Journal of Microbiology and Pathology

Sandeep, J Microbiol Pathol 2018, 2:1

Editorial Open Access

Expansion of Umbilical Cord Blood with IGFBP2 in MSC Co-Culture System – Modest but Long-term Engraftment of Donor Cells

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Received date: September 12, 2017; **Accepted date:** December 26, 2017; **Published date:** January 03, 2018

Abstract

Background: *Ex vivo* expansion of umbilical cord blood (UCB) hematopoietic stem and progenitor cells (HSPCs) could reduce transplant related mortality by enabling faster donor cell engraftment. Our previous study showed that addition of insulin-like growth factor binding protein 2 (IGFBP2) to mesenchymal stromal cells (MSCs) co-culture could expand non-selected UCB grafts that gave decreased incidence of graft-versus-host-disease (GVHD) in mice. In this study we carried out a pilot clinical trial to assess safety and feasibility of this UCB expansion protocol.

Methods: Eligible patients with at least two UCB units matching on 4 out of 6 human leukocyte antigen loci were recruited for this study. Larger UCB unit that provided at least 2.5×10^7 total nucleated cells (TNCs) per kilogram of bodyweight was infused without any manipulation. The smaller unit which gave at least 1.5×10^7 TNCs/kg was expanded without CD34 selection and supported by MSC co-culture in presence of a cytokine cocktail consisting of 100 ng/mL stem cell factor and thrombopoietin, 50 ng/mL Flt-3L, and 20 ng/mL IGFBP2.

Results: Three patients were recruited and expansion resulted in increase of 3.5 ± 2.5 fold of TNCs, 17.9 ± 23.2 fold of overall $CD34^+$ progenitors, 168.1 ± 114.0 fold of colony-forming unit granulocyte-macrophage and 556.7 ± 949.9 fold of primitive progenitors ($CD34^+CD38\text{-}CD90^+$cells). Sufficient HSPC expansion was attenuated by excessive cell loss of approximately 60.5% during harvesting and washing which unexpectedly gave suboptimal expanded graft dosage. Co-infusion of both units gave median of 6.5×10^5 $CD34^+$ cells/kg. One patient maintained 100% engraftment from expanded unit at follow-up on 33 months with minimal GVHD or disease relapse. Multi-organ failure resulted in the demise of the other two patients at day 38 and day 57 post-transplant. The average time to neutrophil recovery was 38 days (range, 34-44 days). Variable number tandem repeat showed that two out of three patients achieved engraftment from the manipulated unit. The trial was terminated because of excessive mortality, primarily as a consequence of the toxicity of multiple conditioning regimens along with other comorbidities.

Conclusion: IGFBP-2 supplementation and MSC co-culture supported modest but long-term engraftment of donor cells with minimal GVHD symptoms.

Keywords: Umbilical cord blood transplantation; *Ex vivo* expansion; Clinical trial; Hematopoietic stem and progenitor cells; Mesenchymal stromal cells; Leukemia; Lymphoma

Background

Umbilical cord blood transplantation (UCBT) is an established means of performing hematopoietic stem cell transplantation (HSCT) that treats patients suffering from malignant and non-malignant disorders of the blood and the bone marrow (BM) [1-4]. As the outcomes of partially matched UCBT are similar to those of fully matched unrelated or related BM transplantation (BMT), there has been a significant increase in UCBT for patients requiring allogeneic grafts [5,6]. Over the last decade, the number of registry UCBT has doubled from approximately 3,200 in 2006 to 6,400 in 2016. This, coupled with the immediate availability of UCB unit from banks, has made it an ideal graft source for pediatric HSCT [7,8]. However, pediatric HSCTs constitute only a minority (<25%) of the global need for such procedures. UCBT has not been widely conducted in adults (approximately 10% per annum) due to the low number of total nucleated cells (TNC) and its subset of hematopoietic stem and progenitor cells (HSPCs) in each UCB unit. Transplant success has been shown to directly correlate with the infused cell dosage where optimum dose have been defined to be at least 25-30 million cells per kilogram (kg) of recipient's body weight. Double unit UCBT (DUCBT) achieves sufficient cell dosage with minimal change in engraftment times compared to single unit UCBT [9]. Even though DUCBT has increased UCB usage in adults, but still many patients continue to be

ineligible as the combined cell dose of two grafts may still fall short of the recommended value. Also DUCBT is strongly associated with a higher incidence of graft-versus-host-disease (GVHD) due to graft-graft immunoreactions along with complex three-way human leukocyte antigen (HLA) matching process. In recent years, *ex vivo* expansion of HSPC has been used as an approach for obtaining sufficient cell dosage from a single UCB to mediate successful adult transplantation while moderating GVHD [10].

