#437: Novel B7-H3 Targeting Dual-Nanobody NK Cell Engagers Display Robust Activity Against a Broad Spectrum of Solid and Hematologic Malignancies

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Abstract

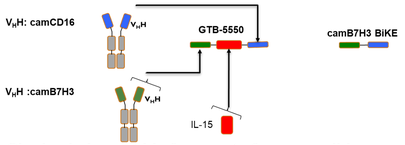
B7-H3 is a checkpoint molecule under intense investigation as an immune therapy target. We previously showed that dual camelid nanobody tri-specific killer engager (TriKE) (GTB-5550) specifically bound B7-H3 on PC3/C4-2 prostate cancer (PCa) cells and activated peripheral blood (PB) NK cells. We have since developed a dual camelid bispecific killer engager (BiKE) targeting B7-H3 and show that both GTB-5550, which harbors wild-type IL-15, and BiKE display broad activity against B7-H3-expressing tumors. Compared to monomeric IL-15, GTB-5550 shows better CD16-dependent metabolic activation of NK cells.

BiKE and GTB-5550 were manufactured in a mammalian expression system and purified from supernatants. We examined a variety of cell lines including PCa cells harboring enzalutamide resistance with divergent mechanisms including 22RV1 (androgen ligand-independent AR-V7 splice variant), a spontaneously resistant LNCaP model (AR hyper activation), as well as a CREB5 overexpressing (epithelial to mesenchymal transition) LNCaP model. These were used to evaluate how the BiKE and GTB-3550 induce NK cell degranulation (CD107a) and interferon gamma production. Metabolic stimulation was measured in NK-92 cell lines.

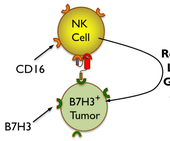
PB NK cells were robustly activated, compared to controls, when treated with GTB-5550 or BiKE and cultured with enzalutamide resistant PCa, osteosarcoma (U2OS, SaOS2), rhabdomyosarcoma (RH30), ovarian carcinoma (MA148, OVCAR8), AML (MV4;11, THP-1) and multiple myeloma (MM1S) cell lines. GTB-5550 was approximately 2x more potent than NCI IL-15 in terms of metabolic stimulation of CD16+ NK-92 cells, but not CD16- NK-92 cells. Spheroid killing assays and deeper metabolic analysis is in progress.

Our data shows that the novel dual camelid nanobody BiKE and GTB-5550 induce NK cell activation against a broad spectrum of tumors expressing B7-H3. Furthermore, B7-H3 is expressed at high levels on prostate cancer cell lines demonstrating enzalutamide resistance, thus inducing efficient targeting of these therapy PCa refractory lines. This B7-H3 targeting NK platform demonstrates broad translational potential. GMP production of GTB-5550 has been initiated.

GTB-5550/BiKE Structure and Function

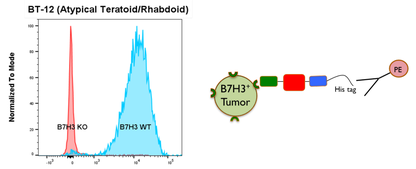


Single chain variable fragments from camelid nanobodies (cam) targeting CD16 (blue) and B7H3 (green) joined by IL-15 (red) and two flexible linker regions to form a single peptide with molecular weight of ~46 kDa. BiKE consists of camCD16 and camB7H3 with a single flexible linker region to form a single peptide of approximately 35kDa.



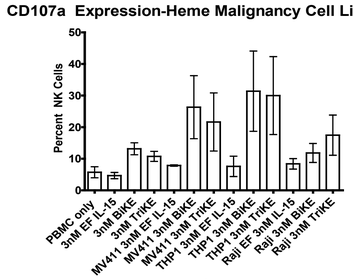
NK cell-mediated target lysis is directed towards B7H3-expressing tumor cells via formation of a direct physical link by the GTB-5550 or BiKE. IL-15 then stimulates the NK cell, inducing activation and proliferation.

