the stirring regime in spinner flasks was 15 min on and 45 min off at 35 rpm and. After 24 h the stirring regime of both the Scinus and the spinner flasks were set on continuous stirring at 35 rpm with a 1 h pause every 8h. ASCs from all conditions were harvested during culture for cell count and visual inspection.

Visual inspection showed cell attachment and a good cell distribution over the MCs (see Figure 1). After 4 days, cell distribution was more distinct on the MCs in the Scinus bioreactor when compared to the spinner flasks. In spinner flasks many MCs were confluent even though empty MCs were observed. This was not observed during both runs in the Scinus bioreactor. Cell proliferation was monitored during the whole culture time (see Figure 2). After 13 days of culture a total cell number of approximately 260 million ASCs was reached.

The necessity to obtain high cell concentrations for cell therapy, while cultivating cell identity, potency and safety, which is essential for success of new bioreactor technology. Our results showed that within the Scinus bioreactor high ASC concentrations can be quickly reached, and easily and efficiently harvested.

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QUALITY OF UMBILICAL CORD BLOOD STEM CELLS FROM INDONESIAN POPULATIONS

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Umbilical Cord Blood (UCB) is one of common source of Hematopoietic Stem Cells (HSC) populations to treat a variety of hematological disorder. It is important to produce high quality stem cells for better therapy result. Many factors can affect UCB stem cell quality, such as collected blood volume, total nucle-

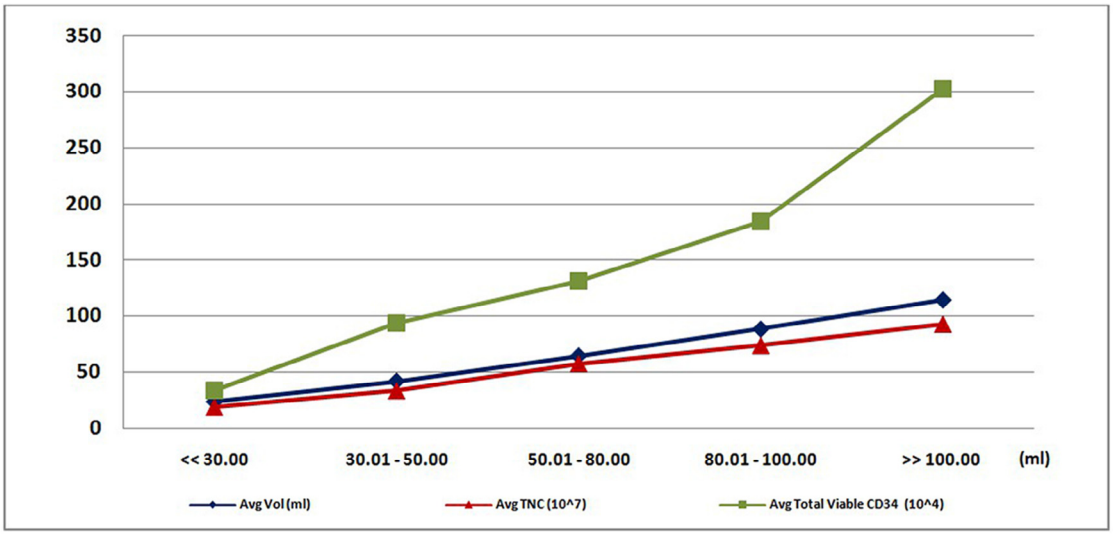

Figure 1. Correlation between collected UCB volume, Total Nucleated Cell (TNC), and Total Viable CD34. Average value are 62.14 ml for UCB volume (min = 9.62 ml; max = 139.62 ml), 52.95×10^7 cells for TNC (min = 3.16×10^7 cells; max = 140.76×10^7 cells), and 131×10^4 cells for Total viable CD34 (min = 0.76×10^4 cells; max = 928.02×10^4 cells).