In our previous study, we demonstrated that bone marrow-mesenchymal stromal cells (BM-MSCs) co-culture supplemented with a cytokine cocktail comprising of 100 ng/ml stem cell factor (SCF), 50 ng/ml FLT-3 ligand (FL), 100 ng/ml thrombopoietin (TPO) and 20 ng/ml insulin-like growth factor binding protein 2 (IGFBP2) supported *ex vivo* expansion of cryopreserved UCBs without the need for a prior CD34 enrichment. DUCBT in mice showed that the expanded unit boosted in vivo hematopoiesis of the unexpanded graft, with increased regulatory T cells content and decreased incidence of GVHD [11,12]. In this current study, we performed a pilot clinical trial to assess the safety and feasibility of this approach and hereby report the results.

Methods

Cell preparation

Cryopreserved clinical-grade UCB was obtained from Singapore Cord Blood Bank. Allogeneic bone marrow (BM) was obtained from Singapore General Hospital with donor's informed consent. The use of UCB and BM were reviewed and approved by the Institutional Review Boards of National University of Singapore, Singapore General Hospital as well as the Singapore Cord Blood Bank's Research Advisory Ethics Committee. BM-MSCs were used as feeder layer to support the *ex vivo* expansion of UCBs as described above.

Ex vivo expansion of CBU

Cryopreserved clinical UCB mononuclear cells (MNCs) were thawed, washed and suspended at a concentration of 4×10^5 cells/ml in Stem Span serum free expansion medium (SFEM) (STEMCELL Technologies, Canada) supplemented with recombinant human cytokine cocktail of 100 ng/ml SCF stemgen (Ancestim, Amgen, Australia), 50 ng/ml FL (Peprotech, Isreal), 100 ng/ml TPO (Peprotech, Isreal) and 20 ng/ml IGFBP2 (R&D systems, USA). The UCB-MNCs were inoculated on 80-90% confluent BM-MSC stromal layer (4^{th} to 6^{th} passage) and cultured at 37°C with 5% CO_2 saturated incubator for 11-13 days. Media and cytokine replenishment was done on day 7. At end of expansion culture, the non-adherent UCB cells were harvested by aspiration, while the adherent UCB cells and BM-MSCs were harvested by detaching with 1,000× diluted trypsin containing 1.0 μM EDTA (Roche, USA) for 5 minutes in 37°C incubator. All the harvested cells were washed with saline (B. Braun, USA) to remove any trace amounts of the human cytokines and growth media. Appropriate sterility tests (for microbial contamination) were performed to assess safety of the expanded graft while cell count was performed by an automated differential cell counter (Beckman Coulter, USA). BM-MSC was cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, USA) medium containing 20% characterized fetal bovine serum (Hyclone, USA). Quality assurance of BM-MSC was performed by checking positive phenotypic expression of CD90, CD73 and CD105 while ensuring that it lacked expression of hematopoietic markers such as CD45 and CD34. Prior to inoculation of the UCB cells, the BM-MSC growth media was completed removed, followed by washing of the confluent MSC layer with saline.

Flow cytometric analysis

All data were acquired using the cytomics FC500 flow cytometer in clinical diagnostic laboratory where 10,000 events per sample were collected. Phycoerythrin (PE) conjugated CD34, allophycocyanin (APC) conjugated CD38, phycoerythrin-Cy7 (PE-Cy7) conjugated CD45 and fluorescein isothiocyanate (FITC) conjugated CD90 were used for phenotypic analysis of the hematopoietic stem and progenitor cells [12,13]. Viability of $CD45^+$ leukocytes was studied with Annexin-V-PE and 7-amino-actinomycin D (7-AAD).

Methylcellulose colony assays

Quantification of granulocyte-macrophage colony-forming unit (CFU-GM) was performed before and after expansion. Briefly, CFU-GM from freshly thawed UCB-MNC or 11-13 days expanded cells of the mentioned cell cultures was evaluated. Duplicates of freshly thawed UCB-MNC (5,000 and 10,000 cells) and expanded cells (1,000 and 5,000 cells) were cultured in 35 mm petri dishes (BD Falcon, USA) in 1.1 ml of HSC-CFU complete media with EPO (Miltenyi Biotec) without any further media manipulation. After 14-16 days of culture in a humidified environment at 37°C and 5% CO_2, colonies were scored and pictured using a SZ61 Olympus microscope equipped with CCD camera.

