Novel γδ T cell engager platform for cancer immunotherapy promoted T cell expansion with a persistence-indicating phenotype

Lei Ding, PhD, Yanjie Li, PhD, Mariska ter Haak, Kate Rochlin, PhD, Sadhak Sengupta, PhD, Lawrence S. Lamb Jr., PhD
IN8bio, Inc., New York, NY 10118, USA, www.IN8bio.com, Email: info@IN8bio.com

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Introduction

Gamma-delta (γδ) T cells initiate a broad, MHC-independent immune response to cancer, owing to their ability to overcome the challenges of heterogeneity by engaging an array of cytotoxic receptors against multiple stress-associated tumor antigens. The use of an activating T cell engager (TCE) that induces substantial proliferation of γδ T cells would increase the availability of these cytotoxic effector cells for both direct and bystander anti-tumor activity in an environment where γδ T cells are generally limited in both number and activity. Here, we present initial findings from a novel activating γδ T cell engager platform (γδ-TCE) targeting CD19+ B cell hematologic malignancies (INB-619).

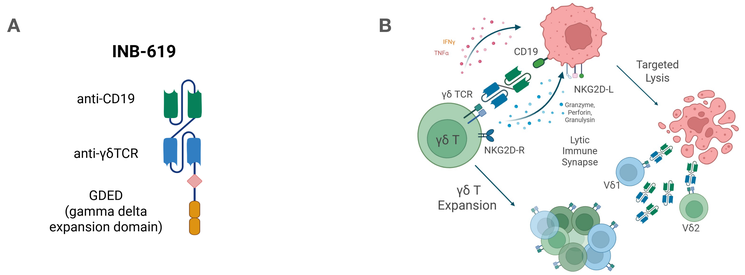


Fig. 1. INB-619 is a pan-γδ T-cell engager targeting CD19+ normal/malignant B cells.

(A) Schematic representation of the INB-619 molecule. (B) INB-619 binds CD19+ cells and drives potent activation and expansion of pan-γδ T cells, including both Vδ1+ and Vδ2+ subsets, leading to efficient target cell depletion.

INB-619 triggers potent, CD19-specific γδ T cell cytotoxicity

Fig. 2. INB-619 induces dose-dependent and target-specific cytotoxicity of γδ T cells.

(A) INB-619 elicits dose-dependent γδ T cell–mediated killing of NALM-6 (CD19+) target cells, when cocultured at E:T=1 (n=3). (B) Cytotoxicity is restricted to CD19+ NALM-6 cells The minimal killing of the CD19− MOLM-13 AML cells at a 1:1 E:T ratio is due to the expected activity of γδ T cells against AML-associated stress antigens.

INB-619 induces controlled, robust γδ T–cell activation

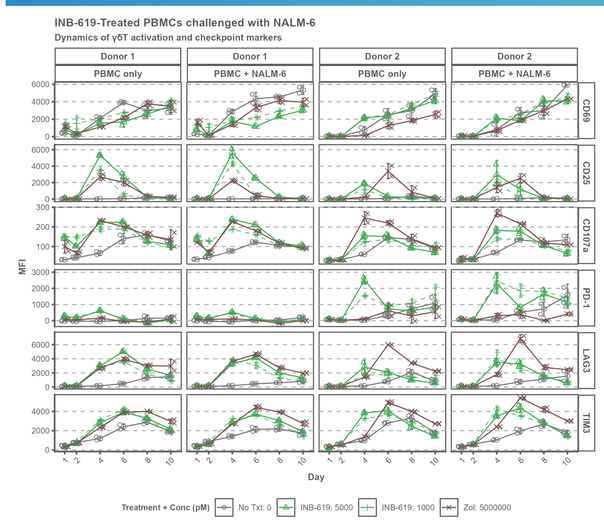
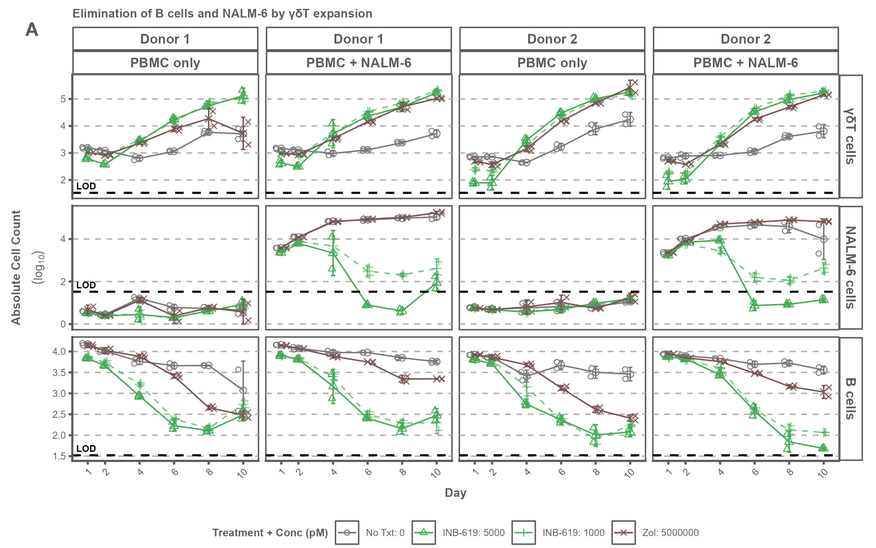


