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 μg/mL) and TIGIT (50 μg/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.

**REFERENCES**


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0123

---

**124 OPTIMIZING THE GENERATION FROM UMBILICAL CORD BLOOD OF ‘OFF-THE-SHELF’ CD19-CHIMERIC ANTIGEN RECEPTOR (CAR) EXPRESSING T CELLS**

1Cristina Mascelli, 1Asma Al Sulaiti*, 1Moza Al Khulaifi, 1Mohammed El-Anbari, 1Mohammed Toufiq, 1Rebecca Mathew, 2Chiara Bonini, 1Monica Casucci, 1Chiara Cugno, 2Suruchi Mohan, 1Sara Deola, 2Damién Chassabbel, 1Sara Tomeli. 1Sidra Medicine, Doha, Qatar; 2San Raffaele Scientific Institute, Milan, Italy

**Background** T lymphocytes expressing CD19-chimeric antigen receptor (CAR) showed the improvement of overall survival of patients with B-cell malignancies. Allogeneic CAR-T cells can overcome the limitation of the availability of patient's lymphocytes, reducing the waiting time for the treatment and decreasing the cost of manufacturing. This study is aimed at the optimizing the generation of 'off-the-shelf' CAR-T cells utilizing Umbilical Cord Blood (UCB) to isolate T lymphocytes.

**Methods** UCBs have been collected at the time of childbirth from volunteer pregnant women at Sidra Medicine. Following the magnetic depletion of non-T cells, UCB-T lymphocytes were activated in vitro for 48 hr. by agonistic CD3/CD28 mAbs either conjugated to magnetic beads (Dynabeads) or to a colloidal polymeric nanomatrix (TranAct; Miltenyi Biotec). T cells generated in vitro were either i. untransduced (UT), or transduced with lentiviral encoding for ii. CD19-CD28z or iii. CD19-4-1BBz CARs. N=32 T cell cultures have been generated from fresh UCB (N=3) and, as control, from the peripheral blood lymphocytes of healthy donors (PBL; N=3) and used for deep phenotype analyses (28 markers) at different time points (Day +9 and Day+14) of the in vitro culture. Cytokines, perforin and granzyme B release (EliSpot or...
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+CD127+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-CART 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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0124

Abstract 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, 1Dania Tantalo, 1Niko Thio, 2Paul Ekert, 1Michael Kershaw, 1Joe Trapani, 1Phillip Darcy, 1Paul Neeson. 1Peter MacCallum Cancer Centre, Melbourne, Australia; 2Children’s Cancer Institute, Sydney, Australia

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 EP58L2 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 pMHIC-directed CARs, targeting the same peptide as the clinical TCR. These CARs have high selectivity, and avoid cross-reaction with the EP58L2 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


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0125

Abstract 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 EP58L2 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 pMHIC-directed CARs, targeting the same peptide as the clinical TCR. These CARs have high selectivity, and avoid cross-reaction with the EP58L2 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


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0124

Abstract 125 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 vs Early-CAR-T cells vs Early-CD8-CAR-T cells. Data for one donor representative of 3 different donors.