5131: B7H3-Targeted Tri-specific Killer Engagers deliver IL-15 to NK cells but not T cells, and specifically targets solid tumors as a pan-tumor antigen strategy mediated through NK cells

J. Miller[1], N. Zorko[1], A. Merino[1], G. Phung[1], M. Khaw[1], P. Howard[1], H. Hamsher[1], Z. Davis[1], F. Cichocki[1], G. Berk[2], M. Felices[1]

[1]Medicine, Masonic Cancer Center - University of Minnesota, Minneapolis, MN, United States of America, [2]GT Biopharma, Brisbane, CA, United States of America

Abstract

Background

IL-15, the homeostatic factor for NK cells is being clinically developed but it has little antitumor activity alone. We hypothesized that targeted delivery of IL-15 to NK cells along with ADCC would impart NK cells with specificity to tumor antigens. As proof of concept, a clinical trial of GTB-3550 (a CD33- targeted Tri-specific Killer Engager [TriKE] in AML) induced endogenous NK cell expansion and activation in refractory AML patients. Here we developed GTB-5550 (a B7H3 TriKE) as a novel dual camelid (cam) TriKE containing WT IL-15 and comprised of two cam engagers: targeting CD16 on NK cells and B7H3 on multiple solid tumors.

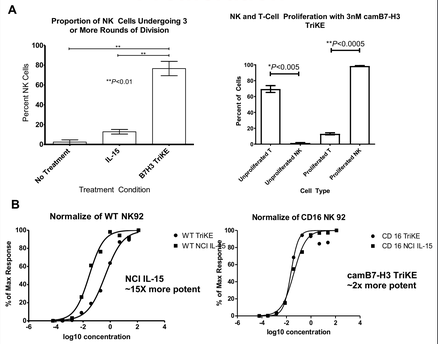
Methods

The IL-15 activity of the B7H3 TriKE was measured in proliferation assays. Tumors were incubated with NK cells with or without B7H3 TriKE. In some tumors, CRISPR was used to knockout B7H3 to serve as a specificity control as well as B7H3 negative hematologic tumors. NK cell function was measured by flow cytometry and in live tumor imaging assays.

Results

B7H3 TriKE was titrated onto lymphocytes resulting in a dose-dependent proliferation of NK cells but not T cells. This was in marked contrast to rhIL-15, that stimulated both suggesting different biologic activity of IL-15 when delivered through the camCD16 engager. camB7H3 was broadly expressed on prostate, head and neck, ovarian and glioblastoma cancers as well as multiple myeloma. We observed a B7H3 TriKE dose-dependent increase in CD107a degranulation and inflammatory cytokines to all B7H3 positive targets that was highly specific, with no response seen with B7H3 negative hematologic targets and control lines created with a CRISPR KO of B7H3. Compared to rhIL-15, GTB-5550 given at molar equivalent dosing induced B7H3 killing in in a dose-dependent manner above that seen with rhIL-15 induced natural cytotoxicity. In vivo activity in xenogeneic models of human tumor is underway and already validating our in vitro studies.

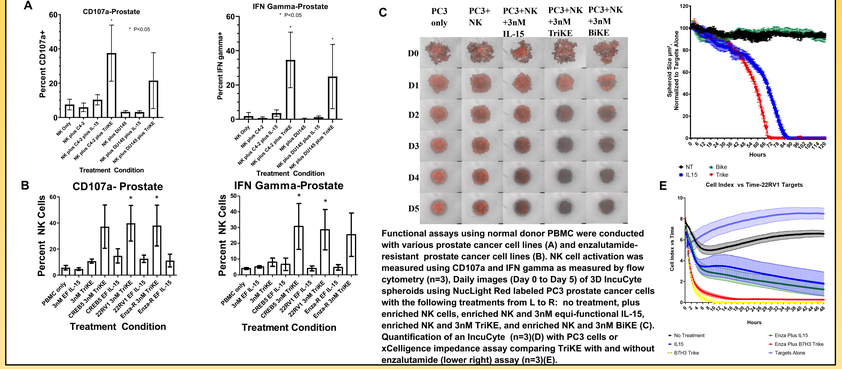
GTB-5550 Promotes NK-Specific Proliferation



PBMCs were pulsed with CellTrace Violet and then incubated for 7 days with 3nM GTB-5550 or NCI IL-15. Cells were analyzed by flow cytometry for dye dilution indicating proliferation and CD3/CD56 expression (A). NK-92 cells without or with wildtype CD16 were incubated for 48 hours with dilutions of NCI IL-15 and camB7-H3 TriKE. Metabolic activity was then measured using resazurin (n=4) to determine EC50 of GTB-5550 versus NCI IL-15 (B).