Inclusion criteria

Patients were eligible for this study if they met all the following criteria: (1) aged between 12 to 60 years, (2) acute and chronic leukemia, MDS with 5% BM blasts or lymphomas (Follicular, Diffuse large B-cell and Hodgkin's), (3) requiring unrelated allogeneic HSCT, and (4) absence of an unrelated donor considered to be acceptable by the transplant center on the basis of HLA compatibility and donor availability. In addition, eligible patients had at least two UCBs that were 4 out of 6 HLA identical to the patient with one unit containing 2.5×10^7 TNCs per kilogram of recipient's bodyweight and the second unit providing at least 1.5×10^7 TNCs/kg. The smaller unit was used for *ex vivo* expansion and the larger unit was co-infused without manipulation. All patients provided written informed consent. This study was approved by the Health Sciences Authority (HSA), Singapore. This trial was registered at www.clinicaltrials.gov as #NCT01624701.

Transplant procedure

Patients underwent myeloablative conditioning with a Flu/Cy/TBI regime as follows: IV Fludarabine (Flu) 25 mg/m^2 on D-7 to D-5, IV cyclophosphamide (Cy) 60 mg/kg on D-6 to D-5, total body irradiation (TBI) 200 rad BD D-3 to D-1. GVHD prophylaxis was oral cyclosporine A at 6.25 mg/kg with a trough target of 300-500 μg/L and oral mycophenolic acid (myfortic) at 720 mg both of which started on D-4. Infective prophylaxis included ciprofloxacin at 500 mg from BD D-8 until engraftment; trimethoprim and sulfamethoxazole at 960 mg/BD from D-14 to D-2 and then at 960 mg BD on Saturday and Sunday of D+21 to D+180; acyclovir 800 mg BD from D-8 to D0, then valaciclovir at 2 g TDS for 4 weeks followed by acyclovir at 800 mg BD; posaconazole at 200 mg TDS from D-4 till D+28 or engraftment which ever was later followed by conversion to itraconazole. Sinusoidal obstruction syndrome prophylaxis was ursodeoxycholic acid at 250 mg

q8H from D-8 to D+21. G-CSF at 300 mcg was given subcutaneous from D+1 until engraftment. Patients were monitored for cytomegalovirus and human herpes virus 6 using peripheral blood (PB) twice per week from D+14 to D+100 days post-transplantation. BK virus in PB was monitored weekly. Donor chimerism was measured by variable number tandem repeat (VNTR) every 2 weeks in the peripheral blood and at D+28 in the bone marrow. Neutrophil engraftment was defined by the first of three days with a neutrophil count >0.5 × 10^9/L. Platelet engraftment was defined as platelet count >20 × 10^9/L without transfusion. GVHD was defined by the Glucksberg criteria [14].

Statistical analysis

The results are expressed as Mean ± Standard Deviation (Mean ± SD). Data processing and statistical analysis was performed using Graph pad Prism 6 software.

Results

IGFBP2 supplementation and MSC co-culture allowed modest expansion of non-enriched UCB

Culturing of the three UCB units in the above described expansion protocol resulted in 3.5 ± 2.5-fold expansion of TNCs; 17.9 ± 23.2 fold expansion of general progenitors ($CD34^+$ cells); 168.1 ± 114.0 fold expansion of CFU-GM; and 556.7 ± 949.9 fold expansion of primitive progenitors ($CD34^+CD38^-CD90^+$cells) (Figure 1, n=3). However, we experienced significant cell loss during pre-culture thawing and washing and post-culture harvesting which resulted in significantly reduced net expansion of TNCs (1.3 ± 1.1-fold), $CD34^+$ cells (7.1 ± 9.6-fold), CFU-GM (68.0 ± 47.3-fold) and $CD34^+CD38^-CD90^+$ cells (226.5 ± 387.0-fold). The absolute cell numbers before thawing and washing as well as after harvesting and washing (ready for infusion) is summarized in Tables 1 and 2. These findings indicated that IGFBP2 supplementation and BM-MSC co-culture could allow modest expansion of HSPC from non-selected UCB grafts.

