CD19 CAR-NK CONSTRUCTION WITH A NEW HINGE AND GITRL CO-EXPRESSION FOR TREG ACTIVITY INHIBITION

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Introduction: Chimeric Antigen Receptor T (CAR-T) cell therapy has emerged as a promising approach in which autologous T cells are engineered to recognize a specific target. Although the clinical responses are encouraging, nearly 60% of large B cell lymphoma patients have disease progression after anti-CD19 CAR-T cell treatment, and neurotoxicity remains a challenge. NK cells are an attractive resource, given their innate capacity to eliminate tumor cells, and the prospect to use allogeneic sources, unlike T cells. This allows the development of well-characterized "off-the-shelf" CAR-NK cells, which also present a reduced risk of cytokine release syndrome and neurotoxicity. Furthermore, several studies indicate that Tregs play a central role in the tumor microenvironment of CD19+ cancers. Thus, targeting these immunosuppressive cells is a strategy to improve CAR-based therapies. In this study, we designed a novel anti-CD19 CAR (CAR19 Ig) featuring an Immunoglobulin (Ig)-like hinge, providing flexibility to access the target. We also constructed a fourth-generation CAR co-expressing GITRL (CAR19 Ig-GITRL) – a modulator that blocks Tregs inhibitory activity. We hypothesize that this innovative approach could boost the antitumor response encompassing the use of "off-the-shelf" CAR-NK cells. **Objectives:** This study aims to evaluate the cytotoxic potential of CAR19 Ig in eliminating CD19+ cells, while also modifying this CAR into a CAR co-expressing GITRL, and assess its potential for Treg blockade. **Methods:** Lentiviral vectors with CAR19 Ig or CAR19 Ig-GITRL were constructed. NK-92 cells were transduced with lentivirus and the CAR-positive population was enriched by sorting. Then, the *in vitro* potential of CAR-NK92 cells against the RAJI (CD19+) cell line was evaluated by coculture at an Effector-to-Target (E:T) ratio of 2:1 for 5h. Cytotoxicity after coculture was evaluated by flow cytometry. Sorted cells were cryopreserved in a solution of 90% FBS and 10% DMSO. **Results:** NK-92 cell transduction resulted in 18% CAR expression in CAR19 IgGITRL-NK-92 and 13% of CAR in CAR19 Ig-NK-92 cells. At first, the sorting was performed with CAR19 Ig-NK-92, leading to over 90% CAR expression. We evaluated the CAR19 Ig-NK-92 cells *in vitro* cytotoxic potential and coculture of the cells demonstrated 22% cytotoxicity, while untransduced NK-92 cells did not eliminate target tumor cells in the same conditions. Additionally, we conducted an assessment of CAR-NK expression, potency, and proliferative capacity after freezing, which remained unaffected by the cryopreservation procedure. To carry on evaluating CAR19 Ig-GITRL-NK-92 cells, they were also sorted resulting in about 90% CAR expression. **Conclusion/discussion:** Usually, lentiviral transduction of NK cells is challenging, which leads to low transduction efficiency. Hence, our findings show a remarkable achievement, attaining a CAR-positive cell population above 90% via sorting. Besides, our study establishes the feasibility of freezing these cells while maintaining stable CAR expression post-thawing, a valuable advantage for "off-to-shelf" therapy. Moreover, our new CAR, featuring an IgG hinge, exhibited increased cytotoxicity against target cells, underscoring the effectiveness of the CAR construct to recognize CD19 and activate NK cells. Subsequently, the next phases of this project will evaluate the cytotoxic potential of CAR19 Ig-GITRL-NK-92, along with exploring its impact on Tregs.

https://doi.org/10.1016/j.htct.2023.09.1007

COMPARISON OF OUTCOMES AFTER MATCHED RELATED, UNRELATED AND HAPLOIDENTICAL DONOR BONE MARROW TRANSPLANTATION FOR PATIENTS WITH ACQUIRED APLASTIC ANEMIA: A MULTICENTER RETROSPECTIVE ANALYSIS OF REAL-WORLD EXPERIENCE IN BRAZIL

