Targeting Pan-Tumor Associated Antigen B7H3 via Tri-specific Killer Engager Therapy Enhances Specificity and Function Against a Broad Range of Solid Tumors

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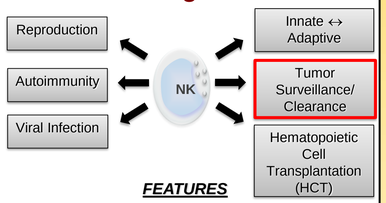
Introduction

- B7H3 (CD276)-is in the same family as PD-1/PD-L1 and has similar function
- Wide range of expression on solid/hematologic malignancies
- Higher B7H3 expression generally correlates with poor prognosis
- Important mediator of immune suppression in tumor microenvironment

Objective: Screen for single domain antibody engagers and discovered a unique sequence to be used in TriKE platform to treat B7H3+ tumors.

NK Cell Background



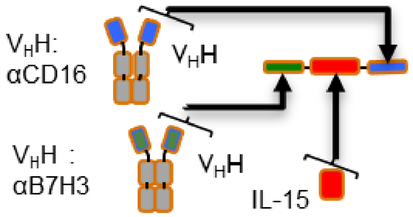
FEATURES

- ~5-15% of circulating lymphocytes
- Not MHC restricted
- No clonotypic receptors
- Don't require priming
  - Contain preformed granules
- Contain broad activating and inhibitory receptors

Methods

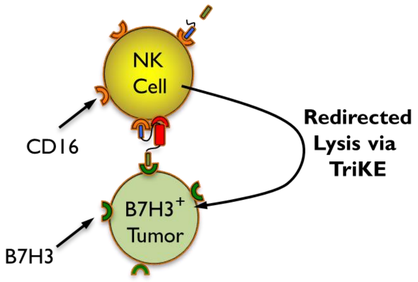
An anti-B7H3 nanobody was developed via biopanning and cloned into a TriKE vector. TriKE was produced in Expi293 cells and affinity purified using poly-His tag. NK cells were co-incubated with B7H3 expression and with 3nM of camelid B7H3 Trike or control. A repeated measures ANOVA was used for statistical comparisons as noted in figure legends.

TriKE Structure



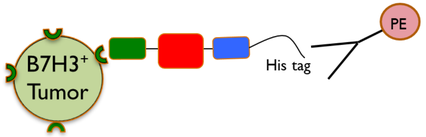
Single chain variable fragments from camelid nanobodies (cam) targeting CD16 (blue) and B7H3 (green) joined by IL-15 (red) and two flexible linker regions to form a single peptide with molecular weight of ~46 kDa.

TriKE Function



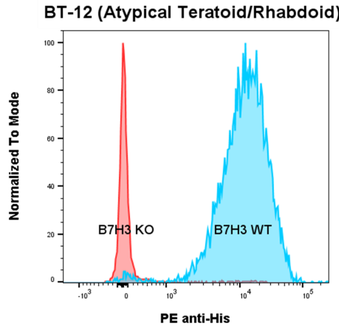
NK cell-mediated target lysis is directed towards B7H3-expressing tumor cells via formation of a direct physical link by the TriKE. IL-15 then stimulates the NK cell, inducing activation and proliferation.

Staining Schema for TriKE Binding Assays



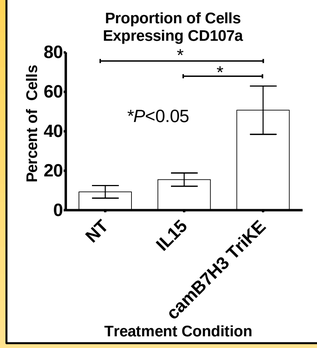
In order to isolate, purify, and assess binding of the TriKE to target cells, a poly-histidine (10X-His) tag was added to the 3' end of the TriKE. Using nickel columns which bind 10X His, the TriKE can then be purified. Using an anti-His antibody conjugated to a fluorochrome, binding specificity can be determined by flow cytometry.

