Table 1. Characteristics of donors (n=7) and bone marrow samples (n=14) from brain-death donors

| Donors | | Median time to BM harvest after brain death,hours (range) | Median weight of BM samples, grams (range) | | Median total CFU-F per BM sample (range) | |
|---|---|---|---|---|---|---|
| Median age, years (range) | Sex (M:F ratio) | | Iliac crest | Femur | Iliac crest | Femur |
| 44 (18-67) | 3:4 | 15 (6-22) | 1.42 (0.32-3.92) | 1.26 (0.15-2.26) | 3591 (208-7107) | 688 (77-1398) |

BM: Bone marrow, CFU-F: Fibroblastoid colony-forming units, F: Female, M: Male

forming units (CFU-F) per gram of BM nor the amount of CFU-F per MNC differ significantly. The comparative analysis of MSC yields showed that there were no significant differences in MSC obtained per gram of IC-BM or F-BM during the first three passages of cell culture. Furthermore, there were no statistical differences in cell doubling time and population duplications. Cultured cells from both sites exhibited the ability to adhere to plastic and express CD105, CD73, CD90 but not CD45, CD34, CD14 markers by flow cytometry analysis. Trilineage differentiation was also achieve, meeting the ISCT minimum criteria to define hMSC. These results show the feasibility to obtain hMSC from two sites in BDD, and underscores the potential of this new source as a suitable allogeneic source of hMSC for cell-based therapies.

Table 1.

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**CHALLENGES IN DEVELOPING AN OFF-THE-SHELF CELL THERAPY FOR ACLF AND NASH**

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**Background & Aim:** Promethera is a biopharma company focused on the research, development and commercialization of stem cell-based therapies and technologies for acute and chronic liver diseases. Our lead clinical program, an allogenic human liver derived mesenchymal stem cell product derived from our patented cell technology platform HepaStem, is currently being used in Phase IIa clinical trials for ACLF (acute on chronic liver failure) with the company also preparing clinical trials for NASH. Non-alcoholic steatohepatitis (NASH), a severe form of non-alcoholic liver diseases (NAFLD), is one of the prominent liver diseases worldwide. There is currently no approved drug for its treatment and liver transplantation is the only therapeutic approach for advanced NASH. Mesenchymal stem cells (MSCs) are promising candidates to modulate the pro-inflammatory and pro-fibrogenic environment of chronic liver because of their immunomodulatory properties. Recent data obtained in preclinical models of early and advanced stage NASH provided significant evidences to open new phase I/II clinical studies in NASH.

**Methods, Results & Conclusion:** The dosing for this indication is likely to be in the order of 100 million cells per infusion. Large numbers of cells thus need to be manufactured and using standard cell culture techniques would make it too expensive and labour intensive. A standard expansion protocol in flasks or cell factories is an open process, and therefore run in expensive clean rooms to avoid the risk of contamination. We have explored several state-of-the art-bioreactor systems, and various types of microcarriers in stirred tanks from different manufacturers. We have tested these systems to optimize the complete workflow of HepaStem culture, including stem cell isolation from livers and expansion up to the scale of many clinical doses. Quality control and comparability studies were performed to verify that the characteristics of HepaStem cells harvested from the different procedures were maintained. We have built a production platform, almost entirely in closed system, that can provide the large numbers of clinical doses that would be needed for the treatment of NASH. This platform functions with a strong reduction of human labour and it also modular and flexible to anticipate growing demand. In parallel we have developed serum-free and xenofree culture methods to be able to abandon the use of animal-derived products, for the related risks and because animal sera become scarce and expensive.

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**WILL NOT BE PRESENTED**

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**EFFECT OF HEPARIN ON PROLIFERATION MESENCYMAL STEM CELL**

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[1]Prodia StemCell, Jakarta, Indonesia, [2]Padjadjaran University, Bandung, Indonesia, [3]Prodia Widyahusada, Jakarta, Indonesia, [4]Prodia, Bandung, Indonesia

**Background & Aim:** Mesenchymal Stem Cells (MSC) have enormous potential as regenrative therapy. Pre-clinical trials and clinical trials have been widely performed. The constraint in therapy using MSC is the number of poorly packed cells, in which the MSC population in various tissues ranges from 0.01-0.0001%. Various strategies have been made to improve the effectiveness of therapeutic use of stem cells, among others by performing ex vivo scale up then after MSC meets an adequate amount applied either autologously or allogenically. It needs MSC propagation culture before it is applied as regenariatif therapy. In this study, heparin addition was added to the MSC culture medium to see its effect on MSC proliferation.

