FluoroSpot) and cytotoxic activity (Delfia assay) have been assessed upon the co-culture with CD19+ or CD19- target cells. 

Results Enrichment of CD4+CAR+ T cells, besides CD8 +CAR+, were observed in UCB-CAR- vs. PBL-CAR-T cells (40–59% of positive cells; as well as of CD45RA+ cells (40–60 vs. 20–30% of positive cells; p<0.05). The preferential selection of early stage of differentiation (CCR7+CD28 +CD27+CD137+CD62L+) for CAR-T cells isolated from both source of lymphocytes occurred. LAG3 and TIM-3 expressing T cells were found with higher frequency in UCB- vs. PBL-CAR-T cells, with superior association with CD4+ UCB-derived cells. CD19-CAR-T cells secreted IFN-g(300–400 N, spot/10 × 104 T cells), regardless the co-stimulatory molecules (CD28z vs 4-1BBz), upon the engagement of CAR by CD19. A minority of IL-4 releasing T cells was found for few CAR-T cells activated with TransAct. IFN-gamma secreting CAR-T cells simultaneously released IL-2, Granzyme B and Perforin but not IL-5 and IL-17, thus belonging to TH-1/effector subset. The cytotoxic activity of these T cells against CD19+ target cells was also determined by europium release assay. Differential gene expression profile was determined in UCB-CAR-T vs. PBL-CAR-T cells bearing the different CARs following the co-culture with either CD19+ or CD19- target cells.

Conclusions The deep characterization of CD19-CAR-T cells contributed to validate the generation of anti-tumor ‘off-the-shelf’ CAR-T cells from UCB.

Ethics Approval The study was approved by Sidra Medicine Ethics Approval Board, approval number 1812044429.
**Background** Chimeric antigen receptor (CAR-T) cells are a promising new therapy for patients with cancer. However, in contrast to their success in B cell malignancies, CAR-T cells targeting solid cancers have had limited success so far due to their poor proliferation and poor long-term persistence in vivo. To address this issue, we used naïve T cells to generate second-generation CAR-T cells recognizing the tumor antigen Lewis Y (LeY), termed ‘early’ CAR-T cells.

**Methods** Purified naïve T cells were activated by CD3/CD28 soluble tetrameric antibody complex, retrovirally transduced (LeY scFv-CD3z-CD28 CAR) and expanded in IL-7/IL-15. The early LeY CAR-T cell function was tested in vitro for cytotoxicity (Cr-release and degranulation), proliferation, and cytokine secretion by CBA, either de novo or following chronic stimulation for 1 month. Finally, early CAR-T cell persistence and anti-tumor efficacy was assessed in the OVCAR3-NSG model, in the presence or absence of anti-PD-1.

**Results** The early-CAR-T cells comprised stem cell memory-like (CD95+, CD62L+, CD45RA+) and central memory phenotype (CD95+, CD62L+, CD45RA-) T cells with increased expression of ICOS, Ki67, TCF7 and CD27 (Figure 1). The early-CART cells retained potent antigen-specific cytotoxicity, and secreted significantly higher levels of cytokines (IFN-γ, TNF-α and IL-2) and increased proliferation compared to conventional CAR-T cells. Importantly, early-CAR-T cells had a significantly higher proliferative capacity after long-term chronic stimulation compared to conventional CAR-T cells (figure 2), and CD4+ CAR-T cells were critical for effective early CD8+ CAR-T cell proliferation capacity in vitro (figure 3). Early CAR-T cells had significantly better in vivo tumor control compared to conventional...
Abstract 126 Figure 5   Anti-PD1 treatment enhance the efficacy of the Early-CAR-T cells. A) Upregulation of PD-L1 on OVCAR3 when expanded in the supernatant from co-culture of OVCAR3 with LeY-CAR-T cells. B) Design of the in vivo experiment (n=7 mice per group). C) T-cell persistence, phenotype and anti-human IgG4 in peripheral blood were measured by FACS. D) Tumor kinetic of OVCAR-bearing NSG mice treated with Early-CAR-T cells or Early-CAR-T cells + Nivolumab

CAR-T cells (Figure 4), this was associated with increased CAR-T cell persistence. Because chronically stimulated early-LeY-CAR-T cells expressed PD-1 (figure 2), and OVCAR3 cells expressed PD-L1 when co-cultured with LeY-CAR-T cells (figure 5), we combined early LeY-CAR-T cells with anti-PD-1 therapy and observed complete tumor regression in these mice. Interestingly, early LeY-CAR-T cell plus anti-PD-1 treatment also enhanced the percentage of circulating stem-cell memory like CAR-T cells in vivo (figure 5).

Conclusions Our early CAR-T cells have better cytokine secretion and proliferation than conventional CAR-T cells. Early CAR-T cells also have superior anti-tumor efficacy in vivo, they have better persistence and maintain the circulating T cell memory pool. Importantly, low dose early-LeY-CAR-T cells combined with anti-PD1-treatment leads to complete clearance of LeY+ solid tumors in vivo. The early CAR-T cell production protocol is directly translatable for improving CAR-T cell efficacy in clinical trials for patients with solid tumors.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0126

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