

were evaluated by flow cytometry. Frequency was evaluated on Treg expressing CD3, CD4, CD25, CD45RA, and FOXP3. Serum samples were assessed for TNF-, IFN-, IFN-, CCL2, CCL3, IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, IL-21, IL-31, IL-33 by multiplex assay. **Results:** Most participants were female (78%) with a median age (range) of 32 (16–59) years. Skin fibrosis assessed by mRSS improved from median (range) 27 (11–41) at baseline to 18 (9–32) at 12-months and 16 (6–31) at 24-months after AHSCT. Forced vital capacity and DLCO percentages remained stable after AHSCT. The frequency of naive Tregs (CD45RA+FoxP3^{high}CD4+) decreased at 3 and 24-months post-transplantation compared to baseline ($p < 0.05$). However, there was a significant increase in their frequency at 36- and 42-months compared to 3-months after AHSCT ($p < 0.05$). When comparing the levels serum cytokines, there was a decrease in IFN- levels at 12-months ($p < 0.05$) and CCL-2 and IL-8 decreased at 24-months ($p < 0.05$). Serum levels of TNF- α , IL-13, IL-31, and IL-33 remained unchanged after transplantation in SSc patients. Of all serum cytokines analyzed, only the anti-inflammatory molecule IL-10 showed an increase at 12 ($p < 0.05$) and 24 ($p < 0.01$) months compared to baseline; there was also an increase from 12- to 24-months ($p < 0.01$). **Conclusion:** The results showed an increase in naive regulatory T cells, indicating the generation of new regulatory cells. Notably, serum levels of key proinflammatory cytokines such as IFN- γ , CCL-2, and IL-8 showed significant reductions post-transplantation, while serum levels of IL-10, a crucial immunoregulatory cytokine, significantly increased. This suggests that AHSCT can modulate the inflammatory milieu and improve immunoregulatory mechanisms, contributing to the clinical improvements observed in these patients.

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GENERATION OF DENDRITIC CELLS EXPRESSING CAR FROM INDUCED PLURIPOTENT CELLS: AN ALTERNATIVE ADVANCED CELL THERAPY FOR CANCER?

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Objective: Immunotherapy based on T cells expressing Chimeric Antigen Receptor (CAR-T) has been shown to be a very promising strategy to treat hematologic tumors. However, the same results are not reported for solid tumors. Therefore, other immune cells have been explored to receive CARs. Dendritic Cells (DC) are a heterogeneous population specialized in

the antigen presentation, with great migratory capacity, being good candidates to receive CAR. Due to their scarcity in peripheral blood and low proliferative potential, an “off-the-shelf” source such as induced pluripotent cells (iPSCs) is needed to enable their use in cell therapy. Thus, our aim is to develop iPSC-DCs expressing CAR as an alternative or supporting therapy for solid tumors. **Methodology:** To insert the CAR plasmid into the iPSC we use the non-viral piggyBac transposon system by electroporation. To differentiation of iPSC into dendritic cells we are using the differentiation media X-vivo 15 media supplemented with glutamine, sodium pyruvate, non-essential aminoacids and 2-mercaptoethanol and different combination of cytokines among the days of differentiation including BMP4, SCF, VEGF, GM-CSF and IL-4. We are characterizing the generated cells by flow cytometry and our ongoing assays include to perform functional tests of migration ability, cytokine production and iPSC-DC potential to stimulate T cells in vitro. Next, we will perform iPSC-CAR-DCs injections in NSG tumor bearing mice to evaluate its in vivo antitumoral activity. **Results:** We transfect iPSC with GFP or CAR and obtained a population with 95% of purity expression with stable CAR and GFP expression. **Discussion:** Although 5 days after electroporation we obtained only 2% of CAR efficiency and 6% of GFP expression, two subsequent cell sorting isolation of CAR and GFP positive iPSCs population resulted in 95% of purity expression with stable CAR and GFP expression after more than 35-days. Distinct iPSC-CAR clones have been selected for expansion aiming to validate CAR stability during distinct differentiations' protocols. Culture of iPSCs followed classical established step-by-step phases: embryoid bodies obtention at day 1–2; hematopoietic Stem-like cells at day 10–16; and the first immune myeloid-like cells at day 13–18. Subsequently, using a combination of GM-CSF plus IL-4 in the differentiation media after around of 30-days of differentiation, we obtained differentiated cells with a typical myeloid immune cell morphology, including few macrophage-like cells, with big cytoplasmatic vacuoles, and numerous cells with dendrite projections close to DC morphology. Also, by multicolor flow cytometry we noted cells expressing HLA-DR+CD1c-CD141+XCR1+ phenotype suggestive of cDC1-like cells. **Conclusion:** We could differentiate the iPSC into cDC1-like cells and their functionality it's been tested. We produced iPSC expressing CAR and GFP by electroporation, in the next step we are going to differentiate these cells into DCs to produce cDC1-like cells expressing CAR.

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A IMPORTANCIA DA AVALIAÇÃO NUTRICIONAL PRÉ-HOSPITALAR DO PACIENTE CANDIDATO AO TRANSPLANTE DE CÉLULAS TRONCO-HEMATOPOIÉTICAS

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Introdução: O Transplante de Células Tronco-Hematopoiéticas (TCTH) é uma modalidade terapêutica utilizada para