Patient outcomes

A total of three patients were recruited to this trial from October 2013 to July 2014 and their main findings are summarized as follows:

Unit	TNC (×10^6)	CD34+ cells (×10^6)	CD34+CD38-CD90+ cells(×10^6)	CFU-GM (×10^6)
CB#1	940	3.96	0.094	0.673
CB#2	1292.6	4.16	1.24	0.33
CB#3	1763	1.84	0.61	--

Table 1: UCB unit characteristics before manipulation.

Unit	TNC (×10^6)	CD34+ cells (×10^6)	CD34+CD38-CD90+ cells (×10^6)	CFU-GM (×10^6)
CB#1	2451.4	71.9	63.3	68.3
CB#2	1034.1	11	6	11.4
CB#3	1074.4	0.95	0.75	1.1

Table 2: Expanded UCB unit characteristics prior to infusion.

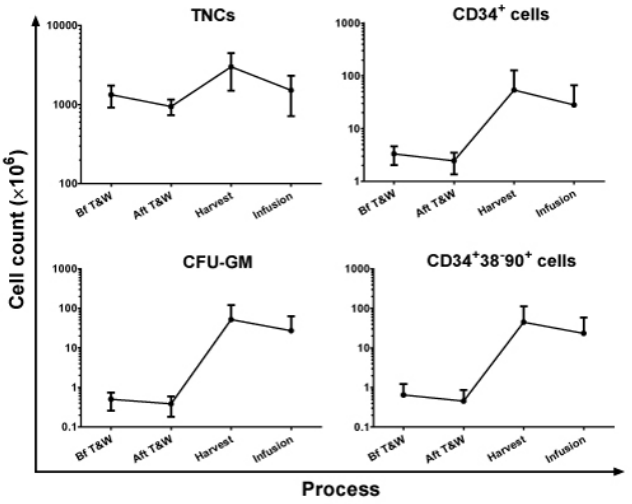

Figure 1: *Ex vivo* expansion of clinical non-enriched UCB in BM-MSC co-culture system supplemented with IGFBP-2. The total number of TNCs, CD34+ cells, CFU-GM and CD34+CD38-CD90+ cells before thawing and washing (Bf T&W) was provided by Singapore Cord Blood Bank. There was cell loss after thawing and washing (Aft T&W) and between post-culture harvesting and washing prior to infusion. Three UCB units underwent *ex vivo* expansion and were co-transplanted with second larger unmanipulated grafts for three patients respectively.

Patient 1: A case of multiple relapsed diffuse large B cell lymphoma (DLBCL)

The patient was a 48 year old female with past medical history of Lupus/Rheumatoid Arthritis/Sjogrens overlap which had been treated with immunosuppression, hyperlipidemia, and hypertension, as well as stage 2 DLBCL which had failed two previous lines of chemotherapy and autologous stem cell transplantation. She received a third salvage regimen and was recruited for the trial in third complete remission. The unmanipulated UCB unit 1 was a 6/6 HLA match and had TNCs and CD34 cell dose of 26.00 × 10^6 cells/kg and 1.25 × 10^5 cells/kg respectively. UCB unit 2 was a 6/6 HLA match and provided TNCs and CD34 cell dose of 43.80 × 10^6 cells/kg and 12.83 × 10^5 cells/kg respectively after expansion. Her transplant was complicated by C difficile colitis at D+4, neutropenic fever at D+6, a presumptive fungal pneumonia at D+19, and acute renal injury (likely drug induced) with fluid overload and type 2 respiratory failure at D+25 leading to ICU admission. The ICU stay was complicated by prolonged intubation due to pneumonia and fluid overload. She also developed bleeding gastric ulcer and eventually succumbed to pneumonia on D+57. She had robust neutrophil engraftment at D+44 but did not achieve platelet engraftment by D+57. VNTR showed early major engraftment from the expanded UCB unit 2 at D+8 with 100% mixed donor chimerism (85% expanded UCB unit 2 and 15% unexpanded UCB unit 1). Subsequent VNTR at Day 17, Day 31, and Day 45 showed nearly

identical results with persistence of the expanded cord. There were no observed symptoms of GVHD (Figure 2).

