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 × 10^4 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/effectector 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.

**References**


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**Abstract 126**

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

**Authors**

Deborah Meyran*, 1Joe Zhu, 1Jeanne Butler, 1Sean Macdonald, 1Daniela Tantalo, 1Niko Thio, 1Kevin Sek, 2Paul Ekert, 1Michael Kershaw, 1Joe Trapani, 1Phillip Darcy, 1Paul Neeson, 1Peter MacCallum Cancer Centre, Melbourne, Australia; 2Children’s Cancer Institute, Sydney, Australia

**Conclusion** 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.

**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.