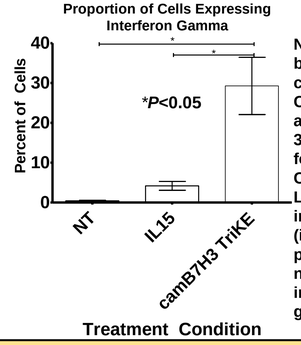
camB7H3 TriKE Demonstrates Specific Binding



BT-12 pediatric brain tumor lines highly express B7H3 (WT, blue)). A B7H3 KO BT-12 (red) cell line was produced using CRISPR (Theruvath *et al*). Similar specificity was noted using Raji (negative B7H3) and prostate cancer cell lines C4-2 (positive B7H3) and multiple other lines.

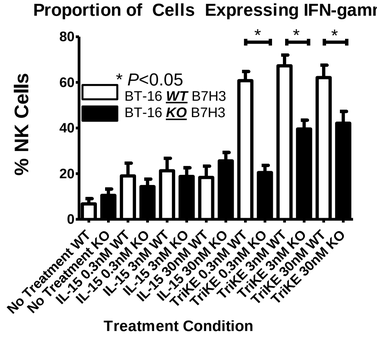
camB7-H3 Activates NK Cells in Presence of B7-H3+ Targets

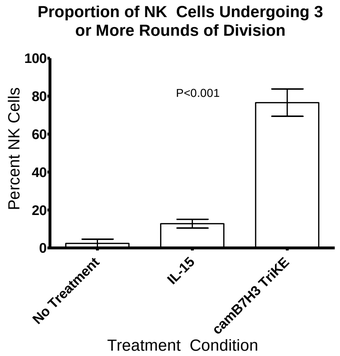




Normal donor peripheral blood (PB) NK cells were co-incubated with B7H3+ C4-2 prostate cancer cells and either no treatment, 3nM IL-15, or 3nM camB7H3 for 4 hours then stained for CD107a (degranulation, LEFT)) or intracellular interferon gamma (inflammatory cytokine production, RIGHT). B7H3 negative cells did not induce CD107a or IFN-gamma production.

camB7-H3 TriKE Demonstrates Specificity and Activity at a 100X Range of Concentrations and Induces Proliferation of NK Cells





(LEFT) Functional assays were conducted with BT-16 WT and BT-16 CRISPR KO cells and either 0.3nM, 3nM, or 30nM of camB7H3 TriKE. Significantly increased killing of WT cells occurred at all 3 doses. (RIGHT) Human PB NK cells were incubated with CellTrace Violet and either no treatment, 3nM IL-15 or 3nM camB7H3 TriKE for 1 week and analyzed for dye dilution (representing cell division).

Results

NK cells co-incubated with camB7H3 TriKE and C4-2 prostate cells significantly increased degranulation (CD107a) and cytokine production (IFN-gamma) compared to controls (P<0.05, n=3). camB7H3 TriKE directly bound C4-2 cells with an estimated EC50 of approximately 3nM. camB7H3 TriKE increased percentages of NK cells dividing robustly (3 or more times) compared to corresponding IL-15 doses at 3 nM (P<0.001, n=3).

Conclusions

camB7H3 TriKE dramatically increases function and activation on endogenous NK in patients with a broad range of cancers, including prostate cancer. TriKE activity was potent across a broad concentration spectrum and corresponded directly with B7H3 target expression.

References

1. Miller J, Zorko N, Kodal B, Davis Z, Lenvik A, Lenvik T, et al. 470 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. Journal for ImmunoTherapy of Cancer. 2020;8(Suppl 3):A287-A8.
1. Theruvath J, Sotillo E, Mount CW, Graef CM, Delaidelli A, Heitzeneder S, et al. Locoregionally administered B7-H3-targeted CAR T cells for treatment of atypical teratoid/rhabdoid tumors. Nat Med. 2020;26(5):712-9.

Disclosures

Disclosures: Vallera, Felices and Miller receive research support and stock and, with the University of Minnesota, are shared owners of the TriKE technology licensed by the University to GT Biopharma, Inc. This relationship has been reviewed and managed by the University of Minnesota in accordance with its conflict of interest policies.
Miller receives research funding and consultancy from Fate Therapeutics. Cichocki receives research funding from Fate Therapeutics.
Gaidarova, Garcia, Lee, Bjordahl, and Valamehr are employed by Fate Therapeutics.