curativo para ambas condições em pacientes elegíveis e de alto risco.

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A SECOND ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANT (HCT2) MAY CURE MORE THAN 40% PEDIATRIC PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Introduction: Relapse of a malignant hematological disease after allogeneic HCT is associated with poor survival and may not be treated with a curative intent. However, a second HCT (HCT2) may achieve durable remission. **Objective:** To determine the outcomes of pediatric patients who received a HCT2 for relapsed malignant hematological diseases. **Casuistic and method:** Review of the medical records of pediatric patients who underwent a HCT2 for relapsed malignant hematological diseases in two institutions from 2013 to 2023. OS was estimated using Kaplan-Meier survival analysis. The conventional HCT Comorbidity index (HCT-CI) was calculated for all patients (http://www.hctci.org/Home/Calculator) categorized in $<$ or $\geq$ 2. **Results:** Nineteen patients with a median age of 10 years (range, 2–17) underwent HCT2 for B-ALL (n = 8), T-ALL (n = 2), AML (n = 5), CML-BC (n = 2), MDS (n = 1) and JMML (n = 1). Donor types were unrelated (n = 4), haploidentical (n = 11), and unrelated cord-blood (n = 4). Donors were different at HCT2 in all patients and all haploidentical HCT2 used the other haplotype. All nineteen patients received myeloablative conditioning, and 52% (n = 10) were in remission at HCT2. The median remission duration after HCT1 was 12 months (range, 2–22) and the median time between transplants was 16 months (range, 5–30). The median follow-up of surviving patients after HCT2 was 33 months (range, 9–117), with 47% alive at time of analysis. The most common cause of death was disease recurrence (n = 6, 31%). At time of analysis, OS, PFS, relapse, and Transplant-Related Mortality (TRM) were 47% , 42%, 45%, and 21%, respectively. OS was 37% (3/8) for B-ALL. All T-cell ALL, CML-BC, MDS and JMML patients are alive, but all 5 patients with AML have died. None of the latter have used post-HCT maintenance to prevent relapse. Pre-HCT2 remission status did not appear to influence OS and PFS, since of 11 patients in remission, 5 remain alive and disease-free. Of the 8 patients transplanted with active disease, 6 remain in remission. Ten patients had HCT-CI $<$ 2 pre HCT2, and 3 of 10 have died. However, 7 out of 9 patients with HCT-CI score $\geq$ 2 died (HR = 4.8, p = 0.007). **Conclusion:** A second HCT can be feasible for patients with relapsed malignant hematological diseases. Overall survival is higher than 40%, suggesting that this approach may cure a proportion of the patients, particularly those with HCT-CI $<$ 2 pre HCT2.

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SÍNDROME VEXAS (SV): POSSIBILIDADE DE TRANSPLANTE ALOGÊNICO?

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Introdução/objetivos: Descrição de diagnóstico e condução de caso SV. **Material e métodos:** Relato de caso descritivo e observacional, a partir de dados de prontuários eletrônicos, com consentimento do paciente. **Resultados:** Paciente de 73 anos, masculino. Relato de familiares de febres recorrentes, artralgias com prejuízo de deambulação, cerca de 10 "pneumonias" no último ano e três acidentes vasculares encefláicos (o último, há 1 ano da admissão). Seus hemogramas apresentavam anemia e plaquetopenia discreta. Equipe médica externa levantou a possibilidade de SV, confirmada por sequenciamento genético demonstrando variante c121 A>G no gene UBA1, com troca de metionina na posição 41 por valina. Frente quadros de febre e dispneia, recebeu diversos ciclos de corticoterapia, sem melhora e com desenvolvimento de fibrilação atrial, abordada com ablação de apêndice atrial e controle com carvedilol e amiodarona. Iniciado tratamento com tocilizumab em 8 mg/kg IV. No dia da segunda aplicação do medicamento, referiu dispneia e relatou febres de 39°C aferidas diariamente há 4 dias da admissão. Clinicamente, apresentava dessaturação, estertores bilaterais e hipotensão, sendo optado por internação em leito de terapia intensiva. Tomografia de tórax demonstrou volumosa consolidação no lobo inferior direito. Ressonância de crânio apresentou encefalomalácia em giros pré e pós-central, centro semioval frontal posterior e lóbulos parietais superior e inferior à direita além de focos de isquemia em fase subaguda subcorticais e sequelas vasculares na periferia do hemisfério cerebelar direito e nucleocapsulares. Recebeu suporte fisioterapêutico, ceftriaxona, azitromicina e metilprednisolona 1 mg/kg/dia. Na admissão em nossa instituição, apresentava os seguintes resultados laboratoriais: Hb 11,1 g/dL, VCM 107,6 fL, leucócitos 3.800 mm^3, segmentados 3.268 mm^3, linfócitos 418 mm^3, plaquetas 119.000 μL, creatinina 1.34 mg/dL, IgA 214 mg/dL, IgG 693 mg/dL, IgM 129 mg/dL, DHL 593 UI/L. Suas provas de hemólise (teste de antiglobulina direto, haptoglobulina, bilirrubinas), sorologias virais, perfil reumático (fator reumatoide, FAN, ANCA), coagulograma e perfil tireoideano estavam dentro da normalidade. Foi solicitado avaliação da equipe de Hematologia. Optado em realizar avaliação medular – Mielograma (normocelularidade, normomaturação, discreta hipoplasia megacariocítica, precursores granulocíticos e eritróides vacuolizados; ausência de blastos); Imunofenotipagem (ausência de população celular com imunofenótipo anômalo); FISH (painel SMD/LMA) sem alterações. Ainda em análise

