Tetra-specific antibody GNC-035: Guidance and Navigation Control (GNC) molecule development for treatment of ROR1+ malignancies

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Abstract

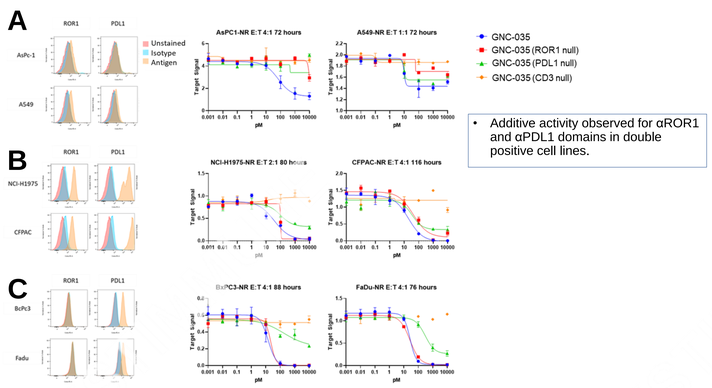
Cancer-intrinsic immune escape mechanisms and immune cell suppression can progressively diminish the curative potential of currently available T cell-based therapies. Barriers to successful T cell checkpoint therapies may be addressed by redirection of T cells toward tumor antigens using T cell engagers that function independently of MHC presented T cell epitopes. Here we demonstrate that an octavalent, tetraspecific Guidance and Navigation Control (GNC) antibody, GNC-035, binds to ROR1, CD3, PD-L1, and 4-1BB and mediates redirected T cell cytolysis of human solid tumor and leukemia and lymphoma cell lines in a ROR1 specific manner.

Experiments using GNC-035 to redirect T cell cytotoxicity toward ROR1+ cancer cell targets show the T cells in PBMC are highly functionalized by pre-exposure to GNC-035. This pre-exposure of PBMC to GNC-035 results in greater tumor cell killing efficacy compared to concurrent exposure of tumor cells in the presence of T cell effectors. This result suggests that the systemic delivery of GNC-035 can condition the T cell compartment to increase the therapeutic impact of T cells migrating to solid tumors, with or without preexisting infiltrating T cells. This beneficial conditioning of T cells by pre-exposure to GNC-035 is not observed with pre-exposure to CD3xROR1 bi-specific T cell engager controls.

To evaluate the potential for GNC-035 to mediate cytokine release syndrome, the molecule is evaluated in soluble formats in the presence of PBMC and the ROR1+ A549 cancer cells, or HUVEC cells. Under these conditions, the cytolysis of A549 target cells is detectable after exposure to GNC-035 at 100 fM concentrations as well as the release of IFN-γ and certain other inflammatory cytokines at 24 or 48 hours post-treatment. However, consistent with Blinatumomab treatment, PBMC exposed to soluble GNC-035 for 24 or 48 hours on a monolayer of HUVEC cells, produced significantly greater amounts of IFN-γ and IL-6 at concentrations greater than 10 pM. These results indicate GNC-035 has a therapeutic window of activity that is ROR1 dependent, spanning cytolytic activity, and IFN-γ release without a production of IL-6 and which is wider than that indicated by Blinatumomab in PBMC.

Collectively, the GNC-035 represents a class of multi-specific and multi-modal immune cell engagers with potential to mediate ROR1+ cancer regression, overcome TCR-based immune escape and reverse T cell immune suppression in tumor microenvironment. The clinical phase I-b study of GNC-035 is under way in breast cancer and hematologic cancers and the available data exhibit strong signals of efficacy with acceptable tolerability.

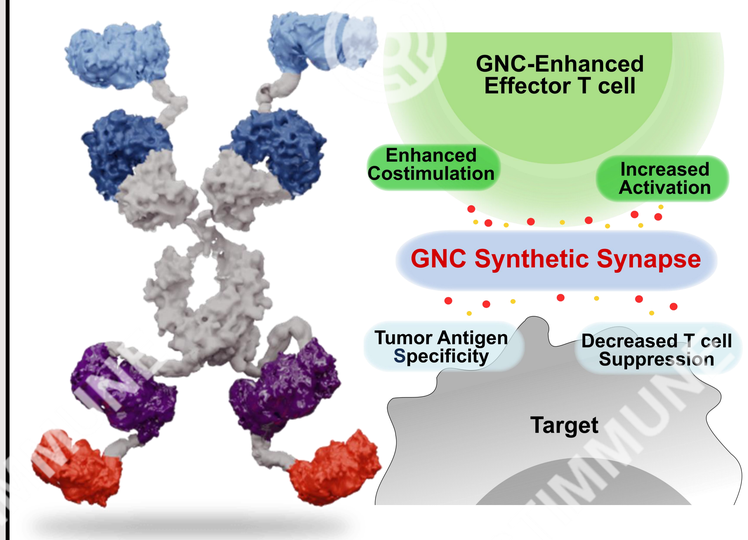
Tetra-specific binding domains mediate increased cytolytic activity against multiple tumor cell lines



- Additive activity observed for αROR1 and αPDL1 domains in double positive cell lines.

Figure 1. GNC Structure mediates specific antigen binding and multi-domain target mediated cytolytic function.
Solid tumor lines in Redirected T cell Cytotoxicity assays using PBMC treated with intact GNC-035, as well as domain-null drug variants against tumor cell lines with phenotypes **A**) ROR1low, PDL1low **B**) ROR1+, PDL1high and **C**) ROR1-, PDL1+. Error bars represent SEM.