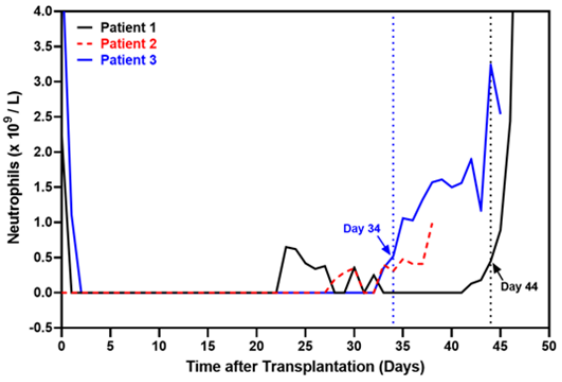

Figure 2: Time to neutrophil engraftment post UCBT in the enrolled patients. Patient 1 (DLBCL) and 3 (Ph^+ ALL) achieved neutrophil engraftment at D+44 and D+34 respectively. Patient 2 (AML) did not reach neutrophil engraftment although neutrophil levels exceeded 0.50×10^9/L on D+36. Patient 1 and 2 died on D+57 and D+38 respectively. Patient 3 is alive without any disease relapse at 33 months post-transplant.

Patient 2: A case of primary refractory acute myeloid leukemia (AML)

The patient was a 21 year old male with no significant past medical history. He was diagnosed with acute myeloid leukemia with, FLT3 ITD negative, and NPM1 positive [6-11]. He had refractory disease after standard IA3+7 induction and FLAG salvage chemotherapy. He underwent treatment with arsenic trioxide, azacytadine and MEC (comprising mitoxantrone, etoposide and cytarabine) chemotherapy, as well as other agents which resulted in complete remission [15]. The unmanipulated UCB unit 1 was a 5/6 HLA match and had TNCs and CD34 cell dose of 33.00×10^6 cells/kg and 1.82×10^5 cells/kg respectively. UCB unit 2 was a 5/6 HLA match and provided TNCs and CD34 cell dose of 21.10×10^6 cells/kg and 2.24×10^5 cells/kg respectively after expansion. His transplant was complicated by Klebsiella bacteremia at D+3 and suspected aspergillus at D+14. Patient eventually developed multi-organ failure with coagulopathy and died of CNS haemorrhage on D+38 post-transplantation. Patient did not reach neutrophil or platelet engraftment although neutrophil levels exceed 0.50 on D+36. VNTR at Day 14 and Day 28 showed 100% donor engraftment from unexpanded UCB unit 1.

Patient 3: A case of Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL)

Patient was an 18 year old male with no significant past medical history. He was diagnosed with Ph^+ all and underwent treatment with HyperCVAD 1A to 2B with imatinib, attaining complete remission. UCB unit 1 was a 4/6 HLA match and had TNCs and CD34 cell dose of 22.00×10^6 cells/kg and 1.44×10^5 cells/kg respectively. UCB unit 2 was a 4/6 HLA match and provided TNCs and CD34 cell dose of 11.80×10^6 cells/kg and 0.10×10^5 cells/kg respectively after expansion. The transplant was complicated by neutropenic fever due to *E. coli* bacteremia on D+4. He had neutrophil engraftment on D+34 and platelet engraftment on D+48. VNTR never showed any engraftment from the unmanipulated UCB unit 1, except for 5% at D+14. However, VNTR showed engraftment from expanded UCB unit 2 (85% at D+14 and subsequently 95-100% at D+28 and D+60 which has persisted for more than 2.5 years). He had molecular recurrence 6 months post-transplant and was treated with dasatinib. He remains in molecular CR at 33 months post-transplant without any GVHD.

In summary, Patient 1 neared engraftment on D+22 but then blood counts dropped again until she had robust neutrophil engraftment on D+44, however, the patient succumbed to pneumonia on D+57. Patient 2 was near neutrophil engraftment on D+37 but demised prior to fulfilling criteria of neutrophil engraftment. Patient 3 had neutrophil engraftment on D+34 and continue to maintain 100% engraftment from the expanded graft without disease relapse at up to 33 months post-transplantation. In this trial study, all patients received a myeloablative conditioning. A retrospective analysis of forty-four haematological malignancy patients who underwent conventional dUCBT (i.e. both UCB units are non-manipulated) after a myeloablative conditioning at Singapore General Hospital and National University Cancer Institute, Singapore (the two main transplant centers in Singapore), it was noted that the median time for neutrophil recovery was D+25 (range: D+14 - D+58) with a 34% transplant related mortality and 12% relapse related mortality [16]. The median TNC and CD34 cell doses of such conventional dUCBT were determined to be 59.00×10^6 cells/kg and 3.50×10^5 cells/kg [16]. Compared to the control cohort, it could be stated that performing dUCBT with an expanded graft (in presence of IGFBP-2 augmented MSC co-culture) could have delayed neutrophil engraftment although there was no significant difference between the two patient groups in terms of the number of infused TNC and $CD34^+$ cells. Although expansion increased absolute TNC and HSPC at the end of culturing, however, harvesting of the graft from the culture-ware resulted in poor recovery of approximately 39.5% of the cells mainly due to loss during centrifugation and washing.

