acute GVHD rodent models (Kovacsovics 2008, 2009, Metheny, 2011).

Methods: This is an open label Phase I clinical dose escalation study with the primary goal to assess safety of MultiStem as an adjunct therapy for adult hematological malignancy patients shortly after allogeneic HSCT. Patients were enrolled for stromal cell administration as a single dose or in multiple weekly doses. Infusional toxicity and RRTs was assessed for 30 days following the last dose. Secondary endpoints included incidence of acute GVHD, infection and survival through day 100. Dose escalation was guided by the Continual Reassessment Method (CRM).

Results: Enrollment was completed for the target of 36 patients from 5 clinical centers for this Phase I clinical trial. 18 patients received a single dose MultiStem IV at 1, 5, or 10 million cells per kg at day 2 and 18 patients received MultiStem IV at 1 or 5 million cells per kg at days 2, 9 and 16 or days 2, 9, 16 and 30 after allogeneic HSCT. There was no observed infusional toxicity in either arm. Two patients in the single dose arm experienced Bearman RRTs (Grade 3 mucositis; Grade 3 renal and pulmonary failure). These events were deemed unrelated to study product. In the multi-dose cohort, one patient experienced liver GVHD, deemed possibly related to study product. Engraftment occurred in all 36 patients and the median time to neutrophil engraftment was 15 days (range, 11-25 days). The 100-day cumulative incidence of Grade II-IV and III-IV GVHD was 28% and 6%, respectively in the single dose arm. Preliminary evaluations of the repeat dose arm showed 21% and 11% Grade II-IV and III-IV GVHD, respectively. The highest tested single and repeat dose regimens showed the lowest GVHD frequencies.

Conclusion: Single dose and repeat dose administration of MultiStem is well-tolerated, without observation of infusional toxicity or graft failure. The observed low incidence of severe acute GVHD supports the concept that this stromal stem cell therapy product is safe and can be harnessed as a novel therapeutic option for GVHD prophylaxis following HSCT.

166

TREATMENT OF STEROID RESISTANT GRADE II TO IV ACUTE GVHD BY INFUSION OF MESENCHYMAL STROMA CELLS EXPANDED WITH HUMAN PLASMA AND PLATELET LYSATE – A PHASE I/II STUDY

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Introduction: For numerous hematological diseases allogeneic HSCT is the only curative therapy. Despite multiple improvements in the last decade in the field of HSCT, aGVHD remains a life-threatening complication. In particular, the outcome of patients with severe steroid-resistant aGVHD is very poor. Therefore, it remains important to search for new therapeutic strategies for the treatment of aGVHD.

Objective: To study the feasibility, safety and efficacy of MSCs expanded with human plasma and platelet lysate (hPPL), in patients with steroid-refractory aGVHD.

Method: In an open-label, non-randomized prospective phase I/II study patients with steroid-refractory aGVHD grade II to IV were treated with ~$2x10^6$/kg MSC. Response rate, TRM, and AE were assessed for up to 1 yr after inclusion. Serum and bloodsamples from were collected.

Results: Between January 2009 and December 2010, 20 patients were included, 2 drop out, and 18 were available for further analysis: 5 children and 13 adults. Median age was 32.5yr (range 1.3-65.9). Organs involved in aGVHD were skin (67%), GI-tract (83%) and liver (28%). Overall grade was II for 22%, III for 72%, and IV for 6% patients. 1 patient received one infusion, all other patients received two or more infusions. Median follow-up was 5.5m (range 0.33-12). Complete response was observed in 11 patients 61%, after a median of 65 days (range 10-184 days). The OS was significantly better in responders when compared to non-responders ($p < 0.001$). Of the 11 patients who reached a CR, 8 patients relapsed approximately 2 months after reaching CR (median 59 days, range: 1-244). Three children relapsed with clinical signs of an allo-immune-lung, auto-immune-cytopenia or limited cGVHD and all 5 adults relapsed with GVHD of the gut (median 98 days after reaching CR, range: 35-302 days). However, GVHD of the gut was then again sensitive to steroids. Overall, 7 patients died, 4 due to progression of aGVHD, 1 patient due to abdominal bleeding and 2 due to sepsis.

Extensive biomarkeranalysis were performed. We found biomarkers who are associated with clinical response of patients.

Conclusion: Generation and infusion of MSCs in steroid-resistant aGVHD grade II- IV is feasible, safe and very effective. In addition, also patients who initially responded to MSCs but develop later a relapse of aGVHD during tapering or cessation of immunosuppressive drugs become again sensitive to the treatment with steroids. Clinical response can be predicted by biomarkers.

167

IN SEARCH FOR MOLECULES INVOLVED IN THE IMMUNOSSUPRESSION INDUCED BY MESENCHYMAL STROMAL CELLS

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Background: Mesenchymal stromal cells (MSCs) improve hematopoietic recovery, contribute to tissue regeneration and posses immunosuppressive properties. Because of these unique properties their applicability in the bone marrow transplantation has attracted much attention, especially as a promising therapy to control graft-versus-host disease. In this way, a better understanding of how MSCs induce immunosuppresion can give us the rational needed for the most appropriate use of these cells.

