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. CD34+ and CD34− cells were detected by flow-cytometry. Progenitor function was determined by colony forming unit assay. Non-viable and early apoptotic cell detection was based on 7-Amino-Actinomycin D/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+ isolated 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 mechanism 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 are being investigated in our laboratory.

**Background:** Autologous hematopoietic stem cell transplantation (auto-HSCT) comprises the manipulation of bone marrow and peripheral blood HSC by cryopreservation and post-transplantation. 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 achieved ≥70% 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.

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


**Background:** Mesenchymal stromal cells are immunomodulatory and may have important role in engraftment 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 ß-thalassemia patients. Between November 2006 and February 2007, 10 ß-thalassemia patients were enrolled. Patients received Cyclophosphamide-based or Fludarabine-based conditioning regimen and short course methotrexate and cyclosporine as GVHD prophylaxis. On day 0, patients were given MSCs intravenously (1.0–2.24 × 10^6/kg) 4 hours before infusion of either bone marrow or peripheral blood stem cells. Outcomes of transplantation were compared between these patients with 50 matched - historical controls group which were transplanted with HSCs in last years.

**Results:** Chills and fever was only notable toxicity in MSC group. The median time to achieve WBC engraftment ≥0.5 × 10^9/L was 12.5 days (range 10–20 days) for MSC group and 12 days (range 6–52 days) in matched-control group (p-value = 0.67).

**Conclusion:** The median time to achieve platelet engraftment ≥20 × 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 comparison group most probably explanation is small number of patients in study group.medical time to pit recovery was shorter in MSC group (p-value = 0.02).
108 MAINTAINING THE IN-VIVO HEMATOPOEITIC NICHE IN-VITRO; A NOVEL APPROACH TO STUDYING HEMATOPOIESIS IN BOTH A MURINE AND A LARGE ANIMAL SYSTEM

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Introduction: Hematopoietic Stem Cells (HSC) are the most extensively characterized stem cell, yet understanding of their niche, and the ability to maintain and expand HSCs in-vitro remains a challenge. In vitro study of HSC has generally been limited to well-based cultures, or use of 3D structures, supplemented with cytokines. As opposed to constructing an artificial niche, we sought to examine whether the existent hematopoietic niche could be maintained in-vitro and support HSC in-vitro, both in murine long bones marrow fragments, & in perfused large animal vertebrae and bone marrow cores.

Methods: Femur and tibia from 3 month old mice were harvested, and sectioned into 1/3rd ends removed to facilitate perfusion of the bone marrow fragments (BMF). In addition, whole marrow 'plugs' were flushed from tibia. These were then cultured in normal or low O2, in regularly changed media +/- cytokines (Flh2, SCF, TPO). BMF were removed over time, and analyzed by CFU assay and FACS for presence of HSC, & transplanted into sublethally irradiated Ly5.2 recipients.

Large animal model: Fresh thoracic and lumbar vertebrae from large juvenile swine were harvested and separated, then maintained in culture as: non-perfused in media, syringe perfused by pump from 0.5 to 1.5 ml/hr of media, or by surgically cannulated vertebral arteries and veins maintained on a bioreactor. Vertebrae underwent core biopsies every 1–2 days and were analyzed for viability and by histology. Results: Murine BMF contained 35–72% viable cells as measured by trypan blue exclusion at up to 14 days. In 4 to 11 day old BMF, a population of Slam +, KLS cells were detectable. In BMF transplanted recipients, donor derived cells were detected 4–7 months post transplant. Mean donor granulocyte engraftment from BMF was 5.2 to 22.1%, compared to 19% from an control BM cells, suggesting long-term engraftment was derived from HSC maintained within cultured BMF. In porcine vertebral bodies, non-perfused vertebrae had < 10% viability by day 4 compared to 20% viability if perfused by pump at < 1 ml/hr. Histology revealed maintained marrow structure and healthy appearing cells in marrow cores from perfused vertebræ in contrast to unperfused marrow samples.

