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The adoptive transfer of T lymphocytes expressing chimeric antigen receptors (CAR) has resulted in impressive complete remission rates in B-cell malignancies. However, the therapeutic efficacy of CAR-T cells is still low or non-existent against solid tumors, which make up the vast majority of neoplasms. One of the main reasons for this failure is the presence of ligands and immunosuppressive cells in the tumor microenvironment. The complete activation of T cells requires the engagement of costimulatory molecules whose expression is temporally segregated and whose nature of biochemical signals are complementary. This is the case of CD28, expressed constitutively, and GITR, expressed right after the initial activation of T cells. Therefore, our hypothesis is that the combined expression of a CAR containing the costimulatory domain CD28 and the GITR ligand (GITRL) will potentiate the antineoplastic action of CAR-T cells. In addition to providing complementary costimulatory signals for effector CAR-T cells, GITRL can suppress the action of regulatory T cells in the tumor microenvironment, thus possibly reducing local immunosuppression. Then, the aim of this study is to evaluate the therapeutic efficiency of anti-GD2 CAR-T cells coexpressing GITRL in a preclinical model of glioblastoma multiforme. In this project, we used as a model a CAR against the ganglioside GD2, which is highly immunogenic and expressed in tumors of neuroectodermal origin, such as glioblastoma. To test our hypothesis, we generated two lentiviral vectors to express CAR anti-GD2 and another one coexpressing GITRL as a costimulatory domain. After that, we produced lentiviral particles to transduced human T lymphocytes and we detected CAR expression in the T cells transduced with both vectors. To verify the functional activity of these CAR-T cells, we performed a coculture assay against T98G cell line, which express high levels of GD2, and HCT-166 cell, that dont express. These cell lines were previously modified to express luciferase to enable the analysis through bioluminescence imaging. We tested two ratios of effector:target cells (E:T) and we observed that both CAR.GD2 and CAR.GD2-GITRL T cells demonstrated powerful capacity of lysis only against cells that express GD2. Then, we interrogated whether these CAR-T cells could keep their lysis capacity after additional rechallenges. So, another two rounds of coculture were performed and the cells showed persistence of cytotoxicity. In addition, tumor cell killing was associated with secretion of IFNy as evaluated by ELISA quantification of coculture supernatants. These data encouraged us to compare the antineoplastic activity and persistence of CAR-T cells anti-GD2 in an orthotopic pre-clinical model of glioblastoma multiforme, which is under way. We expect that the potential results of this project will foster the development of a new advanced cellular immunotherapy strategy for the treatment of solid GD2+ neoplasms and will pave the way to increase the antineoplastic efficiency of CAR-T cells against other malignancies.

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UM171 EXPANDS IMMATURE BONE MARROW CD34+ CELLS FROM PATIENTS WITH TELOMEROPATHIES

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Objectives: Telomeropathies, also referred to as telomere biology disorders (TBD), correspond to a spectrum of diseases characterized by genetic defects in the maintenance mechanisms of telomeres and telomerase. Individuals carrying variants in the genes involved in telomeres machinery may have critically short or dysfunctional telomeres, which leads to poor cell regeneration and predisposition to cancer. The commonly affected tissues are those that renew rapidly, such as the bone marrow, lungs, and skin. The most effective therapeutical approach to treat bone marrow failure in patients with TBD is allogeneic bone marrow transplantation, restricted by the low availability of compatible donors and complications of the conditioning regimen. Fares et al. (2013) demonstrated that the small molecule UM171 expands the $CD34^+$ cell subpopulations from cord blood with superior engraftment potential. We demonstrated that UM171 expands primitive $CD34^+$ cells from patients with immune aplastic anemia. The expanded hematopoietic progenitors showed no observable chromosomal, genetic, or telomeric changes. Here we assessed the potential of the HSC agonist UM171 to expand the HSPC compartment of patients with telomeropathies. **Methods:** Bone marrow aspirate samples were collected from five patients, and $CD34^+$ cells were enriched using immunomagnetic labeling with human CD34 MicroBeads and a magnetic separator. Cells were cultured for 7 days in ACF medium supplemented with cytokines that support expansion of HSPC and UM171 or DMSO (negative control). To assess the capacity of the cells cultured either with DMSO or UM171 to generate hematopoietic progenitors, 1,000 cells/mL were resuspended in Methocult and seeded onto 35 mm dishes in triplicate. After 14 days, the colonies were counted and classified according to their morphology. **Results:** After a 7-day expansion, the percentage of $CD34^+$ cells was higher with UM171 in comparison to control (UM171, 48 $\pm$5.1% vs. DMSO, 25.3% $\pm$6.2% [mean $\pm$standard error] $n = 5$; $p = 0.003$). The cell surface EPCR is a reliable marker for the purification of the HSPC compartment. Therefore, we evaluated the $CD34^+EPCR^+$ subpopulation, which was increased after the UM171 treatment (UM171, 14.4 $\pm$4.1% vs. DMSO, 1.8% $\pm$ 0.6%; $n = 5$; $p = 0.03$). Additionally, cells expanded with UM171 gave rise to more progenitor cells, as observed by the CFU assay (UM171, 142 $\pm$ 18 vs. DMSO, 94% $\pm$ 9; $n = 4$; $p = 0.01$). No significant telomere attrition was observed with the expansion. **Discussion:** Bone marrow failure is a common manifestation in patients with telomere disorders since the telomerase complex is essential for maintaining the self-renewal capacity of the HSPC pool. Our data suggest the possible future use of UM171 to enhance *ex vivo* production of HSPC from TBD patients, making it feasible to use these cells in autologous transplants. These findings should improve our understanding of the effect of UM171 on

these cells and help to develop protocols for scaling up its production for future therapies. **Conclusion:** Our studies reveal UM171 to be a potent molecule to expand HSPC in patients with telomeropathies. Further experiments are required to confirm these results besides the assessment of the engraftment potential of the expanded cells in a murine model.