Discussion

In this first of its kind cell therapy trial in Singapore, we intended to assess the safety and feasibility of transplanting *ex vivo* expanded UCB using MSC co-culture that were augmented with newly identified HSPC regulating cytokine, IGFBP2 [17]. However, there were two deaths out of three patients in this study. Also, the clinical outcomes of these three patients suggest that there was no acceleration of engraftment by this protocol. Thus, the study was halted. Nevertheless, it is interesting that, in the patient who survived, there was long term engraftment from the expanded cord blood unit without the use of any unexpanded "add-back" cells, suggesting that this strategy does not appear to compromise long term hematopoietic repopulation, even if accelerated engraftment did not occur. The protocol is currently being enhanced for future clinical studies by improvement in thawing methods and a study in the use of novel small molecules to enhance cord blood expansion.

As discussed earlier, many of the adverse effects after UCBT are related to the low numbers of TNCs and $CD34^+$ cells. Recipients of 1-2 HLA mismatched UCB grafts containing $<25.00 \times 10^6$/kg and $<1.70 \times 10^5$ cells/kg of TNCs and $CD34^+$ cells respectively lead to significantly poorer clinical outcomes [18,19]. Although our $CD34^+$ cell infusion dose (6.50×10^5 cells/kg) was higher than the recommended threshold, it was still far lower than other recently completed or on-going expansion trial protocols. In the past two decades, several novel

techniques have been developed, and they could successfully expand UCB early progenitor cells while minimizing differentiation. For example, Wagner et al used StemReginin1 (SR1), an aryl hydrocarbon receptor antagonist, to expand UCB $CD34^+$ selected grafts (HSC835 [20]. SR1 protocol gave an average of 330-fold (range: 67-848 fold) expansion of $CD34^+$ in the HSC835 graft which when transplanted with an unmanipulated graft resulted in a median infused TNCs and $CD34^+$ cell dose of 70×10^6/kg and 175×10^5/kg respectively. In this DUCBT trial involving seventeen hematological malignancy patients neutrophil and platelet engraftment occurred at a median of 15 days (range: 6-30 days) and 49 days (range: 28-136 days) respectively which significantly faster than the control (median time to neutrophil engraftment in control cohort was 24 days) [20]. Hematopoiesis was principally derived from the expanded unit in 11 patients and the unmanipulated unit in 6 patients. In HSC835 engrafting patients (n=11), the median time to neutrophil recovery was 11 days and co-related with the transplantation dosage and exhibited durable myeloid engraftment [20]. Horwitz et al expanded purified $CD133^+$ cells from UCB grafts (NiCord) in presence of nicotinamide (NAM) and transplanted to eleven hematological malignancy patients coupled with a second unmanipulated unit [21]. The NAM protocol achieved a median 72-fold (range: 16-186 fold) expansion of $CD34^+$ cells, providing a median infused $CD34^+$ cell dose of 35.00×10^5/kg. For all patients transplanted with NiCord graft, neutrophil recovery was achieved in 13 days (range: 7-26 days) versus 25 days in the center's historical cohort. The median time to neutrophil recovery for the 8 patients who had 100% engraftment with NiCord was 11 days (range: 7-18 days), which was even shorter [21]. The median time to platelet engraftment was 33 days (range: 26-49 days) for all patients transplanted with NiCord and 37 days (range, 20-66 days) for the center's historical control cohort22. Delaney et al expanded UCB $CD34^+$ cells by constitutive induction of Notch signaling and led to significant $CD34^+$ cell expansion [22]. At harvest, there was a median of 91.5-fold (range: 41-471 fold) expansion of $CD34^+$ cells which was transplanted to ten enrolled patients coupled with an unmanipulated UCB unit, resulting in a median $CD34^+$ cell infusion dose of 60.00×10^5 cells/kg. The median neutrophil engraftment time was shortened to 16 days (range: 7-34 days) as compared to 26 days in control cohort although long-term hematopoiesis was imparted by the non-manipulated graft [22]. De Lima et al reported the co-culturing of UCB mononuclear cells with allogeneic MSCs which resulted in a median of 30.1-fold (range: 0-137.8 fold) expansion of $CD34^+$ cells [23,24]. Compared to our current study, this expansion protocol at MD Anderson Cancer Center did not include IGFBP-2 in their cytokine cocktail that primarily consisted on SCF, TPO, FLT-3L and GCSF [23]. Transplantation of the expanded unit along with an unmanipulated UCB to the thirty-one patients resulted in a median of 83.4×10^6/kg TNC and 18.1×10^5 $CD34^+$ cells/kg. It led to engraftment in 9/10 patients at a median of 15 days (range: 9-42 days) for neutrophil and 42 days (range: 15-62 days) for platelet although long term hematopoiesis was imparted by the non-manipulated graft [23]. All these trial data suggest that a negative correlation exists between total nucleated and $CD34^+$ cells with time to neutrophil engraftment. Furthermore, only SR1 and NAM protocols could result in long-term hematopoiesis from the manipulated graft. It must be noted that for both SR1 and NAM trials, the CD34 and CD133 negative fractions of the expanded UCB unit was co-infused along with the expanded fraction and the second unmanipulated UCB. The add back of the T-cell fraction of the expanded UCB unit could have a pivotal role in facilitating engraftment of the expanded cells and modulating the graft-versus-graft reactions between the two UCB units. In both the Notch and MSC co-culture expansion protocol, the T-cell fraction of the expanded unit was either discarded or degraded during expansion culture respectively. When such expanded grafts that were devoid of its T-cell fraction, were co-infused with an unmanipulated UCB containing significantly higher T cells, then long-term hematopoiesis was imparted by the non-expanded graft, likely because of T-cell mediated immunological reactions.

