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

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**Abstract**

Cancer-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 octavent, tetraspecific Guidance and Navigation Control (GNC) antibody, GNC-035, binds to ROR1, CD3, PD-L1, and CD200R1, mediates tetradirectional signaling and mediates redirected T cell cytolytic effects in 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 cytolytic of A549 target cells is detectable after exposure to GNC-035 at 100 nM concentrations as well as the release of IFNγ and certain other inflammatory cytokines at 24 or 48 hours post-treatment. However, consistent with Blinatumomab treatment, GNC-035 exposed to soluble GNC-035 for 24 or 48 hours on a monolayer of HUVEC cells, produced significantly greater amounts of IFNγ 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 IFNγ 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 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**

**GNC-035: Tetra-specific T cell engager**

![Image](https://example.com/image1)

**GNC-035 Exhibits T cell specificity drives T cell activation through α4bb domain**

![Image](https://example.com/image2)

**Interferon gamma mediate GNC RTCC toward PD1 on ASPC-1 cells**

![Image](https://example.com/image3)

**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-α mediate GNC-035 conversion of adaptive resistance to RTCC sensitivity.
- GNC-035 CD3xROR1x4bb domain activity in RTCC highly upregulates PDL1 on ASPC1 target cells.
- Post-cytolytic T cell proliferation is highly dependent on PDL1 domain activity.

**Acknowledgments**

The authors acknowledge the efforts and contributions of numerous staff of Systimmune Inc. and Baili Pharmaceuticals who worked on the development of GNC-035.

**References**

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 Metastatic Melanoma or Nipple Erythema Syndrome A Study of GNC-035, a Tetra-specific Antibody, in Participants With Locally Advanced or Metastatic Solid Tumors...