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

Branch Preclinical Biologics Repository – NCI). T-cells were transduced with a lentiviral vector encoding the anti-GUCY2C CAR. Our CAR utilizes a single chain variable fragment of human origin directed towards the extracellular domain of GUCY2C, the CD28 hinge, transmembrane, and intracellular signaling domain (ICD), 4-1BB (CD137) ICD, and CD3ζ ICD. CAR-T cells were used for experiments between 10 to 14 days after activation in vitro using the xCELLigence real time cytotoxicity assay and intracellular cytokine staining.

Results GUCY2C-directed CAR-T cells specifically lysed the GUCY2C-expressing metastatic CRC cell line T84, while the control CAR did not. GUCY2C-negative CRC cells were not killed by either. In addition to cell killing, GUCY2C-directed CAR-T cells of both the CD8$^+$ and CD4$^+$ co-receptor lineage produced the inflammatory cytokines IFN-γ and TNFα in response to GUCY2C antigen.

Conclusions We demonstrate that human GUCY2C-directed CAR-T cells can selectively target GUCY2C-expressing cancer cells. We hypothesize that GUCY2C-directed CAR-T cells present a viable therapeutic option for metastatic CRC. In vivo animal models to examine this potential are currently ongoing.

Ethics Approval This study was approved by the Thomas Jefferson University Institutional Review Board (IRB Control #18D.495) and the Institutional Animal Care and Use Committee (Protocol #01529).

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106 INCREASING AMPK ACTIVITY IN HUMAN T CELLS ENHANCES MEMORY SUBSET FORMATION WITHOUT SACRIFICING IN VITRO EXPANSION

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Background The ideal adoptive cell therapy consists of memory-like T cells with enhanced oxidative potential. However, current expansion protocols drive T cells towards terminal differentiation, decreasing the number of T cells fit for the in vivo environment. AMP-activated protein kinase (AMPK), whose activity increases in memory cells, is a key regulator of mitochondrial biogenesis and oxidative metabolism, making AMPK activation an attractive candidate to improve adoptive T cell function.

Methods To increase AMPK activity, AMPKγ, which controls the phosphorylation status of AMPKa and therefore activity of the AMPK complex, was cloned into a lentiviral plasmid downstream of the elongation factor 1a (EF1a) promoter and upstream of green fluorescent protein (GFP). An empty vector, containing GFP only, served as a negative control. Human T cells were transduced and expanded in vitro in the presence of IL-2. AMPK activity was assessed via immunoblot for phosphorylation of AMPKa on Thr172 and S555 on downstream target Unc-51-like kinase 1 (ULK1). Memory-marker expression and mitochondrial density (using Mitotracker Red) were analyzed by flow cytometry. Oxidative metabolism and spare respiratory capacity (SRC) were determined using the Seahorse Metabolic Analyzer. Fold changes of in vitro expansion were calculated by adjusting manual cell counts for GFP positivity and CD4+/CD8+ staining.

Results AMPKγ was efficiently transduced and expressed by human T cells, which significantly increased AMPK activity (AMPKa phosphorylation 1.93 ± 0.05 vs 0.6 ± 0.09, p<0.001, ULK1 phosphorylation 1.28 ± 0.11 vs 0.67 ± 0.08, p<0.01). AMPKγ-overexpressing T cells augmented expression of memory markers CD62L, CD27, and CCR7, with an increased yield of stem cell memory-like T cells marked by co-expression of CD45RA and CD62L (figure 1). Mitochondrial density, SRC, and maximal oxygen consumption rates were similarly increased in AMPKγ-transduced cells (figure 2A,B). Further, while enhanced memory cell production is often linked with reduced proliferation, T cells with increased AMPK activity maintained and even trended towards increased rates of expansion compared to empty-transduced controls (figure 3A), with a measurable increase in CD4+ T cell percentages by flow cytometry (figure 3B).
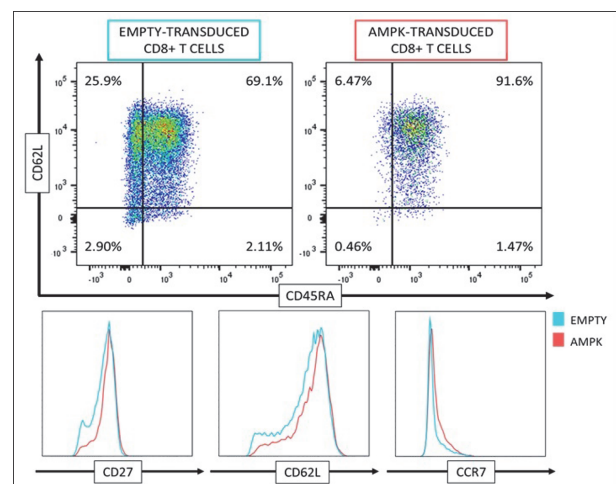
Abstract 106 Figure 1 AMPK-transduced T cells increase expression of memory surface markers. Human T cells were transduced with AMPK-GFP or GFP-only control (Empty). Memory markers were assessed by flow cytometry on Days 7–14 of in vitro culture following expansion with IL-2. Plots are representative of 3 separate donors