and, upon binding, release a CD73-blocking scFv to inhibit the accumulation of extracellular ADO and mitigate immunosuppression of NK cells. Such localized response enhances specificity and reduces off-target effects of NK-based targeting.

**Results** Primary NK cells were successfully electroporated to express our synthetic TIGIT-synNotch construct, as evidenced by increased expression levels of TIGIT (% and MFI) (figure 1). To evaluate the functionality of engineered NK cells against GBM targets, we tested the cytotoxicity of our engineered NK cells against a primary, patient-derived GBM cell line, GBM43. Overall, cytolytic function of engineered NK cells against GBM was significantly higher than that of non-engineered NK cells, with or without CD73 (10 ug/mL) and TIGIT (50 ug/mL) antibodies, for E:T ratios of 5:1 and 10:1 (figure 2), demonstrating the functional efficacy of our genetic construct. Further, engineered NK cells (T-PNK) expressed significantly higher levels of CD107a in response to GBM43 stimulation than non-engineered PNK at E:T ratios 2.5:1 and 10:1 (figure 3).

**Conclusions** Overall, we have shown that co-targeting CD155 and CD73 in a localized, responsive manner can dampen immunosuppression and significantly enhance the killing potential of engineered NK cells against aggressive patient-derived GBM tumors.
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’s Ethics Board, approval number 1812044429.

**Reference**


**Abstract 126**

**Early-phenotype Lewis y CAR-T cell persist better in vivo and induce solid tumor regression in combination with anti-PD1**

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**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.