homing capabilities in patients.

Taken together, PBPC and CB contain very similar phenotypic profiles and polyploidization status with minimal differences in MK populations and MK colony forming potential of the sources. The discrepancies observed in engraftment times in stem cell transplantation may only be a result of the lack of significant numbers of MK progenitors in CB. Further work needs to be done to identify cell surface markers of MK differentiation from the HSC and multipotent progenitor levels to successfully expand CB MK populations. Additionally, understanding which population is the engrafting MK population and the platelet releasing population would better enable future endeavors into generating these populations for successful therapeutic transplantation.

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ABBREVIATIONS

CB	Cord Blood
PBPC	Peripheral Blood Progenitor Cell
MK	Megakaryocyte
CFU-MK	Colony Forming Unit Megakaryocyte

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Figure 1. Phenotypic Characterization of Megakaryocyte Lineage between PBPC and CB
 Fresh PBPC and CB MNCs were collected and stained. A. Representative flow plot of CD34. B. Representative flow plot of CD41a. C. Representative flow plot of CD61. D. Representative flow plot of CD42b. E. Representative flow plot of CD41a/CD61. Bar graphs depict mean % expression $\pm$ SEM of MNCs from 6 different sources. F/G. Flow cytometric phenotype of immature/progenitor MKs and early and late stage MKs. F. Represents % of CD34+ populations. G. Represents % of total MNC population. Bar graphs represent mean % $\pm$ SEM from 6 different sources.

Figure 2. Polyploidization of PBPC and CB MNCs

MNCs were collected and surface stained for MK markers followed by PI staining of DNA.

A. Ploidy analysis of CD41a+ MNCs. B. Ploidy analysis of CD61+ MNCs. C. Ploidy analysis of CD34+ MNCs. D. Total platelets collected in buffy coat fraction as % of total events acquired by flow cytometer. Graphs represent mean $\pm$ SEM of all samples (n=4). Flow is representative of one experiment.

Figure 3. CFU-MK colony forming potential

A. CFU-MK assay of CD34- cells from PBPC and CB at 0.4e6, 0.2e6 and 0.1e6 cells plated. B. CFU-MK assay of CD34+ cells from PBPC and CB at 0.1e6, 0.25 e5 and 0.5 e4 cells plated. Figures are representative of two independent experiments.

Figure 4. Megakaryocyte lineage differential expression

A. Representative flow plots of CD34 and CD61 PBPC and CB lineage subpopulations. B. Representative flow histograms of CD41a and CD42b expression within the CD34/CD61 subpopulations of CB MNCs. C. Proposed diagram of MK lineage progression. D. Graph depicts % MK subpopulations within the MNCs of PBPC or CB. (n=6) Results depicted as mean $\pm$ SEM.

Figure 5. Correlating mPB and CB cell transplantation

A. Graphs depict assumed transplantation of PBPC numbers and the corresponding number of CB cells transplanted in clinical settings. B/C. MK populations are calculated based on percentages of MK subpopulations identified in Figure 4D. D. Human CD45+ concentrations observed in peripheral blood of NSG mice after transplantation of fresh 10.0×10^6 , 1.0×10^6 or 0.1×10^6 PBPC or CB MNCs. Results depict mean $\pm$ SEM. N=5. E. Platelet concentrations in PB of NSG mice. Platelets were phenotyped as human CD61+, human CD41+ and human CD45-. Results depict mean $\pm$ SEM. N=5. F. CD45+ concentration comparison of 1.0×10^6 PBPC or 0.1×10^6 CB MNCs with regard to scid-repopulating cell (SRC). G. Platelet concentration comparison of PBPC or CB MNCs with regard to SRC.

Table 1
Expression of Megakaryocyte Lineage

Table depicts percent $\pm$ SEM of various MK lineage markers and the MK lineage populations in PBPC and CB. Results were obtained from flow cytometry as depicted in Figure 1.

	% PBPC	% CB	P-value
CD34	1.49 $\pm$ 1.31	1.51 $\pm$ 1.05	0.98
CD41a	53.3 $\pm$ 16.8	32.4 $\pm$ 22.7	0.11
CD61	49.3 $\pm$ 19.9	31.9 $\pm$ 20.5	0.19
CD42b	51.56 $\pm$ 17.78	36.23 $\pm$ 21.94	0.23
CD41a/CD61	53.9 $\pm$ 17.78	32.58 $\pm$ 22.79	0.12
% Of CD34+ CD34+CD41a+CD42b-	3.57 $\pm$ 2.5	6.44 $\pm$ 4.57	0.46
% Of CD34+ CD34+CD41a+CD42b+	91.1 $\pm$ 10.9	86.9 $\pm$ 13.2	0.58
% Of CD34- CD34-CD41a+CD42b+	52.5 $\pm$ 17.4	27.7 $\pm$ 19.8	0.06
% Of MNC CD34+CD41a+CD42b-	0.046 $\pm$ 0.034	0.056 $\pm$ 0.041	0.74
% Of MNC CD34+CD41a+CD42b+	1.38 $\pm$ 1.32	1.37 $\pm$ 1.04	0.99
% Of MNC CD34-CD41a+CD42b+	35.98 $\pm$ 13.31	18.57 $\pm$ 15.12	0.07

Table 2
Expression of Megakaryocyte Sub-Populations

Table depicts percent $\pm$ SEM of each MK subpopulation within PBPC and CB as seen in Figure 4D.

	% PBPC	% CB	P-value
CD34+CD61–	0.30 $\pm$ 0.14	0.51 $\pm$ 0.37	0.48
CD34+CD61+	0.27 $\pm$ 0.23	0.34 $\pm$ 0.16	0.61
CD34+CD61++	0.25 $\pm$ 0.14	0.24 $\pm$ 0.09	0.90
CD34–CD61++	27.92 $\pm$ 10.88	17.41 $\pm$ 14.56	0.20
CD34–CD61+	18.8 $\pm$ 5.61	9.83 $\pm$ 4.23	0.02