Palavras-chave: Células-tronco; Metodologia; Cultivo; Imunofenotipagem; Citometria de fluxo; Diferenciação.

## https://doi.org/10.1016/j.htct.2020.10.702

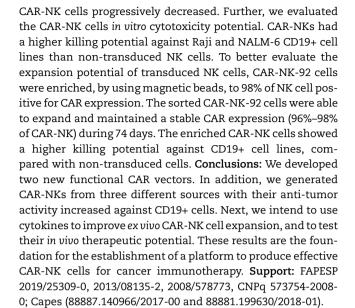
701

## ENGINEERED CD19-CAR NK CELLS AS AN OFF-THE-SHELF ALTERNATIVE TO B CELL LEUKEMIA AND LYMPHOMA TREATMENT

R.N. Silvestre<sup>a</sup>, J. Eitler<sup>b</sup>, D.M.C. Fantacini<sup>a</sup>, K.C.R. Malmegrim<sup>c</sup>, K. Swiech<sup>c</sup>, D.T. Covas<sup>a</sup>, T. Tonn<sup>b</sup>, V. Picanço-Castro<sup>a</sup>

- <sup>a</sup> Centro de Terapia Celular (CTC), Hemocentro de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil
- <sup>b</sup> Experimental Transfusion Medicine, Medical Faculty 'Carl Gustav Carus', Technical University Dresden, Dresden, Germany
- <sup>c</sup> Departamento de Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP), Universidade de São Paulo (USP), Ribeirão Preto, SP, Brazil

Aims: Chimeric antigen receptor-modified T (CAR-T) cells have been successfully used worldwide for the treatment of hematological tumors. In 2019, our group successfully treated the first patient in Brazil. However, their wide application is limited by inherent risks such as graft-versus-host disease and the amount of time it takes to produce CAR-T cells. Allogeneic CAR-Natural Killer (NK) cells can be used as universal products and may be easily available off-the-shelf for clinical application. Considering the importance of CAR-NK for clinical use, the aim of this study is to develop novel therapy to harness the potential of NK cells against leukemia and lymphoma, and to further enhance their effector function by both redefining their specificity and enhancing their potency. For that, we developed a procedure for the transduction and ex vivo expansion of NK cells from three different sources: NK-92 lineage, peripheral blood (NK-PB) and cord blood (NK-CB). In addition, we evaluated if the cytotoxicity of NK cells can be augmented by the expression of a 4<sup>th</sup>-generation CD19-CAR developed in our laboratory. Methods: NK-cell resistance to transduction is a major technical hurdle for developing NK-cell immunotherapy. So, we tested two different backbones to improve the transduction rates and developed lentiviral vectors expressing anti-CD19 CAR and IL-15 or IL-27. The transducing efficiency was measured by flow cytometry using anti-F(ab')2 antibody. To assess NK cytotoxicity, we compared in vitro potential of CAR-NKs to kill Raji and NALM-6 CD19+ cancer cell lines at multiple E:T ratios by using two methods: Europium Solution assay and the Incucyte Live-Cell analysis assay. Results and discussion: NK cells were successfully and stably transduced with two lentiviral backbones. However, the backbone with the promoter SFFV presented better results, and it was used to built our CAR-constructions. The transduction efficiency was assessed 48h after transduction, and it was 28% for CAR.19-IL-15 and 39% for CAR.19-IL-27 in NK-92 cells and for NK-PB and NK-CB cells, the transduction efficiency was around 20% for CAR.19-IL-15. After 21 days in culture, the percentage of



## https://doi.org/10.1016/j.htct.2020.10.703

702

## ESTABELECIMENTO DE UM BIOPROCESSO PARA PROLIFERAÇÃO EM LARGA ESCALA DE CÉLULAS ESTROMAIS MESENQUIMAIS DERIVADAS DE TECIDO ADIPOSO HUMANO



V.A. Simão <sup>a</sup>, J.A.R. Fracasso <sup>b</sup>, M.J. Malagutti-Ferreira <sup>c</sup>, A. Tonso <sup>d</sup>, J.T. Ribeiro-Paes <sup>a</sup>

- <sup>a</sup> Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto, SP, Brasil
  <sup>b</sup> Instituto de Ciências da Saúde, Universidade Paulista (UNIP), Assis, SP, Brasil
  <sup>c</sup> Departamento de Biotecnologia, Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), Assis, SP, Brasil
- <sup>d</sup> Departamento de Engenharia Química, Escola Politécnica, Universidade de São Paulo (USP), São Paulo, SP, Brasil

O emprego de células estromais mesenquimais (MSC) tem sido considerado uma alternativa terapêutica promissora em estudos pré-clínicos e clínicos. Neste contexto, o tecido adiposo destaca-se como uma importante fonte para isolamento e cultivo de MSC. Para emprego de MSC em triagens clínicas é necessário um grande número de células da ordem de  $1,0\times10^8$  células/paciente. Para que se atinja tal concentração é necessária a proliferação celular in vitro, no entanto, o processo de proliferação implica na manutenção das células em um microambiente artificial que pode induzir efeitos genotóxicos e afetar a viabilidade celular, interferindo diretamente na segurança e eficácia da terapia celular. Em função destes aspectos, objetivou-se com este estudo o estabelecimento de um bioprocesso para proliferação de células estromais mesenquimais derivadas de tecido adiposo humano (hADSC) em biorreator tipo tanque agitado, visando estabelecer um sis-