

uso de G-CSF com quimioterapia. A eficácia global do procedimento de coleta de CPHSP por aférese foi de 64,9%. Foi observado que 92 pacientes (22,2%) não atingiram o limiar de 2×10^6 /kg células na 1ª coleta. **Discussão:** A eficácia da coleta de CPHSP por aférese depende de fatores físicos e biológicos. Os fatores físicos estão associados a particularidades do sistema de coleta como raio e rotação, gradiente de densidade dos elementos sanguíneos e, o volume de sangue processado. Já os fatores biológicos são disponibilidade de células CD34+ pré-coleta no sangue periférico e, o movimento das mesmas entre os espaços intra e extravascular. Reconhecidamente, o fator mais importante para sucesso da coleta de CPHSP é o CD34+ pré-coleta. De forma similar a diversos estudos encontramos que o CD34+ pré-aférese se relaciona de forma positiva com o CD34+coletado. **Conclusão:** A necessidade de estratégias de mobilização mais eficazes em pacientes difíceis de mobilizar, reduzir recursos e custos de regimes de mobilização e reduzir o medo do paciente pela dor e a inconveniência de regimes de vários dias, está no cerne dos estudos atuais para entender e direcionar os mecanismos de mobilização e, consequentemente, realizar a melhor coleta de CPHSP possível com toxicidade tolerável e, em um único dia, deixando os dias subsequentes para eventuais complementações.

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EMPOWERING CAR-T CELL THERAPY: GITRL COSTIMULATION FOR ENHANCED ANTITUMOR RESPONSE AND IMMUNOSUPPRESSION RESISTANCE IN SOLID TUMORS

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The treatment of solid tumors with CAR-T cells is challenging due to the complex tumor microenvironment that impairs infiltration and persistence of injected cells. Thus, enhancing persistence and resistance to exhaustion of CAR-T cells is desirable to maximize therapeutic efficacy. GITR, a T cell costimulatory molecule, plays a crucial role in modulating T cell activation and function. When engaged by its ligand GITRL, GITR signaling promotes T cell proliferation, cytokine production, and survival, leading to enhanced effector functions. Additionally, GITR activation can counteract the suppressive effects of Tregs, promoting a more robust and effective immune response. Hence, our hypothesis is that coexpressing a CAR against the tumor antigen GD2 with GITRL will enhance antineoplastic effects and may reduce local immunosuppression and T cell exhaustion. To test our hypothesis, we generated two lentiviral vectors to express CAR anti-GD2 and another one coexpressing GITRL. After that, we produced lentiviral particles to transduce human primary T cells and flow

cytometry was used to verify the transduction efficiency. After transduction, CAR-T cell proliferation was monitored for 8 days. To assess the functional activity of the CAR-T cells, we performed a coculture assay with T98G Glioblastoma cells (GD2+) and the HCT-166 colon cancer cells (GD2-) using two Effector-to-Target cell (E:T) ratios. Both tumor cell lines were previously engineered to express luciferase to enable cell killing analysis through bioluminescence quantification. After coculture, T cells were recovered, stained with antibodies against four activation/exhaustion markers (PD-1, CTLA-4, TIM-3, LAG-3) and analyzed by flow cytometry. The T cell transduction resulted in 33% of CAR+ cells transduced with CAR.GD2 lentiviral particles and 42% with CAR.GD2/GITRL. Moreover, 8 days after transduction, the T cell proliferation ratio increased in 18 fold and 21 fold to CAR-T.GD2 and CAR-T.GD2/GITRL, respectively. In coculture assays, we observed that both CAR-T cells demonstrated a potent cytotoxic activity, with around 90% of T98G cell lysis, while sparing HCT-116 cancer cells, demonstrating GD2-restricted cytotoxic activity. The analysis of exhaustion markers expression profiles revealed an increase in all four markers observed in CAR-T.GD2 cells. Indeed, when comparing the double-positive cell populations for the most expressed markers (PD-1 and LAG-3), we identified a higher concentration of CAR-T.GD2 cells expressing both markers simultaneously (38%) than CAR-T.GD2/GITRL (13%). These data suggest that the inclusion of the GITRL domain in the CAR-T cell construct may enhance T cell resistance to exhaustion and contribute to functional activity. Therefore, the potential results of this project will foster the development of a new advanced cellular immunotherapy strategy for the treatment of GD2+ neoplasms. These findings could also be applied to enhance the cytotoxic activity and persistence for other CAR constructs.

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COMPARAÇÃO ENTRE O TRANSPLANTE ALOGÊNICO DE CÉLULAS TRONCO HEMATOPOIÉTICAS DE MEDULA ÓSSEA APARENTADO E NÃO APARENTADO - UM ESTUDO ECOLÓGICO

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Introdução/objetivos: O transplante de células-tronco hematopoéticas (TCTH) de Medula Óssea (MO) é utilizado no tratamento de várias doenças sanguíneas, malignas e não malignas, hereditárias ou adquiridas. No TCTH alogênico, as células-tronco vêm de um doador, idealmente um familiar