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 (CD28 vs 4-1BB), 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/effecter 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’s Ethics Board, approval number 1812044429.

**Abstracts**

**125 REEXAMINATION OF MAGE-A3 AS A T-CELL THERAPEUTIC TARGET**

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**Background** Recurrent cancer-specific targets are rare. Given the pace of genomic research over the past three decades, few are likely to lie yet undiscovered. In 2013 an innovative MAGE-A3-directed cancer therapeutic of great potential value was terminated in the clinic because of neurotoxicity. The safety problems were hypothesized to originate from off-target TCR activity against a closely related MAGE-A12 peptide.

**Methods** A combination of published and new data led us to test this hypothesis with current technology, including RNA hybridization in situ and further analysis of the clinical TCR's specificity to MAGE-A12 and other antigens.

**Results** We find that a key prediction of the MAGE-A12 toxicity hypothesis, the existence of rare, high-MAGE-A12-expressing cells in the brain, is not supported by the data. Our results imply that an alternative related peptide from the EPS8L2 protein is more likely responsible for the toxicity. Therefore, it may be valuable to reconsider MAGE-A3 as a cancer target using HLA-A*02-restricted TCRs or CARs. As a step in this direction, we isolated MAGE-A3 pMHC-directed CARs, targeting the same peptide as the clinical TCR. These CARs have high selectivity, and avoid cross-reaction with the EPS8L2 peptide that represents a significant risk for MAGE-A3-targeted therapeutics.

**Conclusions** Given the qualities of MAGE-A3 as an onco-testis antigen widely expressed in tumors and largely absent from normal adult tissues, our findings suggest that MAGE-A3 may deserve further consideration as a cancer target. We have identified CARs with selectivity profiles consistent with a cell therapeutic directed against HLA-A*02-positive, MAGE-A3-expressing cancers. The relative merits of TCRs and CARs for this target will be discussed.

**REFERENCE**


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**126 EARLY-PHENOTYPE LEWIS Y CAR-T CELLS PERSIST BETTER IN VIVO AND INDUCE SOLID TUMOR REGRESSION IN COMBINATION WITH ANTI-PD1**

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**Abstract** Early-CAR-T protocol, including Naïve T cells purification and expansion in IL-7 and IL-15 promotes the maintenance of a TSCM and TCM phenotype. A) Scheme of the 7-day production protocol for Early-CAR-T cells. B) Phenotype by FACs of the Early-CAR-T cells and the Early-CD8+CAR-T cells. Pooled data in triplicate for 6 donors. C) Phenotype by Mass cytometry comparing the Conventional-CAR-T cells vs Early-CAR-T cells vs Early-CD8-CAR-T cells. Data for one donor representative of 3 different donors.

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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-CAR-T cells retained potent antigen-specific cytotoxicity, and secreted significantly higher levels of cytokines (IFN-α, 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 2 Early-CAR-T cells are comparable in vitro to conventional CAR-T cells in terms of killing but have a better proliferation capacity that persists after chronic stimulation. The long-term stimulated early- CAR-T cells maintain their memory phenotype and upregulated PD-1. A) Chromium release assay against the LeY+ cell line (OVCAR3), data for one donor representative of 3 other donors. B) Cytokine secretion evaluated by CBA after coculture with the LeY+ cell line (OVCAR3) or with the LeY- cell line (MDA-MB435). C) Division index of CAR-T cells quantified with CTV. D) Evaluation of the differentiation, proliferation and cytotoxicity of the CAR-T cells after chronic stimulation

Abstract 126 Figure 3 Early-CD4+ CAR-T cells are critical for the proliferation capacity of the Early-CD8+ CAR-T cells. A) Scheme of the CD4-depletion protocol to compare Early-CD8-CAR-T proliferation with or without CD4-T cells. B) Division index of CD4-depleted Early-CAR-T cells, CD8-T cells from bulk Early-CAR-T cells, and from CD4+ T cells from bulk Early-CAR-T cells quantified with CTV

Abstract 126 Figure 4 Early-CAR-T cells show in vivo a better persistence and a better proliferation capacity associated with a better tumoral control. A) Design of the in vivo experiment (n=7 mice per group) B) T-cell persistence in peripheral blood was measured by FACS. C) Spearman correlation (Day 13) between Tumor size and% CAR-T cells. D) Tumor kinetic and Kaplan-Meier analysis of survival of OVCAR-bearing NSG mice treated with Conventional CAR-T cells, or Early-CAR-T cells or Low-dose of Early-CAR-T cells
Background Natural killer (NK) cells are highly effective and fast-acting cytolytic cells capable of eradicating target cells with limited adverse effects such as cytokine release syndrome (CRS) or graft-versus-host disease. Chimeric antigen receptors (CARs)-engineered NK cells have been recently used against leukemia with encouraging clinical outcomes. The surface antigen CD19, expressed by B-lymphoblasts, represents an ideal CAR target against B cell acute lymphoblastic leukemia (B-ALL). We developed a highly potent CD19-directed CAR NK cell therapy, NKX019, with an extended in vivo half-life aimed at killing CD19-expressing target.

Methods NK cells isolated from healthy PBMCs were expanded in the presence of NKSTIM cells, IL-2, IL-12, IL-18 and transduced with both a CD19-targeted CAR construct and a membrane-bound form of IL-15 (mblL-15). Control (non-engineered) NK cells were produced in parallel. Cytotoxic activity of NKX019 against CD19+ B-ALL cell line (REH), pre-B ALL cell line (Nalm-6), allogeneic PBMCs was assessed using Incucyte® or flow cytometry. NSG mice bearing either Nalm-6,fluc (Nalm6) or REH,fluc (REH) tumor received different concentrations of NKX019 or control NK cells. In-life analysis of tumor-bearing and naïve NSG mice included: 1) bioluminescence imaging, 2) clinical observations, 3) serum cytokines and 4) CAR+ NK cell persistency.

Results NKX019 showed enhanced cytolytic activity against REH and Nalm-6 tumor cells compared to control NK cells and CAR19+ T cells. The superiority of NKX019 over CAR19+ cells was more pronounced at the earlier time point (24 hours) with near identical calculated EC50 observed at 72 hours for both cell types. Increased cytolytic activity of NKX019 was limited to CD19+ cells in bulk PBMCs. Consistent with our in vitro observations, NKX019 controlled Nalm-6 and REH tumor growth in doses as low as 2x106 cells/kg for up to 30 days with no apparent increase in cytokines commonly associated with CRS. Increased Nalm-6 tumor growth coincided with an apparent decrease in measurable NKX019 in the periphery. In tumor-naïve NSG mice, NKX019 was detectable in the blood for up to 9 weeks post-infusion consistent with its extended half-life.

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.

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PRECLINICAL EVALUATION OF NKX019, A CD19-TARGETING CAR NK CELL

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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 OVCAR-3 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.

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