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