stimulated with autologous LCL with or without MSC for 5 days. The percentage of EBV-pentamer specific CTL was not affected by the presence of MSC (mean 1.59% *vs* 1.52%). MSC did not inhibit IFN-γ production by EBV-CTL (6039 ± 1644 *vs* 5885 ± 1608). Furthermore, cytolytic killing of LCL by EBV-CTL was not suppressed by MSC (mean specific lysis 47 ± 3.6% *vs* 52 ± 4.7% at E:T ratio 30:1). Finally, we studied anti-CMV immunity in 2 patiens who received MSC for acute GVHD of the gut evolving into chronic GVHD, with a good transient response. PBMC from these patients showed persistence of pp65-pentamer positive T-cells and retained IFN-γ response to CMV post MSC infusion (mean SFC $91/10^5$ PBMC pre-MSC, 113 at 2 weeks and 221 at 1 month post MSC infusion). **Conclusion:** MSC have little effect on T-cell responses to CMV, EBV and Ad, which contrasts to their strong immunosuppressive effects on alloreactive T-cells. These data have major implications for immunotherapy of GVHD with MSC and suggest that the effector functions of virus-specific T-cells may be retained after MSC infusion.

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MAINTAINING THE IN-VIVO HEMATOPOIETIC NICHE IN-VITRO; A NOVEL APPROACH TO STUDYING HEMATOPOEISIS IN BOTH A MURINE AND A LARGE ANIMAL SYSTEM

Kraft, D.L., Smith, A., Weissman, I.L. Stanford University School of Medicine, Stanford, CA; DRFV Foundation.

Introduction: INTRO: 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 apposed 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 1/3rds 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 (Flt2, 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 artery and vein and 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 detectable >4 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 .5 ml/hr or 49% viability when perfused at >1 ml/hr. Histology revealed maintained marrow structure and healthy appearing cells in marrow cores from perfused vertebrae 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.

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

Schimieguel, D.M.[2], Silva, L.[1], Dominato, J.A.[3], Silva, M.R.R.[4], Nader, H.B.[3], Borelli, P.[5], Souza, M.K.[2], 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 Federal of Sao Paulo, Sao Paulo, Brazil; [5] University State of Sao Paulo, Sao Paulo, Brazil.

Objective: Successful of autologous peripheral blood stem cell transplantation relies on mobilization's capacity of hematopoetic 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:** Were 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 confluency, 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 establisment 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.

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

Cutler, C.[1], Kao, G.[1], Ho, V.[1], Alyea, E.[1], Koreth, J.[1], Armand, P.[1], Dey, B.[2], Spitzer, T.[2], Soiffer, R.[1], Antin, J.H.[1], Ballen, K.[2] [1] Dana-Farber Cancer Institute, Boston, MA; [2] Massachusetts General Hospital, Boston, MA.

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/m^2 × 6), melphalan (100 mg/m^2 × 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 3.7×10^7 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 5.2×10^7 TNC/kg (range $3.7–7.6 \times 10^7$) and 12.5×10^6 CD34$^+$ cells (range $1.5–29.0 \times 10^6$). 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 transfusion independence by 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 21 days). Acute GVHD was less frequent 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.