

Chagas). Para a execução plena da proposta, foi realizado credenciamento de Laboratório para Coleta das amostras. O protocolo de produção do colírio foi validado pelo Laboratório de Biologia Celular de Botucatu (Laboratório de Biotecnologia Aplicada - LBA) desde 2007. Todos os lotes são liberados após aprovação no Controle de Qualidade, envolvendo análise de conteúdo com teste microbiológico e de rotulagem com todas informações necessárias. Neste período de implantação da terapia para novos pacientes, foram registradas apenas duas observações/queixa, uma não foi justificada e o tratamento foi mantido e outra, na qual houve suspensão do tratamento. Quando aplicado questionário de satisfação para médicos, notou-se total satisfação em recomendar a terapia e significativa melhora do quadro clínico dos pacientes. Já a pesquisa de satisfação dos pacientes aponta que há uma boa adesão ao tratamento, efetivo e imediato alívio dos sintomas causados pela Síndrome do Olho Seco. As perspectivas futuras do tratamento com colírio de soro autólogo envolvem melhoria do produto e uma expansão de mercado para diversas regiões do país.

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ALL-IN-ONE VIRUS-FREE MANUFACTURING PROCESS OF ALLOGENEIC CHIMERIC ANTIGEN RECEPTOR (CAR) T CELLS USING CRISPR/CAS9

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Chimeric antigen receptor (CAR) T cell therapy has emerged as a promising therapeutic opportunity against blood cancers, leading to the six FDA-approved CAR T cell products. All commercially available products are produced following an autologous strategy, which represents a bottleneck for the widespread adoption of this technology due to the poor quality and quantity of patients' T cells. Allogeneic T cells from healthy donors are an excellent alternative over the autologous ones, allowing the production of a ready-to-use therapy besides additional benefits, such as standardization of the final product, cost reduction through manufacturing scale-up, and the possibility of multiple genetic modifications. Safety concerns related to the use of cells from third-party donors can be easily eliminated through T cell receptor (TCR) knock-out with the demonstrated prevention of graft-versus-host disease development. Here we describe CRISPR/Cas9 editing for TRAC (T Cell Receptor Alpha Constant)-targeted genomic integration of the CAR transgene and the consequent TCR knock-out in a fast and efficient virus-free manufacturing process. For the CRISPR/Cas9-mediated knock-in, we designed a plasmid containing the second-generation anti-CD19 CAR flanked by arms homologous to the desired cut site. This sequence was PCR amplified and purified, resulting in a highly concentrated double-stranded DNA (dsDNA)

template for the homology-directed repair (HDR). Human T cells isolated from healthy donors were electroporated with the dsDNA-HDR templates (dsDNA-HDRT) and Cas9 ribonucleoproteins (RNPs) targeting the human TRAC locus. Cells were assayed on days 5 and 11 post-electroporation for CAR expression and killing efficiency, respectively. A mCherry transgene flanked by the same homology arms was also included as a control. Preliminary studies using the mCherry dsDNA-HDRT indicated a 1.2-fold improvement in the editing efficiency by adding the positively charged polymer poly-L-glutamic acid (40 µg) that binds to RNPs and reduces aggregates. Additionally, by culturing the electroporated cells with varying concentrations of an NHEJ inhibitor, we observed a 1.4 to 2-fold increase in mCherry knock-in, suggesting a favorable condition for the HDR. Based on the established optimized conditions, we electroporated T cells with the CAR dsDNA-HDRT (3.1 kb length, 6 µg) and got 88% of CAR+ cells five days after electroporation. The viability was 27% on day 3 and reached about 60% on day 7. On-target CAR gene integration was confirmed through an "in-out" PCR assay on the genomic DNA using primers specific to the TRAC locus and CAR transmembrane domain. We then analyzed the in vitro cytotoxicity of CAR T cells by coculturing them with GFP-expressing tumor cell lines. Complete growth inhibition of the RS4-11 cells (CD19+) was achieved at the 5:1, 10:1, and 50:1 effector-to-target ratios. For the K562 cells (CD19-), conversely, the killing activity was related to the T cells themselves since CAR expression did not further decrease the tumor cell number. These results demonstrate robust cytotoxic function and target specificity of the CAR T cell products. Combined, the data provide a proof-of-concept for the virus-free manufacturing process of allogeneic anti-CD19 CAR T cells, enabling CAR knock-in and TCR knock-out in an all-in-one strategy.

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FATORES ASSOCIADOS À MOBILIZAÇÃO POBRE DE CÉLULAS TRONCO HEMATOPOIÉTICAS PARA TRANSPLANTE AUTÓLOGO EM PACIENTES COM LINFOMAS E MIELOMA MÚLTIPLO

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Introdução: A contagem de células CD34+ no sangue periférico (SP) pré-leucafêrese é um parâmetro para prever o sucesso na mobilização e coleta de células tronco hematopoéticas (CTH). Para uma coleta bem-sucedida, mínima de 2×10^6 CD34+/kg, geralmente é necessária uma contagem de CD34+ no SP do quarto dia (D4) $\geq 10/\mu\text{L}$. **Objetivo:** Analisar