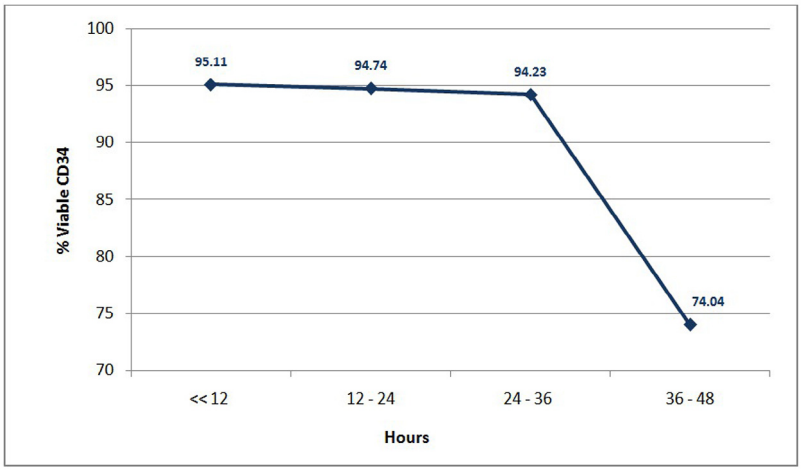

Figure 2. Correlation between Time to Product and % Viable CD34. Time to product is all time needed to deliver UCB unit from collection site to processing facility, continue with processing UCB into the final product.

ated cell (TNC), CD34, and also time to product. Indonesia as an archipelago country with 17,508 islands have stem cell processing lab where located only in capital city. It is very challenging to transport and finished the UCB sample processing before 48 hours.

Method & Results: We have analyzed 206 UCB units from different sites of Indonesia which had collected, processed and cryopreserved. All samples positive for Anti-HIV, HBsAg, Anti-HCV, VDRL/TPHA, CMV IgM and bacterial or fungal contamination were excluded for banking. Average processed UCB volume, TNC, and Total Viable CD34 are 62.14 ml, 52.95×10^7 cells, and 131×10^4 cells (Figure 1). From our data, it shows that as higher collected UCB volume, higher TNC and total Viable CD34 yielded. We analyze the correlation between time to product (≤12 hours, 12–24 hours, 24–36 hours, and 36–48 hours) and % viable CD34. Viable CD34 still stable at ≥ 94% for UCB complete processed below 36 hours and decrease into 74.04% for sample complete processed during 36–48 hours from collection time (Figure 2).

Discussion: FACT Net Cord 6 Ed. already specify the requirements for UCB unit stored for clinical administration are TNC $\geq 50 \times 10^7$ cells, viable CD34 $\geq 125 \times 10^4$ cells, and viability of CD34 cells shall be ≥85%. According to the standard, UCB quality from Indonesian population already meets the international standards criteria for clinical administrations. But for the therapy purpose which need more dosage it is recommended to collect more UCB volume to produce more TNC and Viable CD34. It is also recommended to complete stem cell processing below 36 hours, from collection time, to get >90% viable CD34 cells which more functional for cell regeneration after transplantations.

Conclusion: UCB quality from Indonesian population meets the international standards criteria for clinical applications.

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THERAPEUTIC POTENTIALS OF CORD BLOOD MONONUCLEAR CELLS TRANSPLANTATION FOR LIMB ISCHEMIA. A COMPARISON BETWEEN CD34+ AND CD34– CELLS

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Background: Cord blood -derived $CD34^+$ cells are a well-characterized population of stem cells. Also peripheral blood mononuclear cells (MNCs) improves limb ischemia in patients with arteriosclerosis however technique of isolation $CD34^+$ cells is highly cost in comparison to that of MNCS . Recently, $CD34^+$ cells have also been shown to induce therapeutic angiogenesis in animal models of myocardial, peripheral, and cerebral ischemia. The mechanism by which $CD34^+$ cells promote therapeutic angiogenesis is not completely understood, although evidence supports both direct incorporation of the cells into the expanding vasculature and paracrine secretion of angiogenic growth factors that support the developing microvasculature. Also, the mechanism of action of PBMNCs remains elusive. This work aims at determine cost benefit ratio of using three types of cell therapy in animal model of ischemia reperfusion through assay ischemic, apoptotic and fibrotic changes and how these changes will be ameliorated after cell therapy in ischemic animals.

Material and Methods: Three groups of rats were intramuscularly injected into the unilateral ischemic hindlimb by Cord blood -derived $CD34^+$ cells, cord blood mononuclear cells (MNCs)and CD34–depleted MNCs then 7, 14 and 28 after operation samples were taken from muscle.