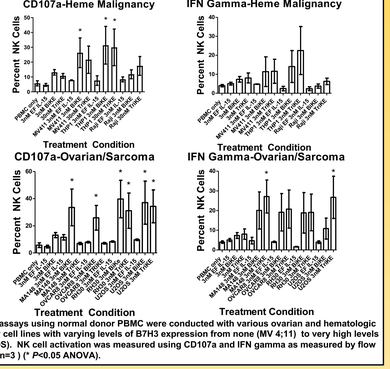
GTB-5550 Robustly Target and Kill A Broad Spectrum of B7-H3+ Cancer Cell Targets

GTB-5550 Targets Prostate Cancer Cells with and without Enzalutamide Resistance



Functional assays using normal donor PBMC were conducted with various prostate cancer cell lines (A) and enzalutamide-resistant prostate cancer cell lines (B). NK cell activation was measured using CD107a and IFN gamma as measured by flow cytometry (n=3), Daily images (Day 0 to Day 5) of 3D IncuCyte spheroids using NucLight Red labeled PC3 prostate cancer cells with the following treatments from L to R: no treatment, plus enriched NK cells, enriched NK and 3nM equi-functional IL-15, enriched NK and 3nM TriKE, and enriched NK and 3nM BiKE (C). Quantification of an IncuCyte (n=3)(D) with PC3 cells or xCelligence impedance assay comparing TriKE with and without enzalutamide (lower right) assay (n=3)(E).

GTB-5550 Targets Sarcoma, Ovarian and AML Subsets

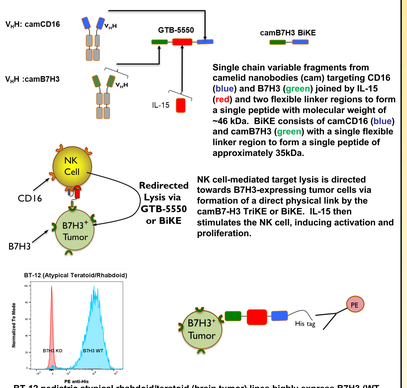


Functional assays using normal donor PBMC were conducted with various ovarian and hematologic malignancy cell lines with varying levels of B7H3 expression from none (MV 4;11) to very high levels (RH30, U2OS). NK cell activation was measured using CD107a and IFN gamma as measured by flow cytometry (n=3) (* $P<0.05$ ANOVA).

Conclusions

- GTB-5550 targets B7-H3 and redirects NK cell function.
- GTB-5550 gives a robust and NK cell specific proliferation signal compared to IL-15 alone.
- GTB-5550 specifically targets B7-H3+ cells.
- GTB-5550 effectively induced NK cell degranulation and interferon gamma production in response to various prostate, brain tumor (atypical rhabdoid/teratoid), HNSCC, multiple myeloma, sarcoma, ovarian, and myeloid malignancies.
- GTB-5550 efficiently kills multiple B7-H3+ solid and hematologic malignancies.
- Clinical manufacturing is underway with an IND planned to open clinical trials in 2023 in a number of solid tumors and multiple myeloma.

GTB-5550 Structure and Binding

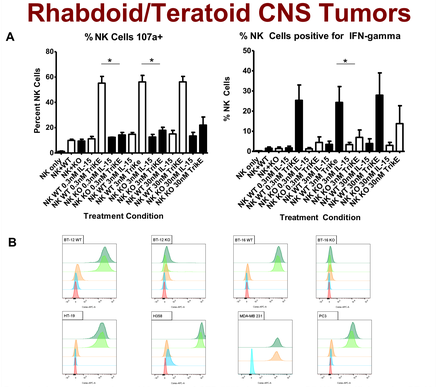


Single chain variable fragments from camelid nanobodies (cam) targeting CD16 (blue) and B7H3 (green) joined by IL-15 (red) and two flexible linker regions to form a single peptide with molecular weight of ~46 kDa. BiKE consists of camCD16 (blue) and camB7H3 (green) with a single flexible linker region 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 camB7-H3 TriKE or BiKE. IL-15 then stimulates the NK cell, inducing activation and proliferation.

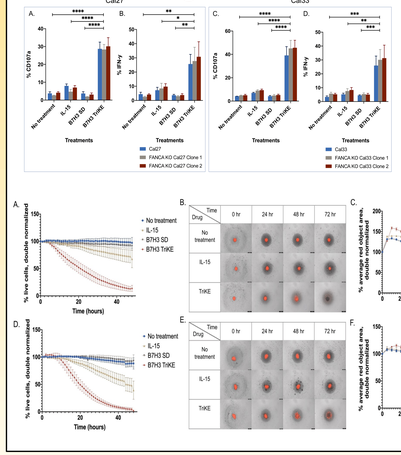
BT-12 pediatric atypical rhabdoid/teratoid (brain tumor) lines highly express B7H3 (WT, blue). A B7H3 KO BT-12 (red) cell line was produced using CRISPR (Theruvath *et al*). Similar specificity was noted using Raji (negative B7H3) and prostate cancer cell lines C4-2 (positive B7H3) and multiple other lines. B7H3 BiKE had similar binding with positive and negative cell lines (Data not shown). Binding assay schema using anti-His antibody to 10X HIS tag on GTB-5550.

