#3435: GTB-5550 (cam16-IL15-camB7H3) Tri-specific Killer Engager (TriKE®) Drives NK Cell Activation and ADCC against Head and Neck Squamous Cell Carcinomas

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Abstract

Worldwide, Head and Neck Squamous Cell Carcinomas (HNSCC) account for about 900,000 cases and 400,000 deaths. In some settings, like Fanconi anemia (FA), patients receive curative treatments (allogeneic stem cell transplantation), only to develop HNSCC in early adulthood at a high rate of incidence. Current treatment strategies for non-FA HNSCC patients include surgery, chemotherapy and radiotherapy. However, these are not viable treatment options for FA HNSCC patients due to their low tolerance for the high toxicity levels of chemotherapy and radiation. Therefore, there is a critical need for novel and targeted therapeutic interventions for the treatment of FA HNSCC patients.

B7H3, a checkpoint member of the B7 and CD28 families, is overexpressed on several solid tumors but is absent or not expressed on healthy tissues. It is a promising target for immunotherapy, and recent basket trials, particularly in prostate cancer, have demonstrated strong clinical signals. Here we developed and tested the ability of GTB-5550, a tri-specific killer engager (TriKE) that includes a B7H3 targeting component, to direct NK cell killing to B7H3-expressing Head and Neck cancer targets. This TriKE molecule includes an NK cell engaging domain containing a humanized camelid nanobody against CD16, a camelid nanobody against B7H3 and a wild type IL-15 sequence between the two engagers. We assessed B7H3 expression by flow cytometry of wild-type HNSCC cells and a paired version with a CRISPER KO of the FANCA gene and determined that the KO had no effect on B7H3 expression. Thus, GTB-5550 activity against HNSCC should be present on both normal HNSCC and FA-HNSCC settings.

NK cell responses against HNSCC lines in the presence of GTB-5550 were assessed through either flow cytometry based functional assays, to evaluate NK cell degranulation and cytokine secretion, or IncuCyte imaging assays, to directly assess target killing. NK cell degranulation and IFN-gamma production of GTB-3550-treated samples were higher compared to that of control samples treated with B7H3 single domain or IL-15 alone. GTB-5550 also induced more HNSCC target cell killing by NK cells compared to treatment with the B7H3 single domain or IL-15 alone irrespective of the FANCA gene, both in 2D and 3D IncuCyte imaging assays. Ongoing experiments will evaluate the functionality and efficacy of GTB-5550 in vivo. Taken together, this data shows that GTB-5550 is able to drive NK cell activity against B7H3-expressing HNSCC cells, which presents potential for a B7H3-targeted TriKE to be used to be implemented clinically to treat HNSCC or FA-HNSCC patients.

HNSCC cell lines do not induce NK cell cytolytic function without treatment

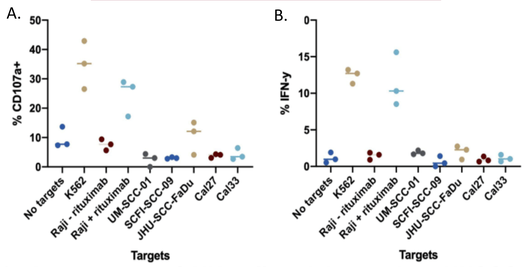


Figure 1: Assessment of NK cell activity against HNSCC without treatment. Frozen PBMCs (N=3) from healthy donors were incubated for 5 hours with 5 HNSCC cell lines: UM-SCC-01, SFCI-SCC-07, JHU-SCC-FaDu, Cal27 and Cal33 to evaluate **(A)** CD107a expression (as a marker for degranulation) and **(B)** intracellular IFN-γ production.

B7H3 is highly expressed on HNSCC but not on healthy immune cells

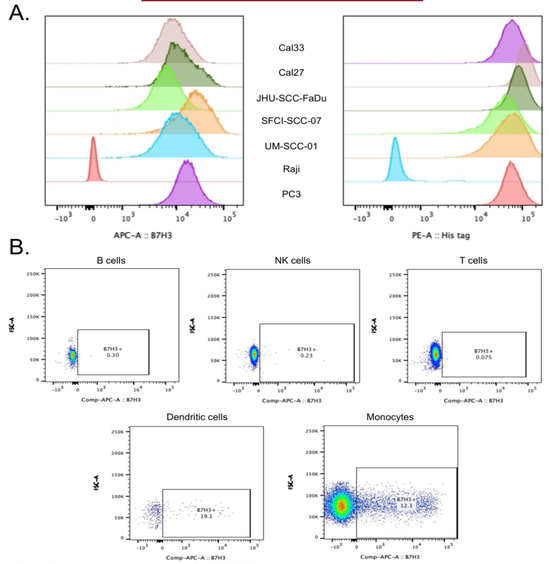
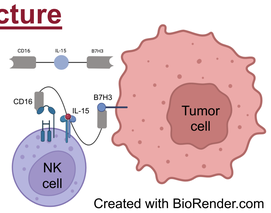


Figure 2: Assessment of B7H3 expression on tumor and immune cells. (**A**) 5 HNSCC cell lines were assessed for B7H3 expression and binding affinity with B7H3 single domain via flow cytometry. (**B**) PBMCs from a healthy donor were assessed for B7H3 expression by flow cytometry.

