Tetra-specific antibody GNC-038: Guidance and Navigation Control (GNC) molecule development for treatment of CD19+ malignancies

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Abstract

B cell malignancies treated with CD19-directed immunotherapies can relapse, in some cases due to clonal selection for reduced CD19 antigen expression or enhancement of immunosuppressive phenotypes. Here we demonstrate that a Guidance and Navigation Control (GNC) tetra-specific antibody, GNC-038, binds to CD19, CD3, PD-L1, and 4-1BB and mediates cytolysis of human leukemia and lymphoma cells by T cells.

Redirected T cell cytolysis (RTCC) occurs in the presence of GNC-038 (Emfizatamab), resulting in the killing of CD19+ leukemia and lymphoma cell lines. The cytolytic functions induced by GNC-038 are similar to Blinatumomab in vitro, as indicated by T cell degranulation and production of IFN-gamma. Human T cells in PBMC exposed to GNC-038 in vitro proliferate in a dose-dependent fashion. Proliferation is further enhanced upon rechallenge with leukemic target cells. Proliferation of T cells from individuals with higher % PD-1+ and Effector polarized compartments is enhanced by GNC-038 compared to Blinatumomab. To evaluate the contribution of each binding domain of GNC-038 in mediating RTCC function, versions of GNC-038 were prepared, replacing each antigen binding domain with anti-FITC binding domains. Under assay conditions using PBMC for RTCC toward the CD19+ target cell line Nalm-6, the results demonstrate the contribution of each domain to the overall, anti-leukemic cytolytic activity.

To evaluate the potential for GNC-038 to mediate cytokine release syndrome, the molecule is evaluated in soluble, and plate bound formats in the presence of PBMC and CD19+ leukemic target cells. In comparison to Blinatumomab, the production of cytokines is comparable, with some notable differences. PBMC exposed to soluble GNC-038 for 48 hours produced more IFN-γ, IL-2 and TNF-α, while showing no significant difference in production of IL-6. Based on these results, the primary indicator of CRS, IL-6, does not suggest increased risk compared to Blinatumomab, while the type of T cell activity induced by GNC-038 in PBMC with leukemia cells is distinct.

Collectively, the GNC-038 represents a class of multi-specific and multi-modal immune cell engagers with potential to mediate CD19+ cancer killing, while also increasing the T cell compartment's therapeutic potential to respond to T cell redirection upon subsequent cycles of therapy. The clinical phase I-b study of GNC-038 is under way and the available data exhibit strong signals of efficacy with acceptable tolerability.

GNC-038 specifically binds target antigens and exhibits anti-tumor activity *in vivo*

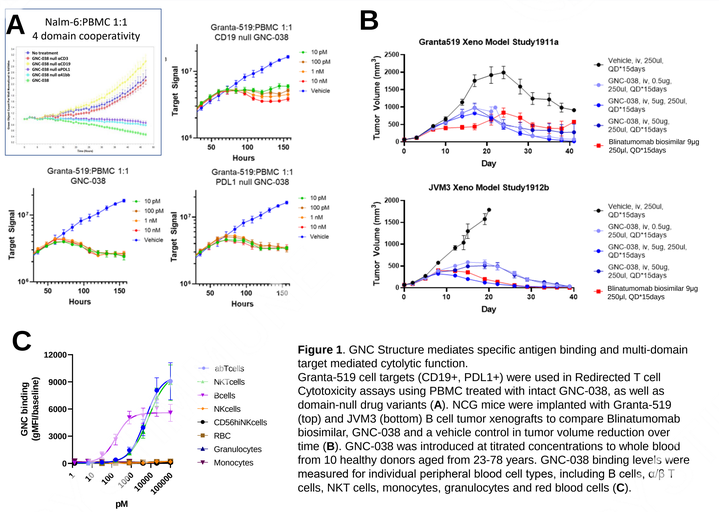
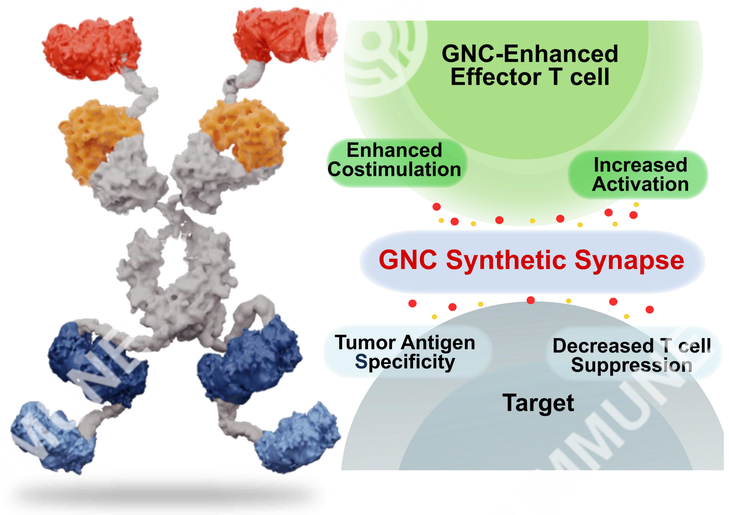


