CONDITIONED MEDIUM DERIVED FROM MURINE BM-MSCS CULTURED AS SPHEROIDS EXHIBIT *IN VITRO* IMMUNOMODULATORY CAPACITY

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Conditioned medium (CM) is defined as the media where cells are cultured, thus contain all the secreted factors and extracellular vesicles (Pawitan 2014). Using CM as new therapeutic treatment offers multiple advantages over the administration of cells: (i) simpler storage, transport, and preservation requirements, (ii) avoidance of the inherent risks of cell transplantation, and (iii) potential application as a ready-to-go biologic (Fuentes et al. 2022). In recent years, CM obtained from the culture of mesenchymal stromal/stem cells (MSCs) has been shown to effectively modulate the immune response *in vitro* and in animal models of lung, renal, cardiac, or hepatic injury as well as in models of burn injury of different organs. Conditions that are typically accompanied by a strong inflammatory response. Dynamic culture conditions, such as fluid flow, shear stress and three-dimensional aggregates cultivation (3D-MSCs), has been demonstrated substantially impact cellular behavior and secretome profile. **For this reason, in this work we evaluate the immunomodulatory capacity *in vitro* of CM derived from 3D-MSCs cultures.** Murine BM-MSCs were cultured as spheroids at two densities: 2.5x10^6 and 5x10^6 cells/mL (D1, D2) in shake flasks with serum-free medium and incubated at 70 rpm, 37°C, 21% O_2 and 5% CO_2 for 96 h. Samples were taken every 24 h to evaluate: morphological parameters of spheroids, re-adherence capacity of MSCs, key metabolites (glucose, lactate, ammonia), viability (secreted LDH), and immunosuppressive potential of the CM by a peripheral blood mononuclear cells proliferation assay. Our results showed that dynamic culture of 3D-MSCs is feasible using basal medium without any type of animal/human supplementation, which represents a clear advantage for commercial production of CM. In both cultures, glucose was depleted at 96 h, while lactate increased and at 48 h began to decrease. In addition, we observed that the CM obtained from spheroids induce a significant decrease in lymphocyte proliferation, displaying an immunosuppression percentage of 79 and 76% (D1 and D2, respectively).

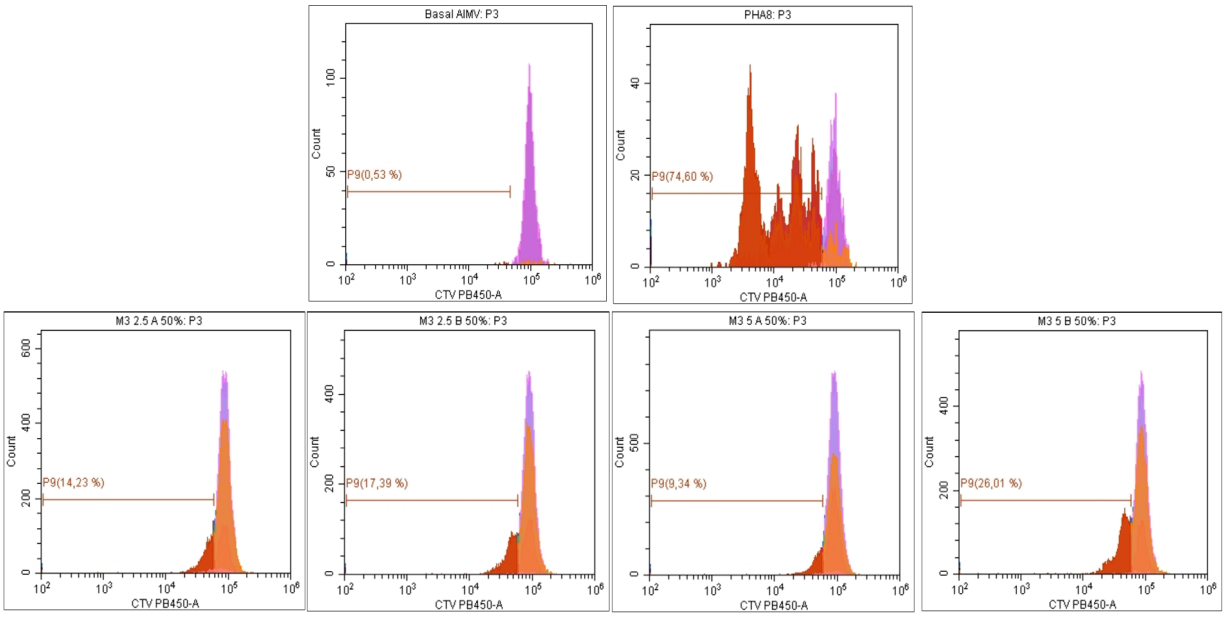

Fig 1. Lymphocyte proliferation: Basal, PHA8 (8 [ug/ml] PHA), M3: Lymphocytes co-cultured with CM obtained from spheroids culture with 2.5x10^6 and 5x10^6 cells/mL.

Poster Number 216