GTB-5550 structure

Figure 3: **Schematic of GTB-5550 TriKE molecule.** GTB-5550 TriKE consists of a humanized camelid nanobody against CD16, a camelid nanobody against B7H3 and a wild type IL-15 sequence between the two engagers, and it functions by bridging the NK and tumor cells.



GTB-5550 induces NK cell activity against HNSCC

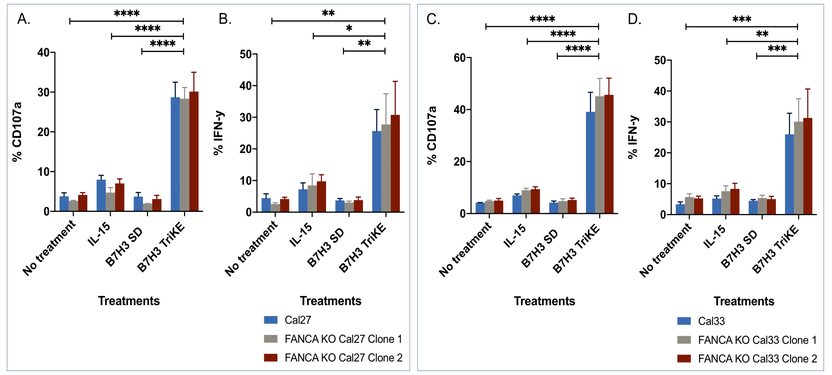


Figure 4: Functional validation of B7H3 TriKE. Frozen PBMCs (N=3) from healthy donors were incubated for 5 hours with **(A-B)** Cal27 trio and **(B-C)** Cal33 trio (each trio consisting of a HNSCC WT line and 2 clones of HNSCC FANCA KO lines) in different treatments: no treatment or 3 nM IL-15, MOPC, B7H3 SD and B7H3 TriKE to evaluate CD107a expression (as a marker for degranulation) and 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.

GTB-5550 induces NK cell killing against HNSCC in real-time imaging assays

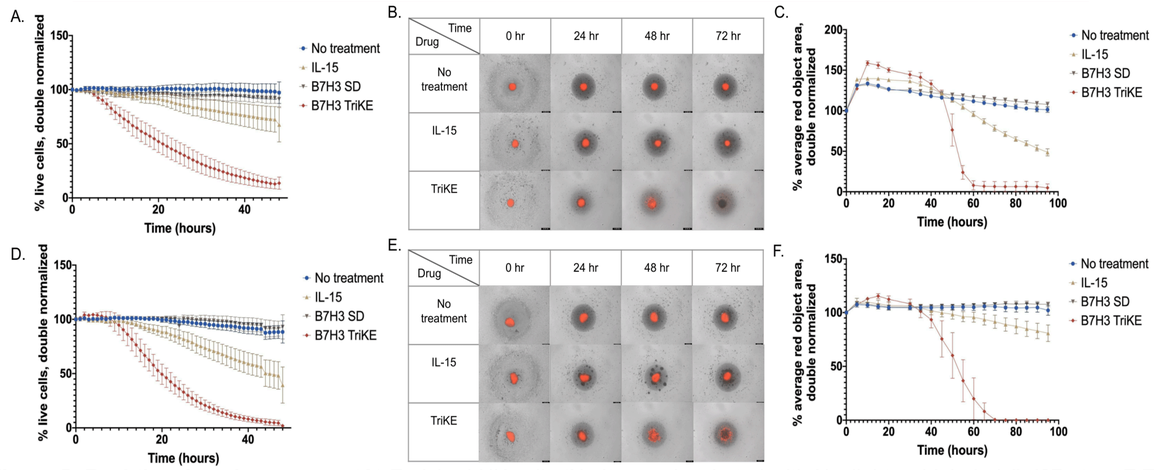


Figure 5: Real-time imaging assays. (A) Enriched NK cells (N=4) were incubated with Nuclight red-labeled Cal27 at an E:T of 5:1 in different conditions: no treatment or 3 nM IL-15, B7H3 SD and B7H3 TriKE for 48 hours in an IncuCyte Zoom imager. Quantifications of percent live cells were done by normalizing hourly counts of red cells to targets alone at t=0. **(B-C)** Spheroids of Nuclight red-labeled Cal27 were formed for 72 hours before incubated with enriched NK cells (N=4) at an E:T of 5:1 in different conditions: no treatment or 3 nM IL-15, B7H3 SD and B7H3 TriKE for 96 hours in an IncuCyte S3 imager. Representative images showing spheroids over time. 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)** Same set of assays were done with Cal33.

Conclusions

There is a critical need for a targeted therapy that can effectively eliminate HNSCC cells while sparing healthy cells. Here, we described the preclinical study of a TriKE molecule against B7H3 ligands that are expressed on HNSCC. We have found that treatment with the B7H3 TriKE effectively induces NK cell degranulation and cytokine production against HNSCC, as well as drives targeted killing of HNSCC in vitro.

Ongoing experiments will evaluate the functionality and efficacy of the B7H3 TriKE in vivo. Future studies will involve investigations of the HNSCC tumor microenvironment, and assessments of the B7H3 TriKE efficacy in the HNSCC tumor microenvironment in addition to evaluating whether HPV status of HNSCC has any implications on efficacy of the TriKE in the HNSCC tumor microenvironment as previous studies have reported differential NK cell activity in HPV+/- HNSCC tumor microenvironment.