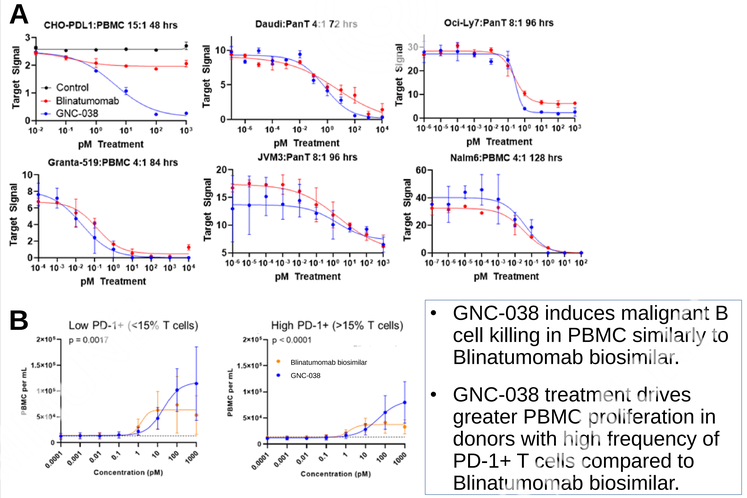
Figure 1. GNC Structure mediates specific antigen binding and multi-domain target mediated cytolytic function.
Granta-519 cell targets (CD19+, PDL1+) were used in Redirected T cell Cytotoxicity assays using PBMC treated with intact GNC-038, as well as domain-null drug variants (**A**). NCG mice were implanted with Granta-519 (top) and JVM3 (bottom) B cell tumor xenografts to compare Blinatumomab biosimilar, GNC-038 and a vehicle control in tumor volume reduction over time (**B**). GNC-038 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 types, including B cells, αβ T cells, NKT cells, monocytes, granulocytes and red blood cells (**C**).

GNC-038: Tetra-specific T cell engager



1st tetra-specific antibody therapy in human trials

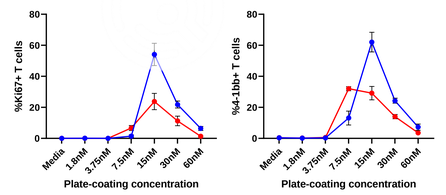
GNC-038 exhibits cytolytic function against multiple malignant B cell lines



- GNC-038 induces malignant B cell killing in PBMC similarly to Blinatumomab biosimilar.
- GNC-038 treatment drives greater PBMC proliferation in donors with high frequency of PD-1+ T cells compared to Blinatumomab biosimilar.

Figure 2. Redirected T cell Cytotoxicity (RTCC) was measured against a panel of leukemia and lymphoma cell lines using PBMC and Pan T effectors during treatment with GNC-038 and Blinatumomab biosimilar (**A**). RTCC assay measuring PBMC proliferation in the presence of Nalm-6 acute B cell leukemia line using an 18-donor set to compare GNC-038 and Blinatumomab biosimilar (**B**). Results are shown for donors who exhibited relatively low (left) and high (right) levels of PD-1+ T cells.