Fig. 3. Dynamics of activation and checkpoint markers on INB-619 expanded γδ T cells.

PBMCs from two healthy donors, with 5% NALM-6 coculture, were incubated with INB-619, zoledronate (Zol), or no treatment media alone (No Txt). Median fluorescence intensities (MFIs) of activation markers (CD69, CD25, CD107a) and checkpoint markers (PD-1, LAG-3, TIM-3) were monitored over a 10-day culture period (n=2).

INB-619 depletes NALM-6/ B cells with γδ T cell expansion



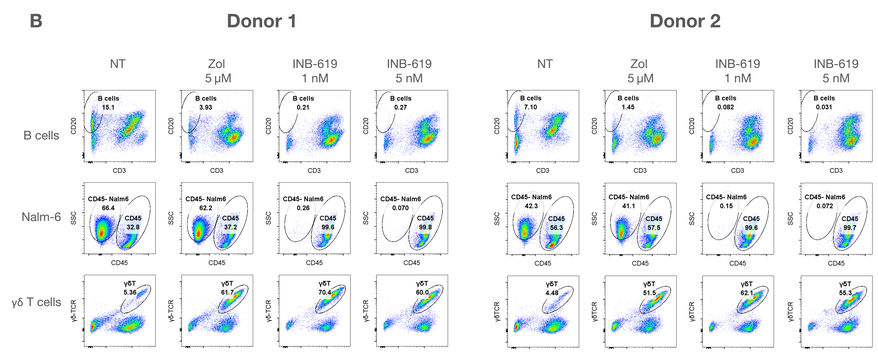


Fig. 4. NALM-6/B-cell depletion and γδ T-cell dynamics in INB-619–treated PBMCs.

(A) NALM-6, B-cell, and γδ T-cell count (log10) over a 10-day culture post treatment were measured by flow cytometry with Trucount beads. (B) Representative flow plots of NALM-6, B-cell, and γδ T-cell populations on day 8 (n=2).

INB-619 expands both Vδ1+ and Vδ2+ subsets

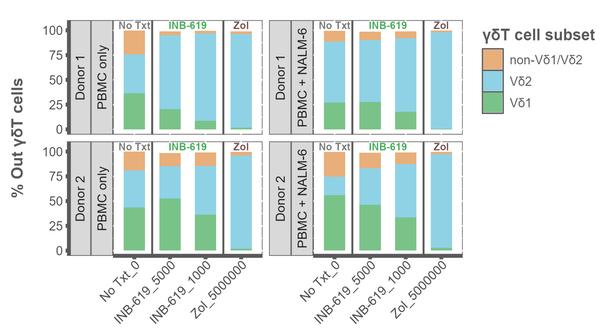


Fig. 5. INB-619 induces robust expansion of both Vδ1+ and Vδ2+ γδ T-cell subsets.

Frequencies of Vδ1+, Vδ2+ and non-Vδ1Vδ2 subsets relative to the total γδT cell population were measured by flow cytometry on day 10 (n=2).