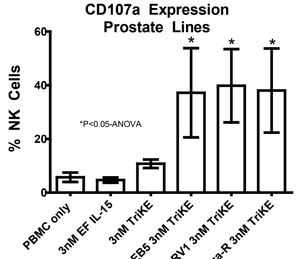
GTB-5550 and BiKE Demonstrate Specific Binding

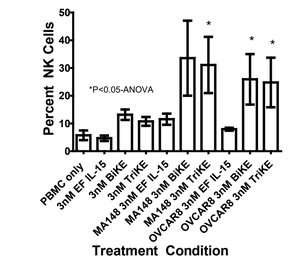


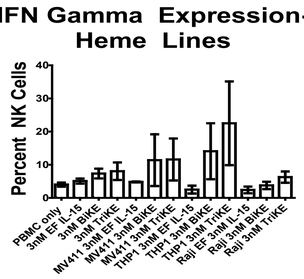
BT-12 pediatric brain tumor lines highly express B7H3 (WT, blue)). A B7H3 KO BT-12 (red) cell line was produced using CRISPR (Theruvath *et al*). Similar specificity was noted using Raji (negative B7H3) and prostate cancer cell lines C4-2 (positive B7H3) and multiple other lines. B7H3 BiKE had similar binding with positive and negative cell lines (Data not shown).

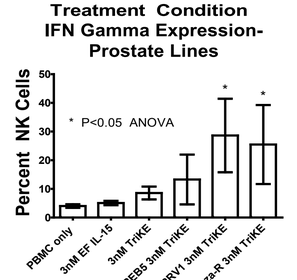
GTB-5550 and BiKE Robustly Activate NK Effector Cells Against B7H3+ Tumor Cell Lines

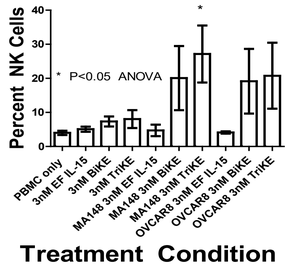


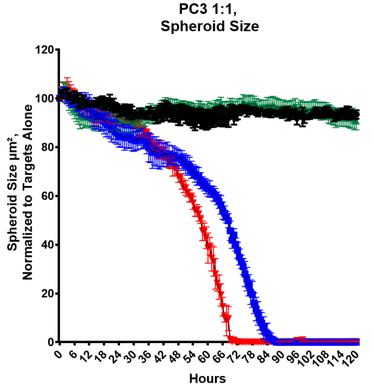


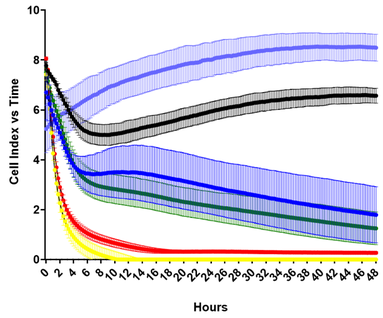






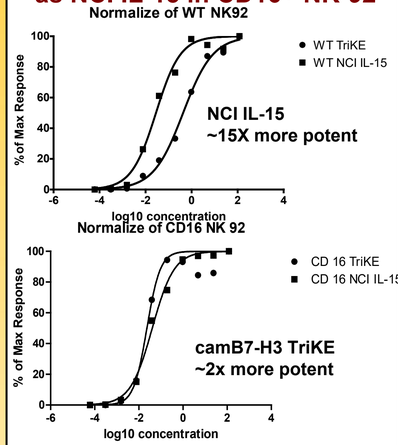






Functional assays were conducted with various ovarian, prostate, and hematologic malignancy cell lines with varying levels of B7H3 expression from none to very high levels. NK cell activation was measured using CD107a and IFN gamma as measured by flow cytometry (n=3), IncuCyte, or xCelligence assay.

GTB-5550 is Twice as Potent as NCI IL-15 in CD16+ NK-92



NK-92 cells without or with CD16 were incubated for 48 hours with dilutions of NCI IL-15 and GTB-5550. Metabolic activity was then measured using resazurin (n=4)

References

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2. 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: 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 GT Biopharma.
Berk is a Board Member and employee of GT Biophrama.