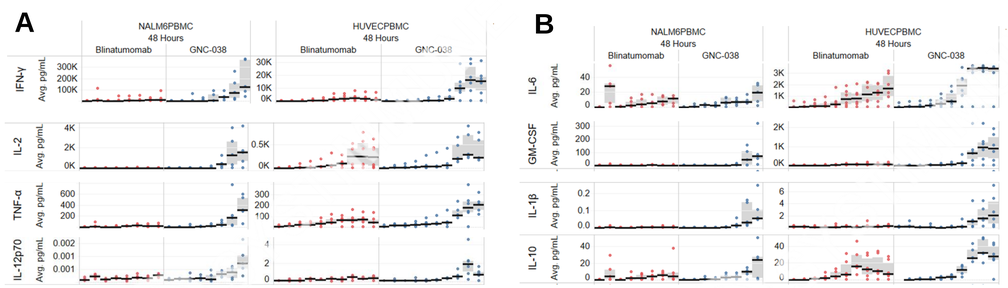
Agonistic α4-1bb domain modulates effector phenotype *in vitro*



Elevated Ki-67 and 4-1bb expression is observed in presence of α4-1bb domain.

Figure 3 Function of agonistic α4-1bb domain *in vitro*
Cell culture plates were precoated in dilutions of GNC-038 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.

GNC-038 induces unique cytokine release profile and higher threshold of IL-6 production relative to Blinatumomab Biosimilar



- Increased production of cytokines linked to anti-cancer effector function with GNC treatment
- Inflammatory cytokine profile does not suggest increased CRS risk
- Concentration at which measurable cytokine is produced is greater than observed EC-50 concentration

Figure 4 GNC-Induced cytokine release in
PBMC cocultured with Nalm-6 acute B cell leukemia line and human umbilical vein endothelial cells (HUVEC) were treated with dilutions of Blinatumomab biosimilar and GNC-038 prior to assessment of soluble cytokine concentrations. Levels of cytokines linked to effector function (**A**) and inflammatory signaling (**B**) were measured at 48 hours by Meso-scale delivery (MSD) multiplex assay (n=6 donors).

Summary

- GNC-038 effectively drives T cell mediated killing of malignant B cells in *in vitro* and *in vivo* models.
- GNC-038 induces greater PBMC cell proliferation in the presence of target cells in donors with higher proportions of PD-1+ T cells, suggesting beneficial proliferation of effector cells may be achieved in donors with more exhausted/effector polarized T cell phenotype (ref 1,2).
- GNC-038 α4-1bb domain polarizes T cells towards activated, proliferative 4-1bb-expressing phenotype.
- GNC-038 induces cytokine prolife linked to anti-cancer effector function without elevated potential for CRS in PBMC co-culture models.

Acknowledgments

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References

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2. Roufaiel et al. Impaired T-cell function in B cell lymphoma: A direct consequence of events at the immunological synapse? *Frontiers in Immunology* 2015

Title	URL
A Study of GNC-038 Injection in Patients With Relapsed or Refractory NK/ T-cell Lymphoma, AITL, and Other NHL	https://ClinicalTrials.gov/show/NCT05627856
A Study of GNC-038, a Tetra-specific Antibody, in Patients With Central Nervous System Lymphoma (PCNSL) and Relapsed or Refractory Secondary Central Nervous System Lymphoma (SCNSL)	https://ClinicalTrials.gov/show/NCT05485753
A Study of GNC-038, a Tetra-specific Antibody, in Participants With R/R Diffuse Large B-cell Lymphoma (DLBCL)	https://ClinicalTrials.gov/show/NCT05192486
Phase I Clinical Study of GNC-038 in Patients With Non-Hodgkin's Lymphoma or Acute Lymphoblastic Leukemia	https://ClinicalTrials.gov/show/NCT04606433
Phase Ib/II Study of GNC-038 Injection in Patients With Relapsed or Refractory Non-Hodgkin's Lymphoma	https://ClinicalTrials.gov/show/NCT05623982