enhancement to determine the role of contact, the effect on proliferation and apoptosis, as well as possible molecular mechanisms. **Methods:** CD34-selected or non-selected UCB cells were co-cultured with MSC under serum-free conditions. CD45+ and CD34+ cells were detected b flow-cytometry. Progenitor function was determined by colony forming unit assay. Non-viable and early apoptotic cell detection was based on 7-Amino-ActinomycinD/Annexin-V staining. Cell cycle analysis was based on propidium iodide staining. **Results:** CD34+ selected as well as non-selected UCB cells had 2 to 5 fold enhancement of growth when cultured on MSCs compared to expansion in cytokines alone. Over 90% of the proliferative activities were contributed by the CD34-selected cells. Progenitor cells expanded rapidly only during the first 7 days of co-culture but declined afterwards. The enhancement on UCB expansion was maximized when the MSC layer was in direct contact with the UCB cells. UCB cells co-cultured with MSC resulted in 2- to 4-fold lower apoptotic cell fraction, and UCB cell-cycling activities were not affected. **Conclusions:** The main mecahnism of enhancement of CBU cultures on MSC co-cultures appears to be due to rescue of cells from apoptosis during the initial few days of culture rather than due to a direct effect on augmenting proliferation. The molecules involved in this interaction aree being investigated in our laboratory.

125

STROMA FORMATION FROM FRESH AND CRYOPRESERVED MONONUCLEAR CELLS OBTAINED AT THE PRE AND POST-MOBILIZATION PHASES FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

Schimiegue, D.M.[2], Silva, L.[1], Dreyfuss, J.L.[3], Zattar, K.C.[2], Nader, H.B.[3], Borelli, P.[4], Oliveira, J.S.R.[2] [1] The University of Texas MD Anderson Cancer Center, Houston, TX; [2] University Federal of Sao Paulo, Sao Paulo, Brazil; [3] University Federal of Sao Paulo, Sao Paulo, Brazil; [4] University State of Sao Paulo, Sao Paulo, Brazil.

Background: Autologous hematopoeitic stem cell transplantation (auto-HSCT) comprises the manipulation of bone marrow and peripheral blood HSC by cryopreservation and posterior thawing. These manipulations can cause cell damage, especially in patients submitted to aggressive induction remission chemotherapy, radiation and auto-HSCT conditioning. **Study Design and Methods:** Thirty-two bone marrow samples (22 patients and 10 controls) were collected at pre-mobilization phase and 27 (20 patients and 7 controls) at post-mobilization, respectively. Fresh or cryopreserved mononuclear cells were cultured for eleven months and had their confluency capacity analyzed. **Results:** In regard to confluency achievement, there was no difference between fresh and cryopreserved samples from pre-mobilization phase. Apart from this, cells from 6 of the 11 (54%) fresh samples collected from patients at pre-mobilization phase achieved ≥70% confluency. Cells from all (5 of 5, 100%) fresh control samples collected at this phase and from 4 of the 5 (80%) cryopreserved, achieved ≥70% confluency. Cells from 5 of the 10 (50%) fresh samples collected from patients during the post-mobilization and 4 of the 10 (40%) cryopreserved samples reached ≥70% confluency. Considering the control group from this phase, cells from half (1 of 2) of the fresh samples and from 1 of the 5 cryopreserved samples, achieved ≥70% confluency. **Conclusion:** Stroma establishment from normal hematopoietic progenitors post mobilization is reduced when compared to samples obtained from patients with hematologic malignancies, most probably due to higher sensitivity of healthy progenitor cells to cryopreservation and thawing-induced damage.

126

CO-TRANSPLANTATION OF MESENCHYMAL STEM CELLS AND HEMATOPOIETIC STEM CELLS IN ß-THALASSEMIA PATIENTS

Ghavamzadeh, A., Valizadeh, N., Alimoghaddam, K., Karimi, A., Bahoush, G., Bashtar, M., Shamshiri, A.R., Jalili, M., Mosavi, A.S., Shahriari, Z., Basirpanah, S., Khalilvand, S., Chardouli, B., Khatami, F. University of Tehran/Medical Sciences, Tehran, Islamic Republic of Iran.