This study has conduct in Prodia Stem Cell Indonesia Laboratory and Prodia Widyahusada Clinical Laboratory at November until December 2017. In this study, the addition of heparin to mesenchymal stem cell culture medium with concentration of each treatment group 0 IU / ml (as control); 0.30 IU / ml; 0.61 IU / ml; 1.25 IU / ml; 2.5 IU / ml; 5 IU / ml; and 10 IU / ml. The culture was carried out for 15 days. On the 1st day; the 3rd; 7th; 10th; and 15th, do MTT test.

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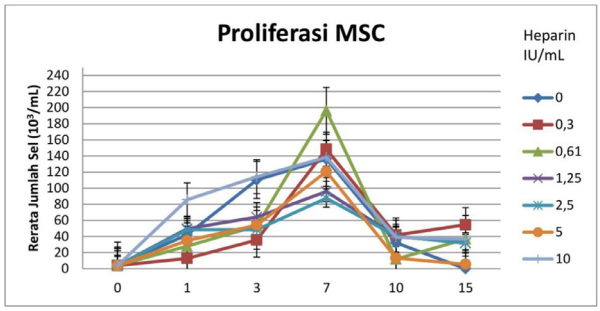
In the study, the highest live cell count was in the heparin treatment group of 0.61 IU / mL (1.9 × 103 cells / mL) p = 0.000.

**Conclusion:** Adding heparin in culture medium, increased the proliferation of mesenchymal stem cells with optimum concentrations of 0.61 IU / mL on day $7^{th}$ after cultures.

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## A SAFE AND SIMPLE APPROACH TO OBTAINING SUFFICIENT CYTOKINE INDUCED KILLER (CIK) CELLS FROM CRITICALLY-ILL CANCER PATIENTS WITHOUT THE NEED FOR APHERESIS OR PERIPHERAL MOBILIZATION

S. Chin[1], W. Kong[2], Q. Lian[3], K. Then[1] & S. Cheong[4]
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**Background & Aim Background:** Cytokine-induced killer (CIK) cells are a group of immune cells that include natural killer (NK) cells, T cells and natural killer T (NKT) cells which can be generated from peripheral blood mononuclear cells (PBMC). NKT cells can be identified by the presence of CD3+ and CD56+ surface markers and are able to target cancer cells directly or indirectly in the absence of antibodies or MHC specific proteins allowing a rapid and non-restricted immune reaction. Treatment with CIK also replenishes immune cells lost during chemotherapy. Studies have shown that $5 \times 10^9$ CIK cells with at least 30% fraction of NKT cells were associated with better outcomes. However most critically-ill cancer patients are unable to tolerate large volume apheresis to obtain sufficient PBMC for CIK expansion.
**Objective:** We explored a new protocol to expand the CIK cells by using only 60ml of peripheral venous blood from stage III-IV cancer patients undergoing chemotherapy and radiotherapy. The benefit of this approach is the usage of smaller amount of blood through simple venipuncture as compared to the more invasive apheresis method.
**Methods, Results & Conclusion Methodology:** 60ml of peripheral venous blood was withdrawn from 9 patients (M=5, F=4) with mean age of 62±11 years old and 1 healthy donor (male, 45 years) using simple venipuncture technique. The samples were isolated and induced using interferon-gamma (IFN-$\gamma$), anti-CD3 antibody, recombinant human interleukin-2 (IL-2) for up to 21 days.
**Results:** We successfully isolated and expanded CIK from all 10 blood samples. The number of CIK was significantly increased from Day 1 to Day 21 of cell expansion ($0.12\pm0.08 \times 10^9$ vs. $7.46\pm1.85 \times 10^9$ cells, $p<0.001$) with average of 84±49 fold increase. The expansion number and rate was similar to the healthy donor. In addition, the proportion of NKT cells (CD16+ and CD56+) was 43.7±11.0%.
**Conclusion:** Using our protocol, we have successfully isolated and expanded CIK to significant and sufficient number of cells using only 60ml of peripheral venous blood. This method may allow more critically-ill cancer patients to consider adjunctive cellular immunotherapy.
Fig. 1.

