

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.

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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