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PERSPECTIVAS INOVADORAS NA ABORDAGEM NA DOENÇA DO OLHO SECO (DOS) E O INTERFACEAMENTO COM BANCOS DE SANGUE DE CORDÃO UMBILICAL HUMANO

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Os bancos de sangue de cordão umbilical e placentário (BSCUP), os laboratórios de processamento de medula óssea/sangue periférico para transplante e os centros de tecnologia celular, passaram a receber a denominação comum de Centros de Processamento Celular – CPC. O país tem BSCUP 14 unidades públicas e 19 de natureza privada, totalizando 33 BSCUP. Uma análise retrospectiva dos relatórios da ANVISA identifica que em 2003 foram coletadas 26 unidades, com desqualificação de 15,38% delas, o crescimento da coleta foi exponencial e dez anos depois, em 2013, foram coletadas 13.995 unidades (5,82% de desqualificação). Em 2020, houve uma diminuição expressiva de coletas, reflexo da pandemia de COVID-19: 4.918 unidades (desqualificação de 12,69%). Este tipo de produtividade compromete a viabilidade financeira destes serviços, e encontrar formas de otimizar bolsas desqualificadas por volume ou quantidade de células para demais finalidades é uma vertente de gestão que deve ser estabelecida. O objetivo deste projeto foi a coleta de segmentos de cordão umbilical das unidades já previamente validadas pelos BSCUP para extração de células tronco, caracterização imunofenotípica e produção de meio condicionado isento de soro fetal bovino. A obtenção do meio condicionado (MC) da cultura de células tronco. Tem crescido cada vez mais o interesse pelo uso dos fatores de crescimento, citocinas e moléculas sinalizadoras livres no MC além das vesículas extracelulares, que se tornaram relevantes, tanto para diagnóstico como para terapêutica, inclusive para aplicações oftalmológicas. Neste campo, identificamos a DOS que impacta profundamente a qualidade de vida das pessoas. Há 15 anos o Laboratório de Biologia Celular tem desenvolvido o soro autólogo, para atendimento dos pacientes refratários aos tratamentos convencionais e farmacológicos disponíveis, em especial aqueles pacientes submetidos ao Transplante de Medula Óssea e que desenvolveram DOS2ário a doença de enxerto *versus* hospedeiro. No entanto, existem pacientes com impossibilidade de acesso venoso ou com sorologias reagentes para doenças infecciosas que são impedidos de utilizar o produto. Diante disto, optou-se por produzir o MC de células tronco de cordão umbilical de parturientes jovens e sem comorbidades para obtenção do secretoma das células para avaliação terapêutica na DOS. Um segmento do cordão umbilical foi retirado e processado seguido de plaqueamento e expansão para posterior identificação de adesão ao plástico, caracterização imunofenotípica por citometria de fluxo utilizando marcadores como CD11b, CD13, CD14, CD34, CD31, CD36, CD45,CD73, CD90, CD 105, CD106 e HLA-DR. Todas as amostras tiveram adesão ao plástico com aspecto fibroblastóides e perfil imunofenpotípico corrobora com o determinado pela SITC. Para a obtenção de MC foram semeadas CTM_{cup} até 70% de confluência e foram submetidas ao *wash out*, recebendo meio de cultura DMEM-F12 aditivado por 48 horas. Após isto, foi coletado 60mL do secretoma das células para o experimento específicos *in vitro*.

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THE POLYOMAVIRUS HEMORRHAGIC CYSTITIS TREATMENT PUZZLE: A SUCCESS CASE AFTER HEMATOPOIETIC CELL TRANSPLANTATION

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Background: BK virus (BKV)-hemorrhagic cystitis (HC) is a well-known complication of hematopoietic stem cell transplantation (HSCT), and contributes to significant morbidity. We report on and discuss the management of a patient with BK viremia, whose post-HSCT course was complicated by severe BKV-HC, combined with grade IV skin and gut graft vs host disease (GVHD). **Case:** A 15 year-old boy diagnosed with leukemia underwent an HSCT with his mother as the haploidentical donor. Graft source was bone marrow, with TNC/kg $4.4 \times 10^{*}8$/kg. Neutrophil engraftment was achieved at day +16; chimerism demonstrated 100% donor cells. Myeloablative conditioning consisted of cclophosphamide (Cy) 50 mg/kg x 2 days (days -5 to -4) and total body irradiation 12 Gy on days -3 to -1. GVHD prophylaxis consisted of post-transplant Cy on days +3 and + 4 along with mycophenolate mofetil x 30 days and cyclosporin. His transplant course was complicated by cytomegalovirus (CMV) infection and grade II skin GVHD. He was discharged on day +20 and readmitted on day +39 with grade IV skin GVHD and transferred to the intensive care unit. Immunosuppressive medications (Ruxolitinib with IV methylprednisolone) were started, and his symptoms started to improve. However, he developed severe lower urinary tract symptoms and hematuria with frequent passage of clots on Day +65. A diagnosis of grade III hemorrhagic cystitis (HC) was made. Quantitative PCR from serum (600 copies/mL) as well as from urine came positive for BK polyomavirus, Cidofovir was started and immunosuppression reduced. Continuous bladder irrigation was initiated without significant improvement. On day +86, a cystoscopy was done for clot