

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.

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

Aaron Martin*, Xueyin Wang, Han Xu, Alexander Kamb, Mark Sandberg, Kathleen Negri, Ming Wu, Dora Toledo Warshaviak, Grant Gabrelow, Michele Mcelvain, Mark Daris, Bella Lee. *A2 Biotherapeutics, Thousand Oaks, CA, USA*

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.¹ 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.

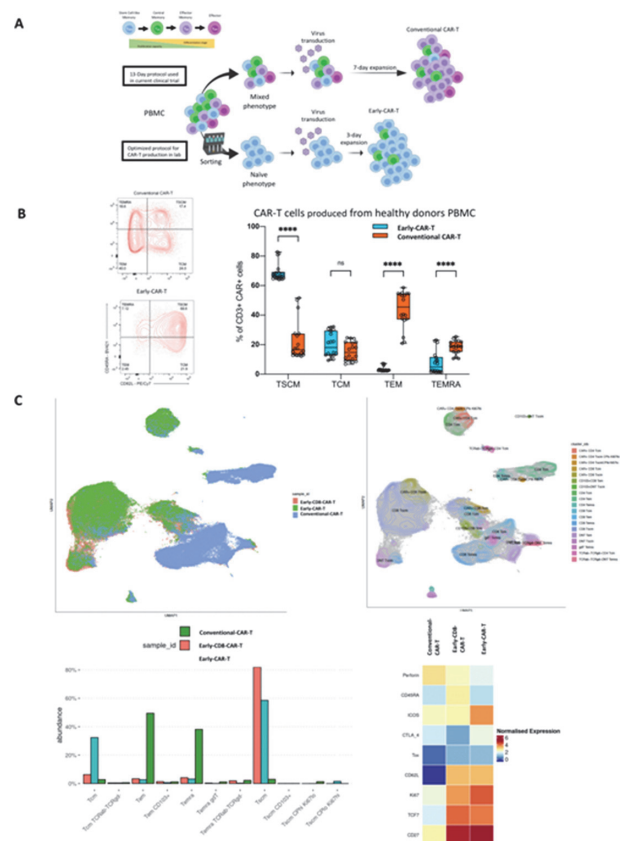
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126 EARLY-PHENOTYPE LEWIS Y CAR-T CELLS PERSIST BETTER IN VIVO AND INDUCE SOLID TUMOR REGRESSION IN COMBINATION WITH ANTI-PD1

¹Deborah 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



Abstract 126 Figure 1 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 conventional CAR-T cells and the Early-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