

0.96 (95% CI 0.86–1.08). The positive predictive value was 20.59% (95% CI 8.7–37.9), and the negative predictive value was 84.33% (95% CI 79.72–88.26), with a calculated accuracy of 77.84% (95% CI 73–82.18). **Discussion:** Reliable methods to assess patient vulnerability to HSCT are crucial for an informed therapeutic decision. The HCT-CI is often used to assess the risk of morbidity and mortality after transplantation. In our study, the HCT-CI showed low sensitivity and high specificity. Its positive predictive value was only 20%, with 16% of the cohort being misclassified as at risk for the event without experiencing it. Both positive and negative likelihood ratios, which represent the test's ability to correctly influence our thinking about the presence or absence of disease, showed uncertain results and a minimal effect on basic pre-test probability estimates. **Conclusion:** The HCT-CI is not a reliable method to predict HSCT-related morbidity. The need to improve a specific risk score for myeloma is highlighted by these findings. Not assessing the HCT-CI as an ordinal variable, potentially missing optimal cut-point identification via ROC analysis, could be a study limitation.

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CHARACTERIZATION OF A NOVEL ANTI-CD19 CAR CONSTRUCT CO-EXPRESSING THE COSTIMULATORY MOLECULE GITRL

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Adoptive therapy using Chimeric Antigen Receptor T-cell (CAR-T) technology has emerged as a groundbreaking immunotherapeutic approach for treating various hematological malignancies. Among the diverse CAR-T targets, CD19, a B-cell surface marker, has demonstrated remarkable success in treating B-cell malignancies. However, despite impressive clinical outcomes, challenges such as efficacy and insufficient in vivo persistence still exist. To address these limitations, preclinical investigations are exploring the integration of additional co-stimulatory molecules into CAR constructs to enhance effector functions and persistence. GITRL (Glucocorticoid-Induced TNF Receptor Ligand) belongs to the TNF family and is a type II transmembrane protein. The interaction between Glucocorticoid-Induced Tumor Necrosis factor Receptor (GITR) and its Ligand (GITRL) reverses regulatory T cell suppression, leading to enhanced immunity against tumors. Therefore, the goal of this research was to develop and validate a novel CAR targeting CD19, incorporating GITRL as a co-stimulatory domain in the CAR construct. Initially, peripheral blood mononuclear cells (PBMCs) from a healthy donor were activated with CD3/CD28 beads and transduced with the lentiviral particles using an MOI (multiplicity of infection) of 5. Subsequently, the frequency of CAR-T cells expressing the anti-CD19 CAR or anti-CD19 CAR-GITRL was

determined by flow cytometry, resulting in 52.4% and 64.9% CAR+ cells, respectively. The CAR-T cells were then assessed for their ability to recognize and selectively eliminate CD19-expressing cancer cells using in vitro cytotoxicity assays. Both anti-CD19 CAR-T cells and anti-CD19 CAR-GITRL-T cells exhibited potent killing of CD19-positive target cells, with lysis percentages of $92.65\pm 0.01\%$ and $84.31\pm 0.01\%$, respectively. In contrast, non-transduced T cells showed minimal or no activity in eliminating the target cells. Importantly, the cytotoxic activity of the CAR-T cells remained strong even upon re-challenge, leading to the elimination of $78.75\pm 0.08\%$ and $63.23\pm 0.17\%$ of CD19-positive cells, respectively. These findings suggest that CAR-T cells did not demonstrate exhaustion, contributing to the durability of the therapeutic effect. To evaluate the in vivo antineoplastic potential of the generated CAR-T cells, we established a disseminated lymphoma model by intravenously injecting Raji cells expressing luciferase into immunodeficient mice. After six days of tumor cell infusion, animals were treated with cellular suspensions containing a suboptimal dose of 2.5×10^6 anti-CD19 CAR-T or anti-CD19 CAR-GITRL-T cells in 200 μ L of PBS (n = 5). A control group received only PBS (n = 6). After 22 days of treatment, all animals in the control group had to be euthanized due to hind limb paralysis caused by lymphoma cell infiltration or due to the loss of at least 20% of initial body weight. Conversely, bioluminescent signal analyses of the groups treated with anti-CD19 CAR-T cells and anti-CD19 CAR-GITRL-T cells demonstrated a reduction in tumor burden by 83.8% and 93%, respectively. This significant reduction highlighted the in vivo antineoplastic potential of the administered cells. The therapeutic effect translated into extended survival of the treated groups, which were monitored for 36 days post-treatment (p < 0.0001, Log-rank test). However, there were no significant differences observed between the treated groups. In conclusion, this study underscores the potential of incorporating GITRL as a co-stimulatory molecule in CD19-targeted CAR-T therapy. Further investigations are warranted to elucidate the mechanisms underlying these positive outcomes and to optimize the utilization of CAR-T cells as a potent tool against neoplastic diseases.

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TRANSPLANTE DE MEDULA ÓSSEA: ANÁLISE COMPARATIVA ENTRE AS MODALIDADES ALOGÊNICA E AUTOGÊNICA

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Introdução: O transplante de medula óssea, também chamado de transplante de células tronco hematopoiéticas, é uma modalidade de tratamento potencialmente curativo para doenças malignas e não malignas hematológicas e para alguns distúrbios não hematológicos. O transplante consiste em fornecer ao paciente células tronco/progenitoras que