quantified by flow cytometry - Cytometric Bead Array (CBA) using the BD™Mouse TH1/TH2/TH17 Cytokine Kit according to manufacturer's instructions. The cytokines measured were IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ and IL-17. The kinetics of these cytokines were monitored on days 3, 6, 9, 12 and 15 of culture. FCAP array software (v3.0.1) was used to calculate the concentrations of each cytokine and the mean fluorescence intensity. **Results and discussion:** An increase in IL-2 and IL-4 was observed between days 9 and 12 of BM-derived SCs culture in groups stimulated with G-CSF. In the culture of PB-derived SCs, the increase in IL-2 and IL-4 occurred between days 3 and 9 in cultures stimulated with G-CSF. The production of IL-6 occurred between days 3 and 9 of culture, both in BM- and PB-derived SCs cultures and reduced until day 15 of culture. In non-G-CSF-stimulated BM-derived SCs cultures, IL-6 levels are elevated in relation to cultures that received G-CSF as a stimulus, on days 6 and 9. IL-10 increased in BM- and PB-derived SCs cultures stimulated with G-CSF on day 3, reducing its concentration until day 15. The same event was observed in BM-derived SCs cultures for IFN-γ and TNF-α, which, like IL-10, were elevated in BM-derived SCs cultures. In PB, an increase in TNF-α is observed on day 3, decreasing progressively until day 15. IL-17 increased on day 12 of BM-derived SCs culture, remaining stable on different days in both BM- and PB-derived SCs cultures. **Conclusion:** G-CSF can modulate or induce the production of different cytokines by SCs derived from BM and PB, contributing to better regulation of immune responses in pathological and therapeutical different contexts. **Funding:** FAPEAM, CAPES and CNPq.

https://doi.org/10.1016/j.htct.2022.09.507

ESTRUTURAÇÃO DO CENTRO DE PROCESSAMENTO CELULAR DO HOSPITAL AC CAMARGO CANCER CENTER PARA IMPLANTAÇÃO DA TERAPIA CAR-T.

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Introdução: A inovadora terapia CAR-T é promissora no tratamento oncológico. No Brasil, os estudos clínicos e CAR-T comercial inicialmente são direcionados para o tratamento de Mieloma Múltiplo e Linfoma difuso de grandes células B. A terapia consiste na coleta autóloga de Linfócitos, modificação genética destas células com o auxílio de um vetor viral que tem a função de alterar o linfócito, produzindo um receptor de superfície capaz de identificar o antígeno específico da doença. **Objetivo:** Compartilhar a experiência do Serviço de Hemoterapia e Terapia Celular na implementação do Projeto CAR-T Cells no AC Camargo Cancer Center. **Metodologia:** O projeto de implementação da terapia CAR-T teve início em 2020 através de auditorias internas e externas, incluindo inspeções realizadas pelas indústrias farmacêuticas, com a finalidade de qualificar o centro para realização da terapia CAR-T. Criamos diferentes fóruns para discussões operacionais e burocráticas. Fez-se necessário a interface entre diferentes áreas da instituição para atender as especificações das indústrias farmacêuticas. Houve mobilização do Serviço de Hemoterapia e Terapia Celular, Inovação, Pesquisa Clínica, Quimioterapia, Qualidade, Práticas Assistenciais, Processos, Manutenção, Educação Continuada, Infraestrutura, Comercial, Suprimentos, Tecnologia da Informação, Laboratório Clínico e Equipe Multidisciplinar. Essa interação entre diferentes áreas deu-se diante da necessidade de criação de fluxo seguro desde o início da identificação do paciente candidato à terapia, até o manejo pós-infusão. A estruturação do Serviço de Hemoterapia e Terapia celular do AC Camargo Cancer Center envolve desde a fase de identificação do paciente canditato à terapia até o pós-infusão. Entre essas duas fases, está a criação da jornada do paciente com definição de novos protocolos para CAR-T cells, acompanhamento junto à pesquisa desde a fase de projeto até a publicação, parametrização de sistemas informatizados e prontuário eletrônico, reestruturação do plano de ensino para treinamento e reciclagem da equipe, adequação de estrutura física do Centro de Processamento Celular com aquisição de novos equipamentos para recebimento e armazenamento do produto final, além da adequação de documentos que já fazem parte do processo de Terapia Celular convencional. Baseado nas auditorias internas e externas, criamos e iniciamos a execução dos planos de ação definidos nos fóruns, onde cada área de interface possui um representante para acompanhamento do projeto e comunicação da equipe das próximas ações. Fomos em busca de parcerias para congelamento programado de Linfócitos até que tenhamos a entrega da área nova com estrutura física autossuficiente. Hoje nosso centro de processamento celular conta com a estrutura para congelamento mecânico de células para Terapia Celular convencional, uma das fases da produção do CAR-T é a realização do congelamento programado e armazenamento em nitrogênio fase vapor. **Conclusão:** Atualmente estamos em novo ciclo de treinamento teórico com as indústrias farmacêuticas para compreender as diretrizes dos fornecedores e alinhar o processo após reciclagem da documentação, a próxima fase é de um novo ciclo de simulações de todas as fases do processo e acompanhamento do cronograma de obras que possui entrega prevista para novembro de 2022. A expectativa é iniciar as coletas de Linfócitos para produção de CAR-T Cells ainda no segundo semestre de 2022. Iniciar a terapia CAR-T vai além da entrega de um projeto inovador, é oferecer para os pacientes o que há de mais moderno para o tratamento do câncer.

https://doi.org/10.1016/j.htct.2022.09.508

GENERATION OF T LYMPHOCYTES EXPRESSING ENGINEERED T CELL RECEPTOR TARGETING THE NY-ESO-1 TUMOR ANTIGEN USING A NEW GENETIC CONSTRUCT