Objectives: This study aimed to search for soluble factors produced by MSCs that can potentially be involved in the mechanism of immunosuppression.

Methods: Mixed leucocyte reactions, were incubated in the absence or presence of 10% MSCs in a contact independent manner using a polycarbonate membrane (transwell) between MLRs and MSCs. After three or seven days, cultures were analysed using flow cytomety or submited to global gene expression analyses.

Results: We found 672 mRNAs increased and 311 mRNAs decreased in at least 2X. 70% of mRNAs increased in MSCs in co-culture were related to immune response. Of these, it was notorious the increased in mRNAs associated with antigen presentation via MHC I and II (8%), chemokine (9%), metabolism / transport of lipids (9%) and regulatory proteins induced by IFN-γ (18 %).

The pathways most likely to be activated in MSCs after co-culture were IFN-γ and IL-17. Among the 672 molecules increased in MSCs we choose COX-2 and the chemokines CCL8 e CXCL8 to evaluate their functions during the immunosuppression induced by MSCs. The use of indomethacin, a COX inhibitor, in co-cultures reversed the inhibitory effect of MSCs. Moreover, when separated from MLRs by a transwell of 0.5mM, which allows the passage of cells, we observed a greater suppression of those lymphocytes that migrated to the MSCs's niche, suggesting that chemokines are important in this process.

Conclusions: After co-culture MSCs changed from an inactivated state, "steady state", to an activated state. This process induced their immunosuppressive phenotype, which involves COX2 and differents chemokines. The specific role of CCL8 and CXCL8 is under investigation.

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168

MESENCHYMAL STROMAL CELLS IMPAIR THE DIFFERENTIATION OF $CD14^{++}CD16^{-}CD64^{+}$ CLASSICAL MONOCYTES INTO $CD14^{++}CD16^{+}CD64^{++}$ ACTIVATE MONOCYTES

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Background: Mesenchymal stromal cells (MSCs) possess immunomodulatory activity both *in vitro* and *in vivo*. However, little information is available about their function during the initiation of immunological responses through their interactions with

monocytes. While many studies have shown that MSCs impair the differentiation of monocytes into dendritic cells and macrophages, there are few articles showing the interaction between MSCs and monocytes and none of them have addressed the question of monocyte subsets modulation.
Methods: To better understand the mechanism behind the benefit of MSCs infusion for graft-*versus*-host treatment through monocytes involvement, we performed mixed leucocytes reactions (MLR) in the presence or absence of MSCs. After three or seven days, cultures were analyzed by flow cytometry using different approaches.
Results: MSCs induced changes in monocyte phenotype in a MLR. This alteration was accompanied by an increase in monocyte counting and in CD14 expression. MSCs induced monocyte alterations even without contact, although the parameters above were more pronounced with cell-cell contact. Moreover, the presence of MSCs impaired MHC I and II, CD11c, CCR5 expression and induced CD14 and CD64 expression on monocytes. These alterations were accompanied by a decrease in IL-1β and IL-6 production by these monocytes but no change was observed taking into account the phagocytosis capacity of these monocytes.
Conclusions: Our results suggest that MSCs impair the differentiation of $CD14^{++}CD16^{-}CD64^{+}$ classical monocytes into $CD14^{++}CD16^{+}CD64^{++}$ activate monocytes, having a role even earlier than the differentiation of monocytes into dendritic cells and macrophages.
Keywords: Immunosuppression, Mesenchymal stromal cells, Monocyte subsets
Financial support: MS, CNPq, and FAPERJ.

169

EFFECTIVE MOBILIZATION OF MESENCHYMAL STEM CELLS IN C57BL/6 MICE UTILIZING SINGLE AGENT PLERIXAFOR (AMD3100) OR IN COMBINATION WITH NEUPOGEN (G-CSF)

