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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0106

107 EFFECTS OF IL-2 AND IL-15 ON THE PROLIFERATIVE AND ANTITUMOR CAPACITIES OF ALLOGENEIC CD20 CAR-ENGINEERED γδ T CELLS IN A 3D B CELL LYMPHOMA SPHEROID ASSAY

Lu Bai, Kevin Nishimoto, Mustafa Turkoz, Marissa Herman, Jason Romero, Daulet Satpayev, Stewart Abbot, Sandra Hayes, Mary Brodey*, Adicet Bio, Inc., Menlo Park, CA, USA

Background Autologous chimeric antigen receptor (CAR) T cells have been shown to be efficacious for the treatment of B cell malignancies; however, widespread adoption and 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 in-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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0107

108 MCY-M11, A CAR-PBMC CELL PRODUCT TRANIENTLY EXPRESSING A MESOTHELIN TARGETED MRNA CAR, EXHIBITS DESIRABLE FUNCTIONAL AND IMMUNE PHENOTYPE ATTRIBUTED TO SUSTAINED ANTITUMOR IMMUNITY IN VITRO
Sashi Kasimsetty, Himavanth Gatla, Dhana Chinnasamy*, CARMA Cell Therapies, Gaithersburg, MD, USA

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
or hMSLNpos human mesothelioma cell line, MSTO-211H, or stimulated with anti-CD3/anti-CD28 antibodies in vitro for 8 days. Distinct cell populations in MCY-M11 were evaluated for kinetics and duration of CAR expression, differentiation, activation, exhaustion, and their ability to secrete various immunomodulatory molecules during in vitro stimulation. Antigen-specific proliferation and cytotoxicity of MCY-M11 against hMSLNpos tumor cells as well as their ability to mount long-term antitumor immunity through epitope spreading mechanisms were studied.

**Results** Individual cell populations in MCY-M11 exhibited a consistent but transient Meso-CAR expression persisting for about 7 days. Cell subsets in MCY-M11 acquired early signs of activation and differentiation within 18–24 hours post-culture, but only attained full activation and lineage-specific differentiation upon specific response to hMSLNpos tumor cells. hMSLN antigen experienced MCY-M11 retained significant fractions of Naïve and Central Memory T cells and increased percentage of Effector Memory T cells along with increased expression of CD62L, CD27, and chemokine receptors (CCR5, CCR7, and CXCR3). MCY-M11 exhibited strong antigen-specific cytotoxicity against hMSLNpos tumor cells with corresponding increase in activation and proliferation of CD4+ and CD8+ T cell subsets and displayed low or no acquisition of known exhaustion markers. NK cells also exhibited a functionally superior molecular signature exhibiting increased levels of NKG2D, NKp44, NKp46, FAS, and TRAIL. The Monocytes and B cells in MCY-M11 also acquired an activated, differentiated, and mature phenotype, expressing molecules required for antigen presentation (HLA-DR, HLA-ABC, and CD205) and T cell co-stimulation (CD80 and CD86) to mount a strong antitumor response. These phenotypic changes in cell subsets of MCY-M11 transpired with simultaneous secretion of potent immunostimulatory molecules and chemokines facilitating an extended antitumor response through epitope spreading.

**Conclusions** We demonstrated that MCY-M11 is a unique cell product possessing a complete built-in immune cellular machinery with favorable phenotype and enhanced functions specialized in mediating an effective and long-term antitumor response.

