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.

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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.

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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.

Financial support: MS, CNPq, and FAPERJ.

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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