Methods: Age-matched rats underwent 1.5 hours of unilateral hind limb ischemia, followed by 7, 14,28 days of reperfusion .Histologic analysis of skeletal muscle fiber injury was assessed. Morphologic evidence of muscular fiber maturation was assessed by myogenin, using IHC. Markers of angiogenesis, as VEGF, and Caspase to assess apoptosis using RT PCR, at 7, 14, 28 days . Also were measured fibrosis using histochemistry and Western blots . RESULTS:, endothelial cell apoptosis and interstitial fibrosis were significantly attenuated by CD34+ cells, This study demonstrates that a low number of CD34+ cells favors reparative neovascularization and possibly myogenesis in limb ischemia, suggesting the potential use of this cell population in regenerative medicine. Lag in muscle r evels of myogenin and an increased level of caspase as well as fibrosis in the rats, also these changes more ameliorated by CD34 treatement in comparison to those treated by MNCs or CD34–ve cells.

Conclusion: The depletion of CD34+ cells attenuated the therapeutic efficiency of MNCs in response to ischemia-induced neovascularization.

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ESTABLISHMENT OF AN AUSTRALIAN CORD BLOOD-DERIVED IPSC HAPLOBANK FOR CLINICAL USE

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Induced pluripotent stem cells (iPSC) are created by reprogramming normal human somatic cells into stem cells that can be differentiated into all cell types within the body, potentially providing a source of cells for therapeutic function. Importantly, iPSCs are immortal and therefore can be expanded and banked, providing an inexhaustible stem cell resource for both research and clinical applications. Technology is now available where Good Manufacturing Practice (GMP)—compliant, clinical grade iPSCs can be created for therapeutic use [1]. Recent publications have promulgated the possibility of global iPSC banks, in which the banked lines have homozygous human leukocyte antigen (HLA) haplotypes and are derived from material selected from donors whose haplotypes are common in the target population. As such, cells derived from these banked iPSCs would be suitable for therapeutic use for many individuals within the population. Cord blood (CB) is an ideal source of starting cells for iPSC generation.

We aim to establish a clinically relevant GMP compliant bank of homozygous HLA haploidentical cord blood derived hiPSC lines for cellular therapies in Australia and globally. In a collaboration between the iPSC core facility at MCRI, the BMDI Cord Blood Bank (BMDI CBB) and Sydney Cord Blood Bank (SCBB), experts in HLA and statistical genomics and experts in GMP and international regulatory compliance, we have embarked upon a project to establish the infrastructure and explore the feasibility of such a bank. HLA tissue typing data from CB banked at the BMDI CBB was interrogated using a purpose-written algorithm; from a total of 13,679 records interrogated at least 143 CBU with homozygous haplotypes at the 2-digit level of HLA-A, B and DRB1 were identified, with at least 10–20 CBU with unique homozygous haplotypes. 17,526 records were interrogated from the SCBB, with at least 10 CBU with unique homozygous haplotypes identified. This preliminary data confirms the feasibility of the establishment of an Australian Cord Blood iPSC haplobank for clinical use. Progress towards this aim will be presented, highlighting the many challenges and considerations ahead.

Reference

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DIFFERENTIAL EFFECT OF PROGNOSTIC FACTORS IN HEALTH AND DISEASE IN PERIPHERAL BLOOD STEM CELL (PBSC) MOBILIZATION AND COLLECTION: A SINGLE-CENTER/ DOCTOR EXPERIENCE IN AUTOLOGOUS HARVEST

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Background and Objectives: Granulocyte–Colony Stimulating Factor(G-CSF) stimulated peripheral blood stem cell collection (PBSC) has effectively replaced bone marrow harvest as a safe stem cell source for autologous transplantation in the past 20 years. Poor collection outcomes are still common in different populations and this study adds important prognostic factor that may affect outcomes.

Material and Methods: PBSC was collected after mobilization with 5 μg/Kg SC daily for 5 days in 216 healthy donors and 131 patients [median donor age at 41 (15–74) years while patient median age was 52 (7–89)] using 2 types of continuous blood cell separator (COBE Spectra or ComTec) on day 6 for healthy donors while some patients needed additional PBSC collection on day 7. Total cell yields were calculated as the number of CD34+ cells/kg body weight (BW). The efficacy PBSC collection compare between day 1 and 2 by using pair T-test. All subjects were monitored for PBSC collection side effects such as hypocalcemia and platelets loss.