**Trial Registration** NCT03608618

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0108

---

**109 DOMINANT-NEGATIVE TGFβ RECEPTOR 2 ENHANCES GPC3-TARGETING CAR-T CELL EFFICACY AGAINST HEPATOCELLULAR CARCINOMA**

Nina Chu*, Michael Overstreet, Ryan Gibbeth, Lori Claree, Christina Gesse, Eric Tu, Letizia Giardino, Gordon Moody, AstraZeneca, Gaithersburg, MD, USA

**Background** Chimeric antigen receptors (CARs) are engineered synthetic receptors that reprogram T cell specificity and function against a given antigen. Autologous CAR-T cell therapy has demonstrated potent efficacy against various hematological malignancies, but has yielded limited success against solid cancers. MEDI7028 is a CAR that targets oncofetal antigen glypican-3 (GPC3), which is expressed in 70–90% of hepatocellular carcinoma (HCC), but not in normal liver tissue. Transforming growth factor β (TGFβ) secretion is increased in advanced HCC, which creates an immunosuppressive milieu and facilitates cancer progression and poor prognosis. We tested whether the anti-tumor efficacy of a GPC3 CAR-T can be enhanced with the co-expression of dominant-negative TGFβRII (TGFβRIIDN).

**Methods** Primary human T cells were lentivirally transduced to express GPC3 CAR both with and without TGFβRIIDN. Western blot and flow cytometry were performed on purified CAR-T cells to assess modulation of pathways and immune phenotypes driven by TGFβ in vitro. A xenograft model of human HCC cell line overexpressing TGFβ in immunodeficient mice was used to investigate the in vivo efficacy of TGFβRIIDN armored and unarmored CAR-T. Tumor infiltrating lymphocyte populations were analyzed by flow cytometry while serum cytokine levels were quantified with ELISA.

**Results** Armoring GPC3 CAR-T with TGFβRIIDN nearly abolished phospho-SMAD2/3 expression upon exposure to recombinant human TGFβ in vitro, indicating that the TGFβ signaling axis was successfully blocked by expression of the dominant-negative receptor. Additionally, expression of TGFβRIIDN suppressed TGFβ-driven CD103 upregulation, further demonstrating attenuation of the pathway by this armoring strategy. In vivo, the TGFβRIIDN armored CAR-T achieved superior tumor regression and delayed tumor regrowth compared to the unarmored CAR-T. The armored CAR-T cells infiltrated HCC tumors more abundantly than their unarmored counterparts, and were phenotypically less exhausted and less differentiated. In line with these observations, we detected significantly more interferon gamma (IFNγ) at peak response and decreased alpha-fetoprotein in the serum of mice treated with armored cells compared to mice receiving unarmored CAR-T, demonstrating in vivo functional superiority of TGFβRIIDN armored CAR-T therapy.

**Conclusions** Armoring GPC3 CAR-T with TGFβRIIDN abrogates the signaling of TGFβ in vitro and enhances the anti-tumor efficacy of GPC3 CAR-T against TGFβ-expressing HCC tumors in vivo, proving TGFβRIIDN to be an effective armoring strategy against TGFβ-expressing solid malignancies in preclinical models.

**Ethics Approval** The study was approved by AstraZeneca’s Ethics Board and Institutional Animal Care and Use Committee (IACUC).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0109

---

**110 IN-DEPTH CHARACTERIZATION OF VARIABILITY IN APERESIS COLLECTIONS FROM NORMAL DONOR POPULATIONS FOR ALLOGENEIC CELL THERAPY**

Shawn Fahl, Shawn Fahl, Shawn Fahl*. Discovery Life Sciences, Huntsville, AL, USA

**Background** The success of autologous CAR-T cell therapies has revolutionized and accelerated development in the cell therapy field. However, the requirement for patient-specific starting material for these therapies remains an impediment to establishing availability for all patients who could benefit, highlighting the need for a highly characterized normal donor pool to generate allogeneic cell therapy material.

**Methods** We have established a network of >2800 normal donors that have been genotyped at the HLA loci (6 digits) and stratified by reactivity to common human viruses, such as cytomegalovirus (CMV) and Epstein Barr Virus (EBV). Furthermore, cell collections from 35 randomly selected donors have been screened by flow cytometry for major immune cell subsets, including T cells, B cells, NK cells, and monocytes. The T cell compartment was further characterized by