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Introduction/objectives: Severe Aplastic Anemia (SAA) is the most frequent indication for Hematopoietic Cell Transplantation (HCT) for nonmalignant diseases in Brazil. Survival after HCT from HLA-Matched Related (MRD), HLA-Matched Unrelated (MUD), and Haploidentical Donors (HID) has improved over the past decades. We aimed to compare the outcomes of a large cohort of patients (pts) submitted to HCT for acquired SAA in Brazil. **Methods:** A retrospective, non-randomized study included 328 pts with acquired SAA who underwent HCT in 5 Brazilian HCT centers between 01/2012 and 12/2022. All pts received bone marrow as the graft source from MRD (n = 154), MUD (n = 96), or HID (n = 78). MUD was defined as HLA 10/10 or 8/8. **Results:** Pts and donors' characteristics are summarized in Table 1. Median follow-up after HCT was 4.7 years. Median age was 18.4 years (1–69 years), most were CMV positive and heavily transfused. 74% had received previous immunosuppression with (n = 09) or without Rabbit-ATG (n = 134). Median disease duration before transplant was 7 months (0.6–184), however pts with a MRD were transplanted earlier. There were some statistical differences among groups: HID were performed more recently and ATG was more frequently used in MUD. Conditioning regimen and GVHD prophylaxis were also different among donor groups. One-year cumulative incidence of Graft Failure (GF) in MRD, MUD, and HID cohort was 4.8%, 10.8% and 18.1% (p = 0.0028), respectively. 27 pts were rescued with a 2nd transplant (MRD, n = 7;MUD, n = 8;HID, n = 12) at a median of 40 days after 1st

HCT, and 14 are alive. In a Multivariate Analysis (MVA), MUD-HCT (HR = 2.35, p = 0.078) and HID-HCT (HR = 4.30, p = 0.0012) were associated with a higher incidence of GF (Table 2). MRD-HCT had a lower incidence of grade II–IV acute graft versus host disease (aGvHD) compared with MUD and HID (12.4% vs. 30.3% vs. 16.9%, p = 0.00175) and lower incidence of chronic GvHD (cGvHD) (8,7% vs. 26,9% vs. 27.2%, p ≤ 0.001).The estimated non adjusted 5-year Overall Survival (OS) for the MRD, MUD, and HID groups were 86%, 72% and 79% (p = 0.02), respectively; event-free survival (event: rejection or death) rates were 85%, 68% and 56%, respectively (p < 0.001) (Figs. 1 and 2). In MVA, OS was increased for transplantations after 2018 (HR = 0.38, p = 0.002) (Fig. 3). MUD-HCT (HR = 3.13, p≤0.001) and HID-HCT (HR = 2.97, p = 0.0039) were associated with decreased OS in comparison to MRD-HCT. The 100-day CI of CMV reactivation and hemorrhagic cystitis were 56% and 8% respectively, with no significant differences between groups. 64 pts died at a median of 102 days (range 2–2328), and the main causes of death were GF, n = 20; sepsis, n = 11; GvHD, n = 9. **Discussion:** This retrospective analysis showcases some significant differences between donor groups, including the fact that MRD pts are transplanted earlier and are subjected to a lower risk of GF, acute and chronic GVHD, which translates into better survival. HID-HCT showed improved survival in recent years, becoming a more viable transplant option, specially in comparison to MUD-HCT. Improved OS in HID-HCT comes at the expense of higher rates of graft rejection and higher rates of aGvHD remain an issue after MUD-HCT. **Conclusion:** These real-world data from a large cohort of SAA pts demonstrate that HCT remains an effective curative treatment with significant improvement in survival in the last 5 years across different donor groups.

