1180 MAXIMIZING NK CELL IMMUNOTHERAPY IN PROSTATE CANCER VIA TRIKES

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Background Natural killer (NK) cells are being leveraged in the clinic due to their safety profile and their ability to mediate tumor killing without prior priming. However, lack of antigen-specific targeting, decreased numbers, and suppressive signals derived from the tumor microenvironment (TME) of Prostate Cancer (PCa), can impact NK cell efficacy. To bypass this issue, we designed a novel tri-specific killer engager (TriKE®) molecule that consists of three parts: an arm that engages with CD16, an activating receptor of NK cells, an arm that binds to tumor antigens express in PCa (PSMA or B7H3), and an interleukin (IL)-15 moiety that is essential for NK cell survival, proliferation, priming and motility (figure 1A).

Methods TriKE molecules were generated in mammalian (Expi293) manufacturing systems. Peripheral Blood Mononuclear Cells (PBMCs) from normal donors or prostate cancer patients were either used directly or magnetically enriched for NK cells in assays co-culturing cells with PCa cell lines in the presence or absence of PSMA TriKE or B7H3 TriKE. Flow cytometry-based readouts evaluated NK cell activation and tumor killing while impedance and imaging assays were also used to dynamically measure tumor killing. Hypoxic (1% oxygen) culture condition and cytokine-induced myeloid-derived suppressor cells (MDSC) were also incorporated in some assays. Pre-clinical xenogeneic mouse models were also used to evaluate efficacy of TriKEs in vivo.

Result Normal donor and PCa patient NK cells display better, specific, degranulation against PCa cell lines in the presence of PSMA (figure 1B) or B7H3 TriKEs. NK cell cytotoxicity is also improved, even in the presence of enzalutamide resistant lines (figure 1C), hypoxia (figure 1D), or Myeloid Derived Suppressor Cells. Finally, the TriKE molecules display improved tumor control, compared to IL-15 control or no treatment, in xenogeneic models of prostate cancer.

Conclusions Our findings indicate that TriKE molecules improve PCa control in several systems and in the presence of varied TME-specific stresses. These pre-clinical studies highlight the potential for using TriKE molecules in the setting of metastatic Prostate Cancer and pave the way for future, targeted, NK cell immunotherapeutic interventions in this setting.

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Ethics Approval Peripheral blood mononuclear cells (PBMCs) from the blood of deidentified healthy donors were obtained, after participants gave informed consent, from Memorial Blood Centers (Minneapolis, Minnesota, USA) and used in compliance with the Committee on the Use of Human Subjects in Research (IRB# 9709 M00134) and in accordance with the Declaration of Helsinki. The in vivo mouse studies were conducted in accordance with the Institutional Animal Care and Use Committee at the University of Minnesota (IACUC# 1908–37330A).

