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.

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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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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 AMPKα and therefore activity of the AMPK complex, was cloned into a lentiviral plasmid downstream of the elongation factor 1α (EF1α) 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 AMPKα 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 (AMPKα 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 2 AMPK-transduced T cells show enhanced mitochondrial density and SRC. (A) Human T cells transduced with AMPK-GFP or GFP-only (Empty) were stained with Mitotracker Red and fluorescence intensity compared between transduced cells and GFP-controls within the same culture to account for variability in Mitotracker dye staining. (B) AMPK and Empty transduced T cells were assessed via Seahorse Metabolic Analyzer using the Mito Stress Test. Results are representative of 3 separate donors. OCR = O2 consumption rate

Abstract 106 Figure 3 Proliferation is maintained in AMPK-transduced T cells, with enhanced recovery of CD4+ T cells. (A) Primary human T cells transduced with AMPK-GFP or GFP-only (Empty) were expanded in vitro in the presence of IL-2. Cells were manually counted and the ratio of day 7 to day 5 cell counts calculated to assess fold expansion over time. (B) At the same, CD4+ and CD8+ percentages were measured in GFP+ cells by flow cytometry

Conclusions Increasing AMPK activity endows T cells with a variety of characteristics ideal for adoptive cell therapy, including increased memory-marker expression, enhanced SRC and oxidative metabolism, equivalent to augmented in vitro expansion, and improved CD4+ T cell yields. Further studies are ongoing to assess the activity and function of AMPK-transduced CAR-T cells both in vitro and in vivo.

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MCY-M11, A CAR-PBMC CELL PRODUCT TRANSIENTLY EXPRESSING A MESOTHELIN TARGETED MRNA CAR, EXHIBITS DESIRABLE FUNCTIONAL AND IMMUNE PHENOTYPE ATTRIBUTED TO SUSTAINED ANTITUMOR IMMUNITY IN VITRO

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Background MCY-M11, an anti-mesothelin CAR (Meso-CAR) mRNA transfected PBMC cell product manufactured through <1 day-process is under clinical evaluation for the treatment of advanced ovarian cancer and peritoneal mesothelioma. In this in-vitro study, we characterized the phenotypic and functional status of immune cell populations in MCY-M11 and their possible role in antitumor immunity.

Methods MCY-M11 cell product were generated using unmanipulated healthy donor PBMCs (n=5) by transfection of Meso-CAR mRNA using MaxCyte’s proprietary Flow Electroporation™ system. Frozen MCY-M11 cell product was thawed and cultured for 18 hours, then co-cultured with hMSLNneg application of CAR T cell products still face a number of challenges. To overcome these challenges, Adicet Bio is developing an allogeneic γδ T cell-based CAR T cell platform, which capitalizes on the intrinsic abilities of Vδ1 γδ T cells to recognize and kill transformed cells in an MHC-unrestricted manner, to migrate to epithelial tissues, and to function in hypoxic conditions. To gain a better understanding of the requirements for optimal intratumoral CAR Vδ1 γδ T cell activation, proliferation, and differentiation, we developed a three-dimensional (3D) tumor spheroid assay, in which tumor cells acquire the structural organization of a solid tumor and establish a microenvironment that has oxygen and nutrient gradients. Moreover, through the addition of cytokines and/or tumor stromal cell types, the spheroid microenvironment can be modified to reflect hot or cold tumors. Here, we report on the use of a 3D CD20+ Raji lymphoma spheroid assay to evaluate the effects of IL-2 and IL-15, positive regulators of T cell homeostasis and differentiation, on the proliferative and antitumor capacities of CD20 CAR Vδ1 γδ T cells.

Methods Molecular, phenotypic, and functional profiling were performed to characterize the in vitro dynamics of the intra-spheroid CD20 CAR Vδ1 γδ T cell response to target antigen in the presence of IL-2, IL-15, or no added cytokine.

Results When compared to no added cytokine, the addition of IL-2 or IL-15 enhanced CD20 CAR Vδ1 γδ T cell activation, proliferation, survival, and cytokine production in a dose-dependent manner but were only able to alter the kinetics of Raji cell killing at low effector to target ratios. Notably, differential gene expression analysis using NanoString nCounter® Technology confirmed the positive effects of IL-2 or IL-15 on CAR-activated Vδ1 γδ T cells as evidenced by the upregulation of genes involved in activation, cell cycle, mitochondrial biogenesis, cytotoxicity, and cytokine production.

Conclusions Together, these results not only show that the addition of IL-2 or IL-15 can potentiate CD20 CAR Vδ1 γδ T cell activation, proliferation, survival, and differentiation into antitumor effectors but also highlight the utility of the 3D spheroid assay as a high throughput in vitro method for assessing and predicting CAR Vδ1 γδ T cell activation, proliferation, survival, and differentiation in hot and cold tumors.

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