Background To overcome the challenges of a hostile solid tumor microenvironment, we have engineered CAR-T cells to co-express mitochondrial enzyme glutamic-oxaloacetic-transaminase 2 (GOT2). GOT2 is hypothesized to improve CAR-T cell fitness by maintaining cellular redox balance under oxidative stress and fueling the tricarboxylic acid (TCA) cycle via glutaminolysis. In vivo preclinical studies have shown improved anti-tumor activity of glypican-3 (GPC3)-targeting CAR-T cells co-expressing GOT2 (BOXR1030) compared to Control GPC3 CAR-T cells. In this study, we have characterized the cellular phenotype and mitochondrial function of BOXR1030 T cells compared to Control CAR-T cells to better understand the contribution of GOT2 to improved CAR-T function.

Methods Phenotype and function of BOXR1030 and Control CAR-T cells (n=3 donors) were assessed at the end of standard CAR-T cell manufacturing process, after repetitive stimulation with anti-idiotype antibody (up to 5 stimulations over 15 days) and co-culturing with GPC3-expressing Hep3B cells (2D) or Hep3B spheroids (3D) in standard or low glucose culture conditions. T cell differentiation status was assessed by flow cytometry using CD45RA, CD45RO, CCR7, CD27, CD28 and CD95. Mitochondrial health was measured by analyzing mitochondrial mass, membrane potential and reactive oxygen species (ROS) generation. Cytotoxic function in terms of tumor cell killing and Granzyme B secretion in culture supernatant were measured by Incucyte and MSD respectively.

Results At the end of manufacturing, BOXR1030 T cells have a higher frequency of T stem cell memory (CD45RA+CCR7+CD27+CD95+) and T central memory (CD45RO+CCR7+CD27+) in CD8+ populations relative to Control CAR-T cells. BOXR1030 T cells also have a lower frequency of terminally differentiated CD27-CD28- T cells. Following in vitro stimulation or Hep3B co-culture in standard and low glucose culture conditions, BOXR1030 T cells showed preservation of early memory populations, reduced CD27-CD28- senescent cells and improved cytotoxic function relative to Control CAR-T cells. Since changes in mitochondrial function are associated with T cell differentiation and senescence, we explored mitochondrial health of BOXR1030 T cells in vitro. In low glucose culture conditions, BOXR1030 T cells showed reduced ROS levels and reduced loss of mitochondrial membrane potential compared to Control CAR-T cells.

Conclusions BOXR1030 CD8+ T cells contain a higher frequency of early memory cells with fewer terminally differentiated senescent cells and show improved mitochondrial health compared to Control CAR-T cells. These data suggest potentially increased fitness of BOXR1030 T cells enabling enhanced in vivo T cell persistence and antitumor activity. BOXR1030 is currently in Phase 1 trials to assess safety and preliminary efficacy (NCT05120271).