Conclusions

In conclusion, addition of IGFBP-2 to MSC co-culture system could boost expansion of $CD90^+$ hematopoietic progenitors from non-selected UCB grafts. In this current trial, we report suboptimal outcome with regards to neutrophil engraftment, primarily due to complicated patient disease status at transplantation and low $CD34^+$ cell infusion dosage which contributed to high mortality and premature trial discontinuation. However, it must be noted that although this trial involved only three patients, there was no observation of acute or chronic GVHD. The current clinical findings are consistent to our previously reported pre-clinical data where the expanded UCB boosted the in vivo hematopoiesis of the unexpanded graft with increased regulatory T cell content and decreased incidence of GVHD [11].

IGFBP-2 supplementation and MSC co-culture supported modest but long-term engraftment of donor cells with minimal GVHD symptoms.

Institutional Review Board (IRB) Statement

The use of umbilical cord blood and bone marrow derived mesenchymal stromal cells were reviewed and approved by the Institutional Review Boards of National University of Singapore (NUS), Singapore General Hospital (SGH) as well as the Singapore Cord Blood Bank's (SCBB) Research Advisory Ethics Committee.

Clinical Trial Registration Statement

The clinical trial protocol was approved by SGH IRB and clinical trial certificate was issued by the Health Sciences Authority (HSA), Singapore. This trial was registered at www.clinicaltrials.gov with identifier: NCT01624701. This was a pilot interventional clinical trial (open label, single group assessment, phase I/II).

Informed Consent

All enrolled patients or their legal guardians provided the necessary informed consent for participation.

Grant Funding

SingHealth Foundation (SHF/CTG026/2009) and Singapore General Hospital (SRG/C4/01/2014) Research Grant.

Author Contributions

Xiubo Fan performed the pre-clinical validations, co-ordinated and supervised expanded graft production, analysed laboratory data and wrote the manuscript; Dixon Grant performed clinical management of study patients, analysed clinical data and wrote the manuscript; Florence Gay provided technical support in carrying out different aspects of the clinical trial; Sudipto Bari analysed laboratory and

clinical data, wrote and revised manuscript and provided technical support; Jeffrey Quek performed clinical management of study patients and analysed clinical data; Madelaine Naim, Melissa Soh, Marieta Chan and Mickey Koh were responsible for developing the workflow and carrying out the clinical expansion of the graft in the GMP facility; Harvey Lodish provided critical information pertaining to the expansion culture; William Hwang conceived and designed the clinical trial, performed clinical management of enrolled patients, analysed clinical and laboratory data, wrote and revised manuscript.

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