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Immunotherapy based on the adoptive transfer of genetically modified T lymphocytes for the expression of engineered receptors has provided impressive results for hematological neoplasms. This is the case of T cells modified to express chimeric antigen receptors (CAR). However, for solid neoplasms, which represent most of the tumors, several limitations still need to be overcome for the development of more effective and safe therapies. In the case of CAR-T cells, one of the limitations in their application for solid tumors is their restricted ability to recognize only surface antigens in their native conformation. Nonetheless, engineered T lymphocytes expressing artificial T cell receptor (TCR) with enhanced affinity for a specific tumor antigen, referred as TCR-T cells, have provided encouraging results against solid tumors. The artificial TCR, as well as the endogenous TCR, is able to recognize intracellular proteins presented by the major histocompatibility complex (MHC), enabling a diversity of options for therapeutic targets, since most tumor-associated antigens are intracellular. The NY-ESO-1 is a potent target in this therapeutic approach and the most used in TCR-T cells studies. This intracellular protein has restricted expression in healthy tissues, found in the placenta and in immune privileged tissues, and has strong and homogeneous expression in different types of tumors. Therefore, the main goal of this study was the generation of TCR-T cells targeting the peptide:HLA complex formed by NY-ESO-1:HLA-A*02 using a new genetic construct of artificial TCR. For this, human T lymphocytes were transduced (MOI 5) with a lentiviral vector for the expression of α and β chains of the engineered TCR. The transduction efficiency as 11% as determined by labeling with NY-ESO-1:HLA-A*02 specific tetramers. Next, TCR-T cells were co-cultured with SW982 sarcoma cells (NY-ESO-1$^+$/HLA-A*02$^+$) or HCT116 colon carcinoma cells (NY-ESO-1−/HLA-A*02$^+$) to evaluate their in vitro antitumor capacity. TCR-T cells were able to eliminate approximately 30% of the SW982 cells after 48h of co-culture in a 1:1 ratio (effector:target cells). Additionally, these cells had no cytotoxic capacity against HCT116 cells. Together, these data demonstrated the in vitro antitumor potential and the specificity of TCR-T cells anti-NY-ESO-1:HLA-A*02 to the target antigen, validating these new genetic construct. Thus, these results are encouraging and promising in view of the development of a new advanced cell therapy for solid neoplasms.

https://doi.org/10.1016/j.htct.2022.09.509

VALIDATION OF LONGER FRESH STORAGE TIME FOR PERIPHERAL BLOOD STEM CELLS INTENDED FOR CLINICAL USE

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Aims: Peripheral blood stem cells (PBSC) storage conditions may influence cell quality and/or functionality, which may directly impact recipients'clinical outcomes. Thus, the Brazilian Health Regulatory Agency (ANVISA) demands validation of fresh storage times greater than 48 (forty-eight) hours, with technical-scientific evidence about the quality and safety of the product. The aim of this study was to verify the effects of longer fresh storage times in the viability and functionality of PBSC intended for Hematopoietic Progenitor Cells (HPC) transplantation. **Methods:** Ten aliquots of PBSC units were analyzed. The samples were storage fresh at 2°C to 8°C. Measurements were performed immediately (I) and 72 h after PBSC collection storage (AS), by flow cytometry analysis and clonogenic assays. CD45+ and CD34+ cells viability, white blood cells (WBC) counts and CD34+ cell concentrations and colony forming units (CFU) were evaluated. All clonogenic assays were performed in duplicate with 1×10^4 cells/mL. The results of these assays were expressed as the arithmetical mean of the duplicates. A descriptive analysis and T-dependent test were performed (SPSS 15 software). **Results:** : According to the in-process controls, the median interval between the end of collection and temporary storage was 72 hours, with the maximum time being 91 hours and 48 minutes and the minimum storage time being 69 hours and 25 minutes. This variation in storage time is due to the end time of some collections that were longer than the laboratory's operating hours, so the samples had to be analyzed within the possibilities of the routine. Median viability of CD34+ cells immediately after collection was 100%, minimum viability was 98.8%, so all cases were in compliance with the laboratory quality indicator target. The median viability of CD34+ cells after fresh storage was 99.75%. There were two cases with viability below 98%, and the minimum viability was 97.1%. The average loss of viability of CD34+ cells in relation to the mean obtained in fresh samples was 0.66%. One case had a 2.99% loss of viability. Despite the significantly decreased CD45+ cells viability after storage (I: 98,3%; AS: 90,4%; p =< 0,0001), no significative changes were noted in CD34+ cell viability (I: 100%; AS: 99,75%), as well as in WBC (I: 248350/μl; AS: 242705/μl) and CD34+ cells (I: $5{,}24 \times 10^6$/kg; AS: $4{,}67 \times 10^6$/kg) concentrations. Moreover, no significant decrease in colony-forming unit numbers was observed in units stored for 72 hours (I: 34,5; AS: 21,5). **Discussion:** Despite the significant decrease in CD45+ cell viability after 72 hours storage, no significant changes were observed in CD34+ cell viability, as well as in WBC and CD34+ cell concentrations in the same period. These results are reinforced by the functional assays, in which no significant decrease in colony-forming unit numbers was observed and it also demonstrated that the cells maintain their proliferation and differentiation potential. **Conclusion:** These results lead to the assumption that the fresh storage time of 72 hours had no significative impact in variables of concern (CD34+ cells and clonogenic assays) and thus does not impact the quality and safety of the product. Nonetheless, despite the strong recommendation of infusing fresh graft as soon as possible, our data suggest that, when necessary, it is safe to use PBSC until 72h after collection.

https://doi.org/10.1016/j.htct.2022.09.510