painel NGS de neoplasias mielóides. Indicado anticoagulação plena, melhorou do quadro respiratório após cuidados em suporte intensivo e recebeu alta hospitalar. Ambulatorialmente sendo avaliado a possibilidade de Transplante de Células Progenitoras Hematopoiéticas (TCTH) alogênico. **Discussão:** Paciente apresentou quadro clínico descrito na SV (masculino, idade avançada, febre, artrite, tromboses, anemia macrocítica, plaquetopenia, inflamação pulmonar) confirmado com vacuolização eritro-granulocítica medular e mutação de UBA1. O tratamento da SV ainda não está bem estabelecido. Considerando o risco de evolução clonal, infecções e tromboses recorrentes, além de complicações terapêuticas iniciais, o TCTH está sendo avaliado. **Conclusão:** o TCTH pode ser cogitado como abordagem curativa frente respostas subótimas a tratamentos pregressos e complicações decorrentes dos mesmos em pacientes elegíveis com SV.

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ENHANCED ANTI-TUMOR ACTIVITY OF CD19. CAR-NK CELLS BY INCORPORATING IL-27

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Autologous T-cells expressing Chimeric Antigen Receptor (CAR) have shown great efficacy against hematologic malignancies, however, severe side effects limit their clinical use. Natural Killer (NK) cells are an attractive alternative to T-cells as carriers for CARs, since they could be used as an allogeneic treatment, and have no, or minimal, risk for Cytokine Release Syndrome (CRS). CAR-NK cells have recently shown promising effect against leukemia without serious side effects after adoptive transfer. Possible allogeneic application of CAR-NK cells could dramatically reduce the costs of such adoptive cell therapy, nevertheless sufficient *ex vivo* expansion of NK cells remains challenging. Transgenic expression of cytokines in CAR-NK cells was shown to partially improve pro-survival or proliferative properties (e.g., IL-15), however what is the optimal cytokine for CAR NK cells is not clear to date. Here, we compared 2nd generation CD19-targeted CAR (CAR.19) with a fourth generation CAR.19 coexpressing Il-27 (CAR.19-IL27) in promoting NK-92 cell line proliferation, activation, and cytotoxic activity against B-cell cancers. CAR constructs were inserted into lentiviral vectors and subsequently transduced into the NK-92 cell line. Effects of CAR-NK cells were assessed against CD19-expressing B-cell lines NALM-6 or Raji *in vitro* and *in vivo* in a murine model of B-cell neoplasia with Raji cells. Tumor burden was measured by bioluminescence and by the curve of survival. We have demonstrated that CAR.19-IL27 exhibits a superior expansion capacity in comparison to both NK-92wt and CAR.19 cells. Moreover, CAR.19-IL27 cells showed higher cytotoxicity towards NALM-6 and Raji compared to CAR.19 *in vitro* (**$p < 0.001$). Degranulation assay, assed by CD107a, showed that CAR.19-IL27 have higher expression of CD107a compared to CAR.19 cells (**$p < 0.001$), which indicates increased secretion of perforin and granzymes. Our systematic transcriptome analysis of activated NK-92 CAR variants shows that IL-27 may be involved in the PI3K/AKT, and MAPK signaling pathways, as genes related to proliferation from these pathways were increased, e.g., PDK1, RPS6, MYC, and PGK1. *In vivo* studies with a xenograft mouse model of B-cell neoplasia showed that CAR.19-IL27 reduced tumor burden compared to CAR.19-treated. Altogether, CD19-CAR NK cells co-expressing Il-27 showed potent and selective antitumor activity with the potential to improve clinical use of CAR NK cells.

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MAGE-A4-CAR-NK CELL THERAPY FOR THE TREATMENT OF SOLID TUMORS

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Introduction: Natural Killer (NK) cells are a promising source for Chimeric Antigen Receptor (CAR) cell-based therapiesdue to their MHC independency for activation, intrinsic ability against tumor cells, rapid cytokine production, and low potential to cause graft-versus-host-disease. Conventional CARs only recognize cell surface antigens, sparing numerous intracellular tumor-associated antigens. MAGE-A4 intracellular antigen is highly expressed in different cancers and has low expression in normal tissues. Following its expression within cells, MAGE-A4 is presented on the cell surface in association with HLA-A*02, conferring a cancer-specific antigen profile. This project uses a TCR-like CAR, and an innovative human GITR signaling domain, developed by our collaborator, Prof. Shiku Hiroshi at Mie University. This CAR construct demonstrates the ability to recognize the intracellular tumor-associated antigen MAGE-A4 when presented by HLA-A*02 molecules. This anti-MAGE-A4 CAR is presently undergoing evaluation in a multi-institutional phase 1 study. **Objectives:** The primary aim of this study is to create an off-the-shelf CAR-NK cell product, using the anti-MAGE-A4 CAR, designed to target the HLA-A*02 complex along with a specific epitope of the MAGE-A4 antigen and to and to evaluate the therapeutic efficiency of these cells in the treatment of MAGE-A4+ solid tumors including melanoma. **Material and methods:** LentiX-HEK293T cells were transfected with MAGE-A4 CAR DNA and helper plasmids to produce LV viral particles. K562 cell line were used for viral titration. NK-92 cell line were transduced using lentivirus particles with MOIs 10, 5, and 3. After