GNC-035: Tetra-specific T cell engager



3rd tetra-specific antibody therapy in human trials

GNC-035 Exhibits T cell specificity drives T cell activation through α4-1bb domain

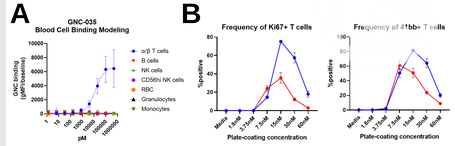
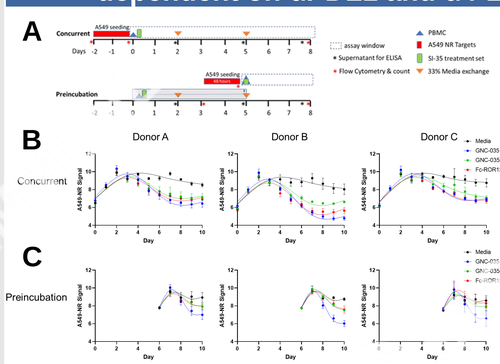


Figure 2. GNC exhibits T cell-specific binding and activation through 4-1bb
GNC-035 was introduced at titrated concentrations to whole blood from 10 healthy donors aged from 23-78 years. GNC-038 binding levels were measured for individual peripheral blood cell (**A**). Cell culture plates were precoated in dilutions of GNC-035 or a structural variant of the drug lacking an α4-1bb domain prior to 96-hour co-culture with isolated naïve T cells and measurement of activation phenotype (**B**).

GNC-035 primed PBMC functional superiority is dependent on αPDL1 and α4-1bb domains



- GNC-035 exhibits similar antitumor activity to PDL1-null and RORE1-CD3 bispecific with concurrent treatment.
- Preincubation of PBMC with GNC-035 result compared to control conditions in 3 donors tested.
- Enhancement s in superior activity of activity in pre-treatment is dependent on presence of αPDL1 domain.

Figure 3. Functional enhancement of Solid tumor cancer line A549-NR are cultured with PBMC and treatment with or without preincubation of PBMC effector with drug treatments (**A**). Time-series imaging data of tumor spheroids for Concurrent (**B**) and Preincubation (**C**) experimental timelines.

Interferon gamma mediate GNC RTCC toward PDL1 on ASPC-1 cells

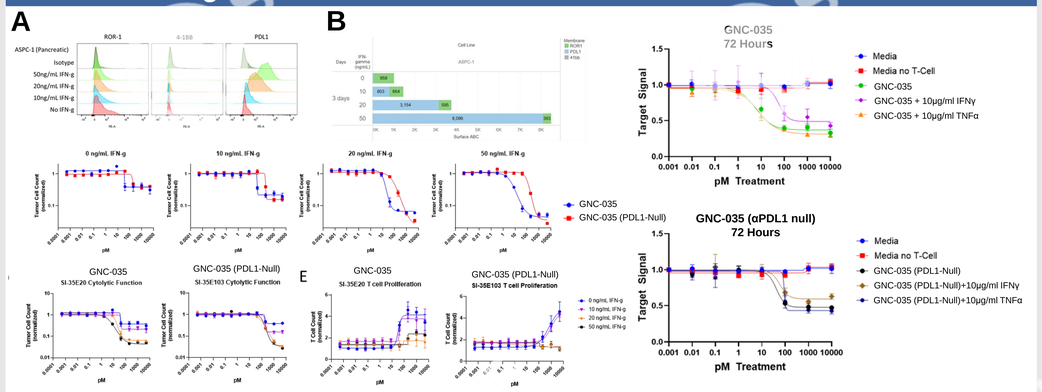
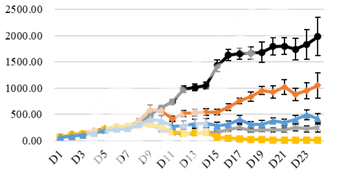


Figure 3. GNC-038 .
Solid tumor lines with in Redirected T cell Cytotoxicity assays using PBMC treated with intact GNC-038, as well as domain-null drug variants (**A**). NCG mice were implanted with individual peripheral blood cell types, including B cells, α/β T cells, NKT cells, monocytes, granulocytes and red blood cells (**C**).

GNC-035 is exhibits anti-tumor activity in mouse xenograft model



- Partial reduction in tumor volume observed in low-dose treatment with GNC-035
- Mid to High dose treatments result in greatest size reduction using HS-746.T xenograft

Figure 4 GNC-035 treatment in mouse xenograft model
NCG mice were implanted with HS-746.T Gastric Cancer tumor xenografts used to measure reduction in tumor volume during treatment with multiple doses of GNC-035.

Summary

- T cells in PBMC are highly functionalized by pre-exposure to GNC-035
- GNC-035 PDL1 binding domain increases drug potency 36-48 hours after GNC treatment
- IFN-g but not TNF-a mediate GNC-035 conversion of adaptive resistance to RTCC sensitivity
- GNC-035 CD3xROR1x41bb domain activity in RTCC highly upregulates PDL1 on ASPC1 target cells
- Post-cytolytic T cell proliferation is highly dependent on PDL1 domain activity

Acknowledgments

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References

Title:
A Study of GNC-035, a Tetra-specific Antibody, in Participants With Locally Advanced or Metastatic Breast Cancer
A Study of GNC-035, a Tetra-specific Antibody, in Participants With Relapsed/Refractory Hematologic Malignancy
A Study of GNC-035, a Tetra-specific Antibody, in Participants With Locally Advanced or Metastatic Solid Tumors

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