Importantly, TAC-engineered gamma/delta T cells displayed robust cytotoxicity at very low effector:target ratios (<1) and caused regression of human tumor xenografts that were otherwise resistant to non-engineered gamma/delta T cells. Curiously, gamma/delta T cell manufacturing was sensitive to the quality of the lentivirus product, where products with low titers were associated with outgrowth of conventional alpha/beta T cells. Outgrowth of alpha/beta T cells was not observed with gamma-retroviruses. We are presently evaluating the anti-tumor activity of gamma-retrovirus-engineered gamma/delta T cells.

Conclusions Off-the-shelf engineered gamma/delta T cells represent a strategy to reduce manufacturing cost and may represent the next generation of engineered T cell therapies.TAC receptors provide a robust tool for directing gamma/delta T cells to attack tumors that are otherwise resistant to gamma/delta T cells and should be evaluated further.

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Ethics Approval The study was approved by McMaster's Animal Research Ethics Board, AUP#19-02-10.

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102 CORD-BLOOD DERIVED NK CELLS, AND CAR-T CELLS, AN ATTRACTIVE IMPROVED IMMUNOTHERAPY TREATMENT TO BE CONSIDERED FOR HEMATOLOGICAL MALIGNANCIES

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Background Multiple myeloma (MM) remains an incurable hematological malignancy where a proportion of patients relapse or become refractory to current treatments. Administration of autologous T cells modified with a chimeric antigen receptor (CAR) against B cell maturation antigen (BCMA) has achieved high percentages of complete responses. Unfortunately, the lack of persistence of CART-BCMA cells in the patient leads to relapses. On the other side, cord-blood derived natural killer cells (CB-NK) is an off-the-shelf cellular immunotherapy option to treat cancer patients with high potential due to their anti-tumor activity. However, clinical results in patients up to date have been sub-optimal. Whereas CB-NK are innate immune cells and their anti-tumor activity is developed in a few hours, CART cells are adaptive immune cells and their activity develops at later time points. Moreover, we previously described that CB-NK secrete inflammatory proteins that promote the early formation of tumor-immune cell clusters bringing cells into close contact and thus, facilitating the anti-tumor activity of T cells. Therefore, we hypothesized that the addition of a small number of CB-NK to CART cells would improve the anti-tumor activity and increase the persistence of CART cells.

Methods T cells transduced with a humanized CAR against BCMA and CB-NK were employed at 1:0.5 (CART:CB-NK) ratio. Cytotoxicity assays, activation markers and immune-tumor cell cluster formation were evaluated by flow cytometry and fluorescence microscopy. In vivo models were performed in NSG mice.

Results The addition of CB-NK to CART cells demonstrated higher anti-MM efficacy at low E:T ratios during the first 24h and in long-term cytotoxicity assays, where the addition of CB-NK to CART cells achieved complete removal of tumor cells. Analysis of activation marker CD69 and CD107a degranulation from 4h to 24h of co-culturing proved differences only at 4h, where CD69 and CD107a in CART cells were increased when CB-NK were present. Moreover, CB-NK accelerated an increased formation of CART-tumor cell clusters facilitating the removal of MM cells. Of note, CB-NK addition did not increase total TNFα and IFNγ production. Finally, an in vivo model of advanced MM with consecutive challenge to MM cells evidenced that the addition of CB-NK achieved the highest efficacy of the treatment.

Conclusions Our results suggest that the addition of 'off-the-shelf' CB-NK to CART cells leads to a faster and earlier immune response of CART cells with higher long-term maintenance of the anti-tumor response, suggesting this combinatorial therapy as an attractive immunotherapy option for MM patients.

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103 INCLUSION OF A DAP10 COSTIMULATORY DOMAIN ENHANCES ANTI-TUMOR EFFICACY OF CHIMERIC PD1-EXPRESSING T CELLS IN MULTIPLE TYPES OF SOLID TUMORS

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Background Adoptive transfer of T cells is a promising anti-tumor therapy for many cancers. To enhance tumor recognition by T cells, chimeric antigen receptors (CAR) consisting of signaling domains fused to receptors that recognize tumor antigens can be expressed in T cells. One receptor that is a prospective target for a new chimeric antigen receptor is PD1 because the ligands for the PD1 receptor are expressed on many cancer types. Therefore, we developed a murine chimeric PD1 receptor (chPD1) consisting of the PD1 receptor extracellular domain and the activation domain of CD3 zeta. In addition, current chimeric antigen receptor therapies utilize various costimulatory domains to enhance anti-tumor efficacy. Therefore, we also compared the inclusion of CD28, Dap10, 4-1BB, GITR, ICOS, or OX40 costimulatory domains in the chPD1 receptor to determine which costimulatory domain induced optimal anti-tumor immunity.

Methods To determine if this novel CAR could potentially target a wide variety of tumors, the anti-tumor efficacy of chPD1 T cells against murine lymphoma, melanoma, kidney, pancreatic, liver, colon, breast, ovarian, prostate, and bladder cancer cell lines was measured.

Results Of the eighteen cell lines tested, all expressed PD1 ligands on their cell surface, making them potential targets for chPD1 T cells. Regardless of the costimulatory domain in the CAR, all of the chPD1 T cells induced similar levels of T cell proliferation and tumor cell lysis. However, differences were observed in the cytokine secretion profiles depending on which costimulatory receptor was included in the CAR. While

most of the chPD1 T cell receptor combinations secreted both pro-inflammatory (IFNγ, TNFα, IL-2, GM-CSF, IL-17, and IL-21) and anti-inflammatory cytokines (IL-10), chPD1 T cells containing a Dap10 costimulatory domain secreted high levels of proinflammatory cytokines but did not secrete a significant amount of anti-inflammatory cytokines. Furthermore, T cells expressing chPD1 receptors with a Dap10 domain also had the strongest anti-tumor efficacy in vivo. ChPD1 T cells did not survive for longer than 14 days in vivo, however treatment with chPD1 T cells induced long-lived protective host-anti-tumor immune responses in tumor-bearing mice.

