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.

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

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**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. MED17028 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 pre-clinical models.

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