TARGETING PAN-TUMOR ASSOCIATED ANTIGEN B7H3 VIA COMBINATION OF TRI-SPECIFIC KILLER ENGAGER AND OFF-THE-SHELF NK CELL THERAPY ENHANCES SPECIFICITY AND FUNCTION AGAINST A BROAD RANGE OF SOLID TUMORS

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Background B7H3 is a tumor associated antigen (TAA), found on numerous malignancies including prostate, lung, and breast cancers. High levels of B7H3 expression are correlated with late stage disease and poor prognosis. Furthermore, B7H3 is minimally expressed on normal tissue, making it an ideal TAA for broad cancer treatment strategy. We developed a tri-specific killer engager (TriKETM) consisting of a nanobody anti-CD16, IL-15, and nanobody anti-B7H3 joined by flexible linkers (camB7H3 TriKE) (figure 1A). The combination of B7H3 TriKE with an off-the-shelf NK cell therapy presents an appealing therapeutic strategy for the treatment of solid tumors with decreased risk of toxicity in allogeneic settings compared to T-cell derived products.

Methods An anti-B7H3 nanobody was developed via biopanning and cloned into a TriKE vector. TriKE was produced in Expi293 cells and affinity purified using poly-His tag. NK cells were co-incubated with cell lines exhibiting a range of B7H3 expression and with 3nM of camelid B7H3 TriKE or control. We have previously derived NK cells expressing high affinity non-cleavable hncCD16, CD38 KO, and IL-15/IL-15R fusion from clonal master engineered iPSC lines. Engineered iNK cells were tested in conjunction with the TriKE. A repeated measures ANOVA was used for statistical comparisons as noted in figure legends.

Results Engineered iNK cells co-incubated with camB7H3 TriKE and C4-2 prostate cells significantly increased degranulation (CD107a) and cytokine production (IFN-gamma) compared to controls (figure 1B/C, P<0.05, n=3). camB7H3 TriKE directly bound C4-2 cells with an estimated EC50 of approximately 3nM. camB7H3 TriKE increased percentages of engineered iNK cells dividing robustly (3 or more times) compared to corresponding IL-15 doses at 3 nM (figure 1D, P<0.001, n=3). Furthermore, camB7H3 TriKE enhanced cytotoxic activity of engineered iNK cells against a variety of tumor cells in 2D and spheroid format independent of cytokine support (figure 1E-F). Engineered iNK cells incorporating an anti-B7H3 chimeric antigen receptor (CAR) is also being developed and will be discussed.

Conclusions camB7H3 TriKE dramatically increases function and activation on endogenous NK cells as well as engineered iNK cell, which can be adoptively transferred to patients with a broad range of cancers, including prostate cancer. TriKE port studies to combine ATRC-101 with agents targeting PD-1 in the clinical treatment of solid tissue malignancies.

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Trial Registration NCT04244552

Ethics Approval The study was approved by WIRB (Western Institutional Review Board) on Jun 11, 2013. The WIRB study number is 20130121.

REFERENCE

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Abstract 470 Figure 1 A) Schematic of TriKE molecule demonstrating spatial relationship of anti-CD16 nanobody, IL-15, and anti-B7H3 nanobody with flexible linker regions. B) Percent of PB NK cells transduced with CD107a+ as a marker of NK degranulation. Unselected PBMCs were stimulated with 3nM camB7H3 TriKE, 3nM IL-15, or no treatment with B7H3-expressing C4-2 prostate cancer cell lines Cells were stimulated for 5 hours and evaluated for degranulation (surface CD107a) by flow cytometry, gating on the NK (CD56+CD3-) population (displayed). Graphs display mean ± SEM. P<0.05 using repeated measures ANOVA. C) Percent of PB NK cells expressing intracellular interferon-gamma. Unselected PBMCs were stimulated with 3nM camB7H3 TriKE, 3nM IL-15, or no treatment with B7H3-expressing C4-2 prostate cancer cell lines. Cells were stimulated for 5 hours and evaluated for degranulation (surface CD107a) by flow cytometry, gating on the NK (CD56+CD3-) population (displayed). Graphs display mean ± SEM. P<0.05 using repeated measures ANOVA. D) Percent of PB NK cells dividing robustly (3 or more times) over a 7 day stimulation with 3nM camB7H3 TriKE, 3nM IL-15, or no treatment. PBMCs were incubated with Cell-Trace Violet reagent prior to stimulation. Graphs display mean ± SEM. P<0.001 using repeated measures ANOVA. E) Representative 2D IncuCyte images of PC3 prostate cancer cell lines transduced with NuLight Red. Cells were co-incubated with iNK alone, iNK with 3nM IL-15 or iNK with 3 nM anti-B7H3 TriKE. iNKS represent remaining NuLight Red transduced PC3 cells after 72 hours of co-incubation as noted above. F) Representative microsphere IncuCyte images of PC3 prostate cancer lines transduced with NuLight Red. Cells were co-incubated with iNK cells alone, iNK with 3 nM IL-15 or iNK with 3 nM anti-B7H3 TriKE. Images represent remaining NuLight Red transduced PC3 cells after 72 hours of co-incubation as noted above.
activity was potent across a broad concentration spectrum and corresponded directly with B7H3 target expression. These studies represent the proof-of-concept of a novel pairing of off-the-shelf, engineered iNK cells with B7H3-directed pancancer engager molecules (TriKEs and CARs) to enhance specificity, persistence and anti-tumor function.