Conclusions Therefore, adoptive transfer of chPD1 T cells could be a novel therapeutic strategy to treat multiple types of cancer and inclusion of the Dap10 costimulatory domain in chimeric antigen receptors may induce a preferential cytokine profile for anti-tumor therapies.

Ethics Approval The study was approved by Longwood University's IACUC.

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104 BCMA-TARGETING CAR-T CELLS EXPANDED IN IL-15 HAVE AN IMPROVED PHENOTYPE FOR THERAPEUTIC USE COMPARED TO THOSE GROWN IN IL-2 OR IL-15/IL-7

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Background Chimeric antigen receptor-T (CAR-T) cells that target B cell maturation antigen (BCMA-CARs) have emerged as a promising treatment for multiple myeloma (MM). Despite impressive initial responses to BCMA-CAR therapy in clinical trials, relapse is common, signifying a need to improve the in vivo efficacy and persistence of BCMA-CARs.[1] The development of unfavourable differentiation or T cell dysfunction, such as exhaustion and senescence, during the ex vivo expansion of the BCMA-CARs could be limiting their therapeutic potential. For CD19-directed CARs, reduced dysfunction and differentiation and improved anti-tumour responses were achieved by expanding the cells with IL-15 instead of IL-2.[2] Therefore, in this study, our aim was to determine whether expanding BCMA-CARs with IL-15 or IL-15/IL-7 instead of IL-2 alters their levels of exhaustion, senescence, differentiation and activity.

Methods T cells stimulated with anti-CD3/anti-CD28-coated beads were supplemented with IL-2, IL-15, IL-15 + IL-7 or no cytokine and transduced with ARI2h, a BCMA-CAR with a 4-1BB co-stimulatory domain produced at our institution.[3] Expanded BCMA-CARs were analysed by flow cytometry for markers of T cell dysfunction, or challenged with MM cell line ARP-1 and then tested for cytokine production, cytotoxic ability and activation signals.

Results BCMA-CARs cultured in IL-15 or IL-15/IL-7 expanded similarly to those grown in IL-2, with comparable CAR transduction efficiencies, CD4:CD8 ratios and proliferation rates. BCMA-CARs grown in IL-15 had low expression of exhaustion marker LAG-3 and high expression of the costimulatory molecule CD27, which is important for T cell survival and persistence, when compared to BCMA-CARs cultured in IL-2. Moreover, BCMA-CARs grown solely in IL-15 were less differentiated than those supplemented with IL-7, and had higher expression of stem cell memory marker CXCR3 within the naïve population than those expanded with IL-2. When challenged with MM cell line ARP-1, IL-15-grown BCMA-CARs upregulated activation marker CD69, exhibited strong cytotoxicity and robust production of IFNγ and IL-2. However, in comparison to BCMA-CARs expanded in IL-2 or IL-15/IL-7, those grown with IL-15 had lower mTORC1 activity and p38 MAPK phosphorylation when activated by ARP-1 cells, suggesting differential regulation of key pathways for T cell metabolism and senescence, respectively.

Conclusions To summarise, BCMA-CARs expanded with IL-15 alone exhibited the most favourable phenotype for therapeutic use compared those grown with IL-2 or IL-15/IL-7. Future experiments using murine MM models will be critical in understanding the in vivo benefits or drawbacks of culturing BCMA-CARs in IL-15 compared to IL-2 or IL-15/IL-7.

Ethics Approval Research involving human material was approved by the Ethical Committee of Clinical Research (Hospital Clinic, Barcelona). Peripheral blood T cells were obtained from healthy donors after informed consent in accordance with the Declaration of Helsinki.

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105 A THIRD-GENERATION HUMAN GUCY2C-TARGETED CAR-T CELL FOR COLORECTAL CANCER IMMUNOTHERAPY

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Background Colorectal cancer (CRC) presents a significant public health burden, responsible for the second most cancer-related deaths in the United States, with an increasing incidence in young adults observed globally.[1,2] While the blockade of immune checkpoints received FDA approval as a CRC therapeutic, only patients with microsatellite instability, accounting for 15% of sporadic cases, demonstrate partial or complete responses.[3] We present a third-generation chimeric antigen receptor (CAR)-T cell directed towards the extracellular domain of the mucosal antigen guanylyl cyclase C (GUCY2C), which is over-expressed in 80% of CRC cases, as a therapeutic alternative for late stage disease. Here, we demonstrate that human GUCY2C CAR-T cells can selectively kill GUCY2C-expressing colorectal cancer cells in vitro and produce inflammatory cytokines in response to antigenic stimulation.

Methods Peripheral blood mononuclear (PBMCs) cells were isolated from leukoreduction filters obtained from the Thomas Jefferson University Hospital Blood Donor Center (IRB #18D.495). Magnetic Activated Cell Sorting (MACS) technology was used to negatively select pan-T cells (Miltenyi Biotec), followed by activation and expansion using anti-CD3, anti-CD28, and anti-CD2 coated microbeads (Miltenyi Biotec) and supplemented with IL-7 and IL-15 (Biological Resources