Conclusion: These results suggest that the intrinsic HSC niche can be maintained in-vitro and further optimization of this approach may provide a novel means to study murine and eventually human HSC and niche in-vitro.

129 ESTABLISHMENT OF BONE MARROW STRUMA FROM PATIENTS AT PRE AND POST-MOBILIZATION FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS TRANSPLANTATION

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Objective: Successful of autologous peripheral blood stem cell transplantation relies on mobilization’s capacity of hematopoietic progenitor cells from marrow to blood stream. The aim of this study was to evaluate the correlation between the velocity of establishment and maintenance marrow’s stroma in vitro from mononuclear cells and bone marrow biopsy data obtained before and after mobilization treatment in hematologic malignancies and controls. Materials and Methods: We evaluated clinical data from 22 patients and 10 healthy donors as controls regarding the velocity of establishment and maintenance of stroma through long-term bone marrow culture on semisolid medium and bone marrow histopathological features. Results: Out of 32 samples harvested at pre-mobilization, 21 (66%) achieve ≥70% confluency while 11 of 27 samples (34%) could do so after mobilization. At pre-mobilization, 91% of samples that did not achieve confluency were from patients. After mobilization, 9 (82%) and 2 (18%) samples from patients and controls reached confluence, respectively, indicating a reduction of stroma establishment potential, especially from controls (p = 0.03). We could not observe any difference between “good” and “poor” mobilizer in both times. Nevertheless, the velocity of establishment was faster from the controls than patients. Conclusions: Patients with fibrotic or poor cellularity demonstrated lower capacity of stroma’s establishing suggesting that damage might affect most probably the marrow microenvironment than the hematopoietic progenitors. However, a more reduction of stroma establishment capacity disclosed in controls than patients after mobilization might be due to a more intense mobilization in these settings.

130 DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION WITH REDUCED INTENSITY CONDITIONING AND SIROLIUMUS-BASED GVHD PROPHYLAXIS

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Single-unit umbilical cord blood transplantation (UCBT) in adults is associated with high transplant-related mortality, largely due to delayed engraftment and infection. Double UCBT (DUCBT) is associated with faster engraftment, but also with high rates of acute GVHD. We studied DUCBT using sirolimus and tacrolimus to improve GVHD outcomes.

Methods: Conditioning consisted of fludarabine (30 mg/m² × 6), melphalan (100 mg/m² × 1), and rabbit ATG (1.5 mg/kg × 4). UCB units were ≥4/6 HLA-A, B, DR allele-matched with each other and the recipient, and contained a minimum combined dose of 3.7 × 10⁷ TNC/kg pre-cryopreservation. GVHD prophylaxis was tacrolimus (5–10 ng/ml) and sirolimus (3–12 ng/ml). Results: 29 patients (median 49 years, range 19–67) with ≥100 day follow-up are reported. Diagnoses include AML(8), NHL(7), HD(5), MDS(4), CLL/PLL(2), ALL(1), MPD (1) and CML(1). The median total cell doses prior to cryopreservation were 5.2 × 10⁷ TNC/kg (range 3.2–7.6 × 10⁷; DUCBT), and 12.5 × 10⁷ TNC/kg (range 1.5–29.0 × 10⁷). Neutrophil engraftment occurred at a median of 21 days (range 13–70) and platelet engraftment occurred at a median of 42 days (range 25–162) after DUCBT. Three subjects did not attain platelet engraftment before day 100 and there were 3 late graft failures. 3 patients developed Gr. II–IV acute GVHD (2 Gr. II and 1 Gr. III). Median time to engraftment was later when compared with a prior DUCBT cohort that received cyclosporine and MMF as GVHD prophylaxis (10.3% vs. 36.9%, p = 0.04). Only 2 patients developed chronic GVHD after DUCBT.

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Poster Session I