https://doi.org/10.1016/j.htct.2023.09.1008

PERFIL DA MOBILIZAÇÃO E COLETA DE CÉLULAS CD34+ EM PACIENTES PARA TRANSPLANTE AUTÓLOGO EM UM INSTITUTO FEDERAL

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Introdução e objetivos: A coleta das células CD34+ para transplante autólogo é possível através da administração de agentes mobilizantes como o Fator Estimulador de Colônias de Granulócitos (G-CSF) associado ou não ao Plerixafor, a depender da quantidade de células CD34+ em Sangue Periférico (SP). Em casos selecionados, há ainda a possibilidade da utilização de quimioterápicos. Este trabalho tem por objetivo analisar o perfil da mobilização e coleta de Células Progenitoras Hematopoiéticas do Sangue Periférico (CPHSP) para Transplante de Medula Óssea (TMO) autólogo. **Materiais e métodos:** foram analisados os registros das coletas de CPHSP por aférese em máquina de fluxo contínuo dos transplantes autólogos no período de janeiro de 2021 a maio de 2023, utilizando estatística descritiva para análise dos dados. Foram consideradas adequadas as coletas que alcançaram o mínimo de 2,0×106 células CD34+/kg no produto final. Adotou-se o critério de ≥10 células/mm^3 em SP para início de coleta. **Resultados:** foram realizadas 102 coletas em 97 pacientes entre D4 e D5 de mobilização, sendo 55,6% do sexo masculino e 44,3% do sexo feminino. A maioria dos pacientes encontrava-se na faixa etária de 40–59 anos (34%), seguido de 18–39 anos (30,9%), ≥ 60 anos (25,7%) e < 18 anos (9,2%). As patologias mais prevalentes foram Mieloma Múltiplo (39,2%) e Linfoma de Hodgkin (28,4%). Das 102 coletas, 59% utilizaram G-CSF e 41% G-CSF + Plerixafor. Com relação às doenças mais frequentes, 41,3% dos Linfomas de Hodgkin e 65% dos Mielomas Múltiplos utilizaram apenas G-CSF. Dentre todas as coletas, daquelas que utilizaram apenas G-CSF, 46 (45%) alcançaram o número de células desejadas de CD34+/kg em uma única coleta, 7 (6,9%) necessitaram realizar uma segunda coleta e 2 (2%) uma terceira. No entanto, 5 (4,9%) coletas não alcançaram o alvo no produto final, sendo 1 suspensa por ser uma coleta complementar (segunda mobilização do paciente) e 1 pelo COVID-19. No grupo que utilizou G-CSF + Plerixafor, 26 (25,5%) atingiram o alvo na primeira coleta e 6 (5,9%) numa segunda. Não alcançaram o alvo na primeira coleta 5 (4,9%), tampouco na segunda coleta (4,9%). Das 15 coletas que não atingiram o estabelecido para o produto final, 6 apresentaram valores próximos ao alvo, viabilizando o transplante. **Discussão:** A maioria dos pacientes eram jovens (40–59 anos) e, dentre as doenças, o mieloma múltiplo foi a entidade mais frequente. Apesar de 85,3% das coletas do estudo alcançarem o número de células desejadas no produto final coletado, 95,8% (93) dos pacientes conseguiram realizar o TMO. O grupo que utilizou apenas G-CSF teve êxito em 53,9% quando comparado àquele que associou G-CSF e Plerixafor (31,4%). Isso pode corroborar a dificuldade deste último grupo em alcançar o mínimo de células periféricas para dar início à coleta, sendo considerados pacientes "maus"respondedores. **Conclusão:** Observamos um número considerável de coletas (41%) onde se fez necessário o uso de um segundo agente mobilizante e, ainda assim, apresentando falha de mobilização em 23,8% destas. Os dados ora apresentados merecem melhor entendimento quanto à interação da patologia de base, os tratamentos prévios e os mecanismos dos agentes mobilizantes.

https://doi.org/10.1016/j.htct.2023.09.1009

DOENÇA RESIDUAL MENSURÁVEL DE LLA-B PÓS CAR-T

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Introdução: O monitoramento da Doença Residual Mensurável (DRM) tornou-se prática clínica de rotina no tratamento das Leucemias Linfoblásticas Agudas (LLA). A avaliação da DRM tem demostrado fator prognóstico, que permite a atribuição de grupos de risco nos diferentes braços de tratamento, variando desde redução significativa do tratamento a intensificação.