GTB-5550 Targets B7-H3+ Atypical Rhabdoid/Teratoid CNS Tumors



Functional assay with using a 100x range of GTB-5550 demonstrates B7-H3 specificity using BT-16 atypical rhabdoid/teratoid CNS tumor cells +/- CRISPR KO (A) .Single-domain camelid nanobody with a 10x His tag was incubated with target cell lines and then co-stained with anti-His APC or equimolar (16.67nM) commercial mAb B7-H3 APC (clone 7-157), isotype, anti-His APC alone, or unstained cells (B). BT-12 and BT-16 B7-H3 WT and KO cells were a gift from Dr. Crystal Mackall.

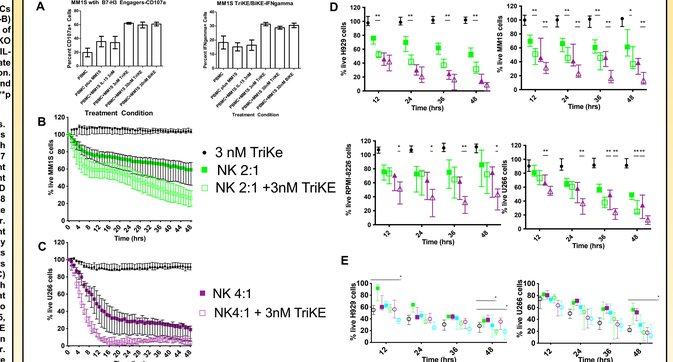
GTB-5550 Targets Fanconi Anemia Patient HNSCC



Functional validation of B7H3 TriKE. Frozen PBMCs (N=3) from healthy donors were incubated with (A-B) Cal27 trio and (B-C) Cal33 trio (each trio consisting of a HNSCC WT line and 2 clones of HNSCC FANCA KO lines) in different treatments: no treatment or 3 nM IL-15, MOPC, B7H3 SD and B7H3 TriKE to evaluate CD107a expression (intracellular IFN-γ production. Error bars indicate standard error of mean, and statistical significance was determined as *p < .05, **p < .01, ***p < .001 and ****p < .0001.

Real-time imaging assays. (A) Enriched NK cells (N=4) were incubated with Nuclight red-labeled Cal27 at an E:T of 5:1 in different conditions: no treatment or 3 nM IL-15, B7H3 SD and B7H3 TriKE for 48 hours in an IncuCyte Zoom imager. Quantifications of percent live cells were done by normalizing hourly counts of red cells to targets alone at t=0. (B-C) Spheroids incubated with enriched NK cells (N=4) at an E:T of 5:1 in: no treatment or 3 nM IL-15, B7H3 SD and B7H3 TriKE for 96 hours in an IncuCyte S3 imager. Representative images are shown. Quantifications of percent average red object area (live cells) were done by normalizing hourly counts of average red object area to targets alone at t=0. (D-F)

GTB-5550 Targets Multiple Myeloma Cells



Functional assays using normal donor PBMC were conducted with MM1S multiple myeloma cell lines. NK cell activation was measured using CD107a and IFN gamma as measured by flow cytometry (n=3)(A). We compared the ability of peripheral blood NK cells with or without B7-H3-TriKE to kill myeloma cells in live imaging IncuCyte Zoom assays with escalating doses of TriKE. Maximal killing occurred with 3 nM concentration and there was statistically significant increase in NK cell mediated killing of all four myeloma lines when 3nM B7-H3-TriKE was added (B, C). We also tested B7-H3-TriKE with the proteasome inhibitor bortezomib (10nM), the immunomodulatory drug lenalidomide (5µM), or the anti-CD38 antibody daratumumab (10 µg/mL). Cytotoxicity curves were performed in triplicate. Combination therapy with B7-H3-TriKE, NK cells, and lenalidomide showed significantly enhanced killing of H929 cells after 48 hours of live cell imaging. Combination therapy with B7-H3-TriKE, NK cells, and daratumumab showed statistically enhanced killing of H929, RPMI-8226, and U266 at various time points compared to B7-H3 TriKE and NK cells alone.

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.
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.
3. Zorko N, Felices M, Merino A, Walker JS, Kodal B, Lenvik A, et al Ann Volume 32, Supplement 7,Pages S1373-S1464 (December 2021).

Disclosures

Disclosures: M. Felices and J.S. 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. G. Berk is an employee of GT Biopharma. The remaining authors have no disclosures.

This work was supported by R35 CA197292 and NCI CA111412 (to M. Felices and J.S. Miller) and the Department of Defense W81XWH-20-1-0659 (M. Felices). N. Zorko is supported by The Randy Shaver Cancer Research and Community Foundation, University of Minnesota Academic Investment Education Program (AIEP) and a Department of Defense Early Investigator Award (W81XWH-22-1-0242). NAZ was previously supported by the Hematology Training grant (5T32HL007062-45, PI Vercellotti) at the University of Minnesota while conducting these studies.

NCI Designated Comprehensive Cancer Center

University of Minnesota Driven to Discover℠