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**471** PANCREATIC CANCER THERAPY BASED ON COMBINATION OF DNA VACCINATION AND PI3KGAMMA INHIBITION

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**Background** Pancreatic ductal adenocarcinoma (PDA) is the 4th leading cause of cancer mortality in developed countries, with one of the poorest prognoses among all cancers. Although 10–15% of patients are candidates for gross total surgical resection, recurrence is frequent, and the overall 5-year survival rate is around 8%. Using a proteomic approach, we have identified alpha-Enolase 1 (ENO1) as PDA-associated antigens. We have shown that ENO1 DNA vaccination efficiently prolongs survival of engineered mice that spontaneously develop PDA (both KC and KPC mice). Recently, we have demonstrated that PI3K gamma play a critical role in PDA by driving the recruitment of myeloid derived suppressor cells into tumor tissues and it’s genetic or pharmacologic inhibition effectively inhibits PDA progression and metastasis. In this study we assessed the hypothesis that targeting myeloid derived suppressor cells, via pharmacological PI3Kgamma inhibition, synergizes with ENO1 DNA vaccination by inducing a strong and sustained immune response.

**Methods** KPC mice were vaccinated 4 times with ENO1 starting at 4 weeks of age; 2 weeks after the last immunization mice were treated with the PI3Kgamma inhibitor TG100-115 (2.5 mg/kg), for further two weeks. At sacrifice neoplastic lesions, immune infiltrate, T and B cell response were analyzed.

**Results** Mice that received ENO1 and TG100-115 therapy showed a significant decrease in tumor size compared to both ENO1 and PBS treated mice. Moreover, the analysis of pancreas tissues indicated that combined therapy induced an increased number of CD8 and F4/80 cells and a decrease of FoxP3, CD31 and NG2 cells compared to control mice. In addition, we extract mRNA from formalin fixed paraffin embedded pancreas tissues of treated mice. We observed an increase of Granzyme B in both ENO1 and ENO1+TG100-115 and a down modulation of genes involved in fibroblast activity was potent across a broad concentration spectrum and corresponded directly with B7H3 target expression. These studies represent the proof-of-concept of a novel pairing of off-the-shelf, engineered iNK cells with B7H3-directed pancancer engager molecules (TriKEs and CARs) to enhance specificity, persistence and anti-tumor function.}

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**Ethics Approval** All animal experiments were approved by the University of Turin, Italian Ministry of Health and performed in accordance with EU laws in the animal facility of the Molecular Biotechnology Center (MBC). Reference no: 378/2015-PR and 597/2019-PR.

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**472** IMMUNOPET-INFORMED SEQUENCE FOR FOCUSED ULTRASOUND-TARGETED MCD47 BLOCKADE CONTROLS GLIOMA

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**Background** The natural disease course for glioblastoma (GB) entails invariably grim outcomes for patients. Phagocytic immunotherapies, such as CD47 blockade (e.g. mCD47), have recently demonstrated promise for GB therapy. However, their efficacy is challenged by presence of the blood brain and tumor barriers (BBB/BBT). Transient disruption of the BBB/BBT via focused ultrasound (FUS) and circulating microbubbles (MB) holds promise for improving therapeutic outcomes in the context of mCD47. However, critical questions regarding the optimal protocol for therapeutic antibody delivery with FUS remain. We herein leverage immuno-PET imaging to spatiotemporally map [89Zr]-mCD47 delivery across the BBB/BBT with FUS in an orthotopic GB model. We then use these insights to design a combinatorial paradigm for mCD47 delivery with repeat FUS BBB/BBT-D.

**Methods** MRI-guided FUS BBB/BBT-D was performed in the presence of systemically circulating MBs in mice with orthotopically implanted GL261 tumors. Mice received i.v. [89Zr]-mCD47 either without FUS, immediately prior to FUS [FUSPRE] or following FUS [FUSPOST]. Subsequently, mice underwent serial PET/CT imaging followed by terminal ex vivo assessment of antibody biodistribution. A therapeutic paradigm was then executed, wherein GL261-bearing mice received i.v. mCD47 (8 mg/kg) either as monotherapy or in combination with FUS BBB/BBT-D over three sessions spaced three days apart. Overall survival was monitored and tumor outgrowth was tracked via serial contrast-enhanced MRI.

**Results** Contrast-enhanced MRI confirmed BBB/BBT-D in GL261 tumors (figure 1A). However, PET/CT imaging revealed a lack of tumor-preferential [89Zr]-mCD47 uptake with or without FUSPRE, suggesting that neither condition improved antibody penetration over that in naïve brain (figure 1B-C). Remarkably, FUSPOST conferred superlative [89Zr]-mCD47 uptake at the site of BBB/BBT-D, boasting between 4.3- to 6.7-fold more uptake relative to other groups (figure 1C). This elevation in uptake was sustained over the time points assessed (0–72 hours post-FUS) (figure 1C-D). Using these insights, we evaluated a rational paradigm (figure 2A) combining mCD47 with repeat FUSPOST BBB/BBT-D (figure 2B-C) for glioma therapy. FUS-mediated delivery of mCD47 across the BBB/BBT significantly constrained tumor outgrowth (figure 2D-E) and enhanced survival (figure 2F) in GL261-bearing mice.