INB-619 expanded γδ T cells display favorable phenotypes

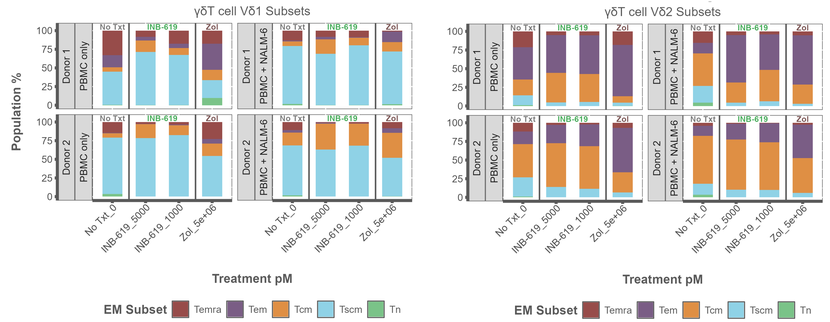


Fig. 6. Maturation phenotypes of expanded γδ T cell subsets from two healthy donor PBMCs treated with INB-619, Zolendronate (Zol) or media alone no-treatment (No Txt).

Frequencies of maturation phenotypes in Vδ1+ and Vδ2+ subset γδ T cells were quantified by flow cytometry on day 10 post treatment (n=2).

Cytokine release from PBMCs treated with INB-619

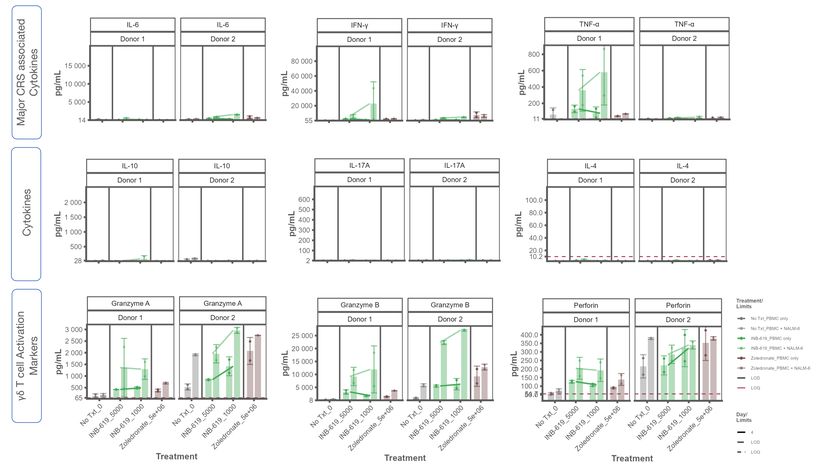


Fig. 7. Cytokine secretion from PBMCs cocultured with NALM-6 and treated with INB-619.

Cytokine levels in culture supernatants were measured on day 4 from PBMCs treated with INB-619 (1 nM or 5 nM), 5 μM zoledronate (Zol), or non-treated (No Txt), during NALM-6 and B cell elimination (n=2). Cytokine levels are consistent with those previously presented using Lupus patient PBMCs with INB-619.

Conclusions

- **Novel pan–γδ T cell engager:** INB-619 selectively expands and activates γδ T cells for potent, dose-dependent target elimination.
- **Robust yet controlled activation:** INB-619 induces strong but well-regulated activation of pan-γδ T cells, avoiding the overstimulation commonly seen with CD3-based TCEs.
- **Robust γδ T cell expansion:** INB-619 expands both Vδ1+ and Vδ2+ subsets and mediates complete depletion of CD-19+ Nalm-6 cancer cells and B cells from donor PBMCs.
- **Favorable maturation phenotypes:** INB-619-expanded γδ T cells show persistent functional capacity and effective cytotoxic potential.
- **Minimal CRS risk:** Target clearance occurs with low/no increase in levels of IL-6, IL-10, IL-4, and TNFα.
- Additionally, the modular design of the TCE allows for potential uses across a wide range of cancer targets by leveraging the innate recognition capabilities of γδ T cells with targeted engagement of specific tumor-associated antigens.