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Introduction: Mesenchymal stem cells (MSC) are a rare population of cells that have the ability to form muscle, bone, cartilage, and adipose. MSC can be obtained from the bone marrow (BM). Strategies to mobilize MSC into the peripheral blood (PB) where they can be easily collected would be of therapeutic benefit but there is a lack of consensus on effective strategies to mobilize MSC.
Methods: During a screen in C57BL/6 mice, subcutaneous (SC) injections of neupogen (G-CSF) or plerixafor (AMD3100) were identified as agents that mobilize MSC. Subsequently, neupogen (G-CSF, 50μg/kg SC twice a day for 4 days) and plerixafor (AMD3100, 5mg/kg SC, once 1 hr prior) were assessed in individual mice (N = 5) as single agents or in combination. PB and BM were collected and plated for colony formation in 5%CO_2 5%O_2 for 7 days. MSC colonies were scored as colony formation units-fibroblast (CFU-F total, large, & small) in parallel with hematopoietic progenitors (CFU-GM, BFU-E, and CFU-GEMM). Data is presented as colonies /mL PB or /femur ± SEM and analyzed using Mann-Whitney *U* test.
Results: Analysis of data showed that, as compared to saline, treatment with G-CSF and to a greater extent AMD3100 resulted in mobilization of total CFU-F (large & small CFU-F) into the PB (5.5±2.5, 22.1±5.3, and 135.8±11.5 CFU-F/mL respectively ($p<0.05$). Combination G-CSF+AMD3100 mobilized at levels (110.5±5.1 CFU-F/mL) not statistically different than single agent AMD3100 ($p<0.05$). Analysis of the large CFU-F colonies revealed that a significant number of large CFU-F were mobilized with AMD3100 or combination G-CSF+AMD3100 ($p<0.05$), but not G-CSF alone. In the BM, the total number of CFU-F, as compared to saline, was decreased in response to G-CSF or G-CSF + AMD3100, but not single agent AMD3100 ($p<0.05$). The response of hematopoietic progenitor mobilization to the agents tested was as expected: G-CSF, AMD3100, or G-CSF+AMD3100 mobilize CFU-GM, BFU-E, and CFU-GEMM into the PB with the greatest level of mobilization resulting from G-CSF+AMD3100 ($p<0.05$).
Conclusion: Single agent AMD3100 administered SC is effective at mobilizing mouse MSC (total CFU-F or large CFU-F) with no additional enhancement from combination G-CSF+AMD3100. G-CSF treatment, alone or in combination, depletes MSC in the bone marrow whereas treatment with AMD3100 does not. This suggests that use of single agent plerixafor (AMD3100) may be an effective strategy to mobilize MSC for use in the context of regenerative medicine.

170

EPAC ACTIVATION REGULATES HUMAN MESENCHYMAL STEM CELLS MIGRATION AND ADHESION

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Currently, one of the challenges confronted by the clinical applications of human mesenchymal stem cells (hMSCs) is how to enhance the homing and engraftment of hMSCs to the target tissues with high efficiency. To overcome such barrier, mechanisms responsible for the hMSCs homing and engraftment are one of the key research foci. As of now, the exact mechanism and soluble factors involved in migration and adhesion of hMSCs have not been completely unfolded. Exchange protein directly activated by cAMP (Epac), a novel protein discovered in cAMP signaling pathway, attracts our attention due to its potential role in regulating cells adhesion and migration by triggering the downstream Rap family signaling cascades. However, the exact biological role of Epac in cells homing remains elusive and even controversial. Our study aimed to evaluate the regulatory effects of Epac in the homing process of hMSCs. We confirmed that hMSCs expressed functional Epac. In addition, Epac activation stimulated by specific analogue enhanced the adhesion and migration capacities of hMSCs significantly. Such homing enhancement effects were associated with corresponding morphological changes induced by Epac. The Epac activation was further found to be contributed directly to the chemotactic responses induced by stromal cell derived factor-1 (SDF-1) which is a known crucial chemokine in regulating hMSCs homing. These findings suggested Epac is connected to the SDF-1 signaling cascades. In conclusion, our study revealed that Epac plays a role in hMSCs homing by promoting adhesion, migration and also by enhancing chemotactic effect induced by SDF-1. Appropriate manipulation of Epac may enhance the homing and engraftment of hMSCs and facilitate the future clinical applications of hMSCs.

171

ROLE OF NFATC2 IN PROLIFERATION AND DIFFERENTIATION OF HUMAN CD34+ HEMATOPOIETIC STEM CELLS

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Background: Cyclosporine-A (CSA) inhibits NFATc2 activation and is effective in management of graft versus host disease (GVHD). Little is known of the role of NFATc2 transcription factor and its inhibition, in affecting differentiation and proliferation of CD34+ HSC's (Hematopoietic Stem Cells).
Methods: We used Ficoll density gradient method to obtain Mononuclear Cell (MNC) from human cord blood units (provided by Pablo Rubinstein, MD, New York Blood Center). MNC's were enriched for CD34+ HSC's using magnetic bead separation (Auto Macs, Miltenyi) which were plated at density of 60,000 cells / ml in serum free media with IL-3 (5ng/ml), FLT-3L (100 ng/ml), SCF (50 ng/ml), G-CSF (30 ng/ml) and GM-CSF (5ng/ml). Cells were cultured under normoxic conditions for 7 days at 37.0^{C} with 5% CO2. HSC proliferation and differentiation was studied in presence and absence of CSA.
Results: Flow cytometry analysis of HSC's grown in absence of CSA showed high CD 34 (84.7%) and low CD33/ HLA-DR (0.57% and 5.71 % respectively) expression on Day 0 of culture. In presence of CSA at 2mM concentration, we noted a more rapid rise of CD33 (41.0% versus 28.1%) HLA-DR (44.4% versus 29.1%) and CD 71 (12 % Vs 24.4%) markers by day 7. No difference was noted in total cell count in CSA treated 701,666.6 ± 7637 (mean and SD) and untreated 643,333 ± 5166 conditions.