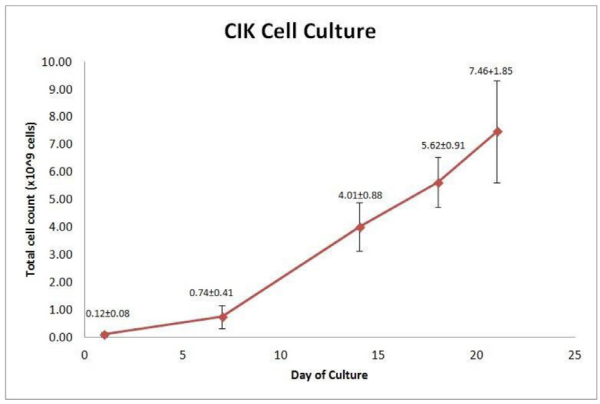


Figure 1. Mean of CIK cells at Day 1 up until Day 21 of expansion. Numbers of CIK cells increased significantly after 7 days of culture from $0.12\pm0.08$ to $0.74\pm0.41 \times 10^9$ cells and continued to grow exponentially up until 21 days of culture.

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## ARTHROSCOPIC EVIDENCE OF CARTILAGE REGENERATION IN SEVERE KNEE CARTILAGE DEFECTS AND OSTEOARTHRITIS FOLLOWING TREATMENT WITH ALLOGENEIC UMBILICAL CORD-DERIVED MESENCHYMAL STROMAL CELLS (CHONDROCELL-EX)

B. Md Yusoff[1], M. Ramlan[1], S. Chin[2], N. Mohamad[1], S. Cheong[3], M. Muhammed Ali Noor[1], A. Mohd Don[1] & S.S. Abdul Wahid[4]
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**Background & Aim Background:** Knee cartilage defect is a difficult condition to treat. Currently, there are no satisfactory treatments for grade 3 International Cartilage Repair Society (ICRS) defect. Allogeneic umbilical cord-derived mesenchymal stromal cells (UC-MSCs) offers a safe and convenient potential therapeutic option for ICRS grade 3 knee articular cartilage defect because of their immunomodulatory functions and ability to promote cartilage differentiation. Despite subjective functional improvements reported by patients in many studies, objective arthroscopic images of gross cartilage regeneration are rarely obtained and it is not known if the improvement is merely due to reduction of joint inflammation or associated with cartilage regeneration.
**Objective:** The aim of this study was to assess the efficacy of allogeneic umbilical cord-derived mesenchymal stromal cells in patient with knee articular cartilage defects and to demonstrate cartilage regeneration using follow-up arthroscopy.
**Methods, Results & Conclusion Method:** This is a single-arm, open-label, compassionate study at the Universiti Kebangsaan Malaysia Medical Centre (UKKMC) with 12 months follow-up period. Subjects with knee articular cartilage defect ICRS 3 diagnosed via diagnostic arthroscopy were treated with allogeneic UC-MSCs (Chondrocell-Ex, Cytopeutics, Malaysia) and assessed in five subsequent visits. During the visits, assessment was done following Visual Analog Score (VAS), International Knee Documentation Committee (IKDC), Tegner Activity Score (TAS), and Knee injury and Osteoarthritis Outcome Score (KOOS) scoring. At the end of the study, subjects underwent another arthroscopy and gross morphology of the knee articular cartilage was directly visually reassessed.
**Results:** Six female subjects (mean age of 46.8±5.4 years) with single knee injury received the treatment. All patients had knee cartilage defect of $2.5cm^2$ or larger. We observe no procedure or stromal cell related adverse events during the 1 year follow up. VAS, IKDC, TAS and KOOS significantly improved in these subjects with VAS/KOOS, and TAS/IKDC score at 3 months and 6 months post therapy onwards until 12 months. Direct visualization during follow-up arthroscopy confirmed total coverage of previously defected articular cartilage in all patients.

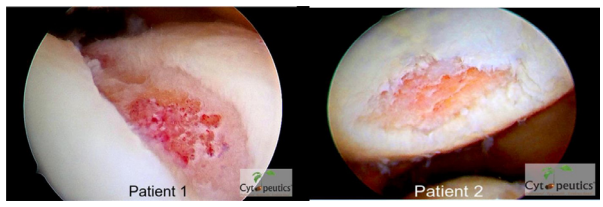


Fig. 1. Figure 1 shows the baseline knee articular cartilage arthroscopic images, prior to intra-articular injection of UC-MSC. At 12 months post-treatment, the injury sites are 100% covered with smooth hyaline cartilage, demonstrating the ability of UC-MSCs Chondrocell-Ex in regeneration of new cartilage.