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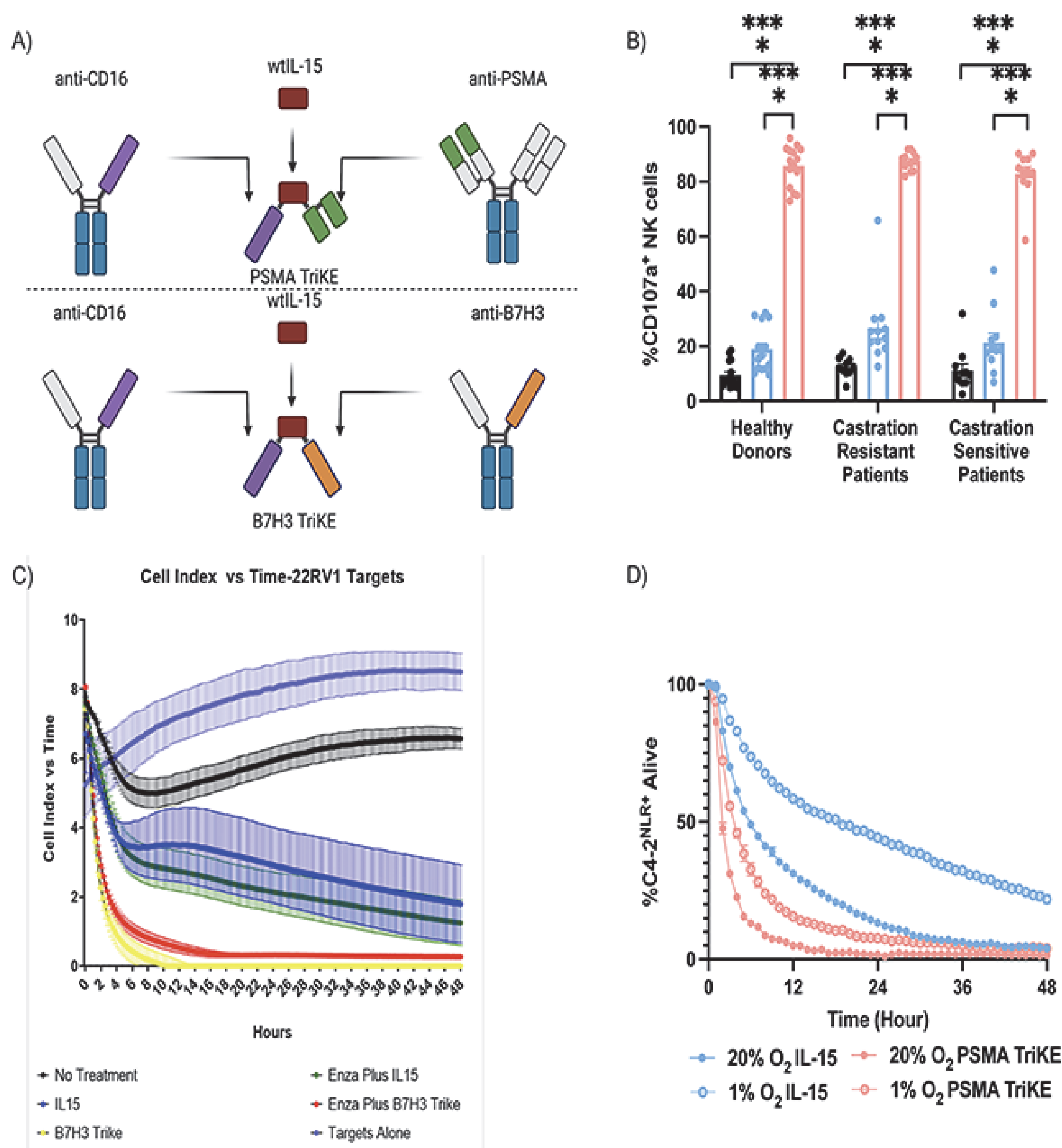

Abstract 1180 Figure 1 (A) Schematic of PSMA (top) and B7H3 (bottom) TriKE structures. (B) Pooled NK cell degranulation (measured by surface expression of CD107a) after 5 hours of co-culture of C4–2 WT with peripheral blood mononuclear cells (PBMC) derived from healthy donors, castration sensitive or castration resistant prostate cancer patients with no treatment (NT: Black), IL-15 (3nM:Blue) or PSMA TriKE (3nM:Red); N=11–15; mean ± SEM; Mixed effects analysis; Tukey's multiple comparisons test); **** $p < 0.0001$. (C) Xcelligence impedance-based cytolytic assay evaluating NK cells co-cultured with 22RV1 targets, alone or in the presence of enzalutamide and B7H3 TriKE or controls. (D) NK cells were negatively selected from fresh peripheral blood mononuclear cells (PBMC) and cultured with equifunctional doses of IL-15 (0.06nM) or PSMA TriKE (3nM) in a specialized incubator chamber with 20% or 1% oxygen (O2). Media is refreshed on day 4 during the culture. On Day 7, NK cells were harvested and used for experiments. Representative graph of live cell imaging assay showing NK cells killing NucLight Red (NLR)-expressing C4–2 at E:T ratio of 1:1 with NT or with equifunctional doses of IL-15 (0.06nM) or PSMA TriKE (3nM). Live cell count is normalized to targets alone control and time=0.

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