

## Investigation of expanded-cells as a medical treatment for bone marrow transplant

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**Background and Aims:** Nowadays, Cord-Blood Hematopoietic Stem Cells (HSCs) are well known as a very valuable source of cell for cell therapy purposes including gene therapy and Bone Marrow Transplantation (BMT). They were used in autologous and allogeneic transplantation by genetic manipulation and stimulating drugs. However, to reduce the risk of rejection should be less genetic manipulation and techniques concerned. Then we used some hormone and growth factor in ideal dosage as stimulating factors to expand cells. After this in-vitro culture (before in vivo phase and clinical trial) we should test these cells for ex-vivo evaluation.

**Methods:** HSCs were isolated from cord blood units and cultured in StemSpan medium and 3cytokines, hormones and growth factors: Stem-Cell-Factor (SCF-100 ng/ml), Thrombopoietin (Tpo-50ng/ml) and FMS-Like Tyrosine-Kinase3-Ligand (Flt3L-50 ng/ml) for 21days. Then cells were analysis in ex vivo phase by Flow-cytometry, Clonogenic-assay, Long-Term Culture-Initiating Cells (LTC-IC) and Karyotyping. For Statistical analysis, results were expressed as mean  $\pm$  SD and analyzed by T-test. Differences were considered to be significant at  $P < 0.05$ .

**Results:** phenotypic level by Flow-cytometry showed more HSCs. In functional level by Clonogenic-assay; we resulted these increases in cell population and colony forming cells have accompany with increase in LTC-IC frequency (primitive cells  $\pm$  20%). Positive tests meant serial dilution cell dose (500, 1000, 1500 cells/ml) had saved their differentiated potential during stimulation period. After this growth factor treatment, for research in genetics and cytogenetics, Karyotyping had development. Chromosome number (46n) and G-banding pattern were like a normal cell and no abnormality in quantity (aneuploidy) or quality was taken and also no fusion between HSCs has been occurred.

**Conclusions:** This approach can be very effective for overcoming limited number of cord blood HSCs for utilizing them for cell therapy purposes and this simple and non-expensive treatment without any cytotoxic effect can be used in clinical trials.

**Keywords:** Hematopoietic stem cells; Ex vivo expansion; Bone marrow transplantation