Background: mesenchymal stromal cells are immunomodoulatrory and may have important role in engraftement and GVHD/HVGD. we studied co-transplantation of ex vivo expanded MSCs and HSC. **Patients and Methods:** In this study, we coadministered culture-expanded MSCs with HLA-identical sibling-matched HSCs in β-thalassemia patients. Between Nowember 2006 and February 2007, 10 ß-thalassemia patients were enrolled. Patients received Cyclophosphamide-based or Fludarabine –based conditioning regimens and short course methotrexate and cyclosporine as GVHD prophylaxis. On day 0, patients were given MSCs intravenously ($1.0–2.24 \times 10^6$/kg) 4 hours before infusion of either bone marrow or peripheral blood stem cells. Outcomes of transplantation compared between these patients with 50 matched - historical controls group which were transplanted with HSCs in last years. Matching criteria were included: recipients' gender, source of stem cell (bone marrow or peripheral blood), thalassemia group (intermediate or major), class of ß-thalassemia major (I, II, III) . **Results:** Chills and fever was only notable toxicity in MSC group. The median time to achieve WBC engraftment $\geq 0.5 \times 10^9$/L was 12.5 days (range 10–20 days) for MSC group and 12 days (range 6–52 days) in matched-historical control group (p-value = 0.67).

The median time to achieve platelet engraftment $\geq 20 \times 10^9$/L was 18 days (range 12–30 days) for MSC group and 22 days (range 10–81 days) for comparison group (p-value = 0.02). Incidence of acute GVHD was 80% and 76% in MSC and historical-matched group respectively (p-value = 1). 3-month overall survival rate was 90% and 91.7% in MSC and matched control group respectively (p-value = 0.29). 100-day disease free survival rate was 80% and 87.8% in MSC group and matched historical control group respectively (p-value = 0.27). **Conclusion:** In this study we demonstrated that co-transplantation of HLA-identical sibling MSCs with HSCs is seems to be safe. we can't find statistical significant difference in acute GVHD incidence, severity, OS, DFS, Median time to WBC recovery between MSC group and compartion group.most probably explantation is small number of patients in study group.median time to plt recovery was shorter in MSC group (p-value = 0.02).

127

MESENCHYMAL STEM CELLS EXERT DIFFERENTIAL EFFECTS ON ALLOANTIGEN- AND VIRUS-SPECIFIC T CELLS

Karlsson, H.[1,2], Samarasinghe, S.[1], Ball, L.[3], Sundberg, B.[2], Lankester, A.C.[3], Dazzi, F.[4], Rao, K.[5], Veys, P.[5], Le Blanc, K.[2], Ringdén, O.[2], Amrolia, P.J.[1,5] [1] Institute of Child Health, London, United Kingdom; [2] Karolinska Institute and Karolinska University Hospital Huddinge, Stockholm, Sweden; [3] Leiden University Medical Centre, Leiden, Netherlands; [4] Imperial College School of Medicine, London, United Kingdom; [5] Great Ormond Street Hospital for Children, London, United Kingdom.

Objectives: Mesenchymal stem cells (MSC) suppress alloantigen-induced proliferation, interferon-γ (IFN-γ) production and cytolytic killing *in vitro* and infusion of third-party MSC appears a promising therapy for acute GVHD. However, little is known about the specificity of immunosuppression by MSC and in particular the effect on cell-mediated immunity to infectious pathogens. We have studied the effect of MSC on virus-specific T-cell responses. **Results:** Peripheral blood mononuclear cells (PBMC) from 6 normal donors were stimulated for with autologous lymphoblastoid cell lines (LCL) (EBV), pp65 peptides (CMV), an adenoviral vector Ad5f35 (Ad) or allogeneic PBMC (Allo), in the presence or absence of third-party MSC (MSC/PBMC ratio 1:10). MSC significantly suppressed proliferation in response to Allo (mean 61% suppression, p = 0.003), but had less effect on the response to EBV (mean 42% suppression, p = 0.016) and no suppression of the response to CMV or Ad. MSC had no effect on expansion of EBV and CMV pentamer-specific T-cells after stimulation with their cognate antigen. ELISPOT assays demonstrated that MSC significantly inhibited IFN-γ production in response to Allo (mean SFC/10^5 cells 1125 ± 274 without MSC, 263 ± 49 with MSC) and to a smaller extent to EBV (3181 ± 548 *vs* 2147 ± 387), but not to CMV (2535 ± 374 *vs* 2532 ± 311). Established EBV-specific cytotoxic T-cells (EBV-CTL) from 5 donors were