INB-600 A Novel T Cell Engager Platform Specific for Gamma-Delta (γδ) T cells

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

Introduction

T cell engagers (TCEs) are molecules that redirect T cells to bind targets by linking T cell receptors (TCRs) with target-associated antigens expressed on malignant or more recently nonmalignant cells responsible for autoimmune disease pathology. Most TCEs are bispecific, designed to bind both an antigen expressed on the cell of interest and CD3, thereby initiating a T cell directed immune response, activation/expansion of T cells, and subsequent lysis of the targeted cell. This approach has led to complications such as T cell exhaustion, cytokine release syndrome (CRS) and neurotoxicities (ICANS). Gamma delta (γδ) T cells offer a promising alternative due to their innate MHC-independent recognition and decreased production of CRS-associated cytokines. We present INB-619, a novel pan-γδ TCE that selectively binds, activates, and expands γδ T cells. INB-619 demonstrates specific binding to target cells, including SLE B cells from patient PBMCs, induces potent cytotoxicity, and supports robust expansion of Vδ1+ and Vδ2+ T cells. These findings position INB-619 as a promising therapeutic candidate with potential applications in both oncology and autoimmune indications.

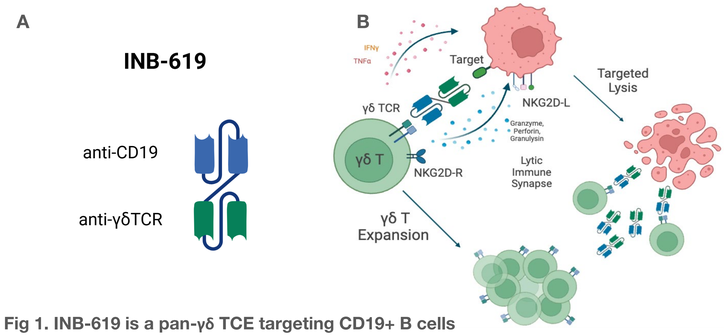

Fig 1. INB-619 is a pan-γδ TCE targeting CD19+ B cells

INB-619 (A) targets CD19+ hematologic malignancies and autoimmune B cells, with robust activation and expansion of pan-γδ T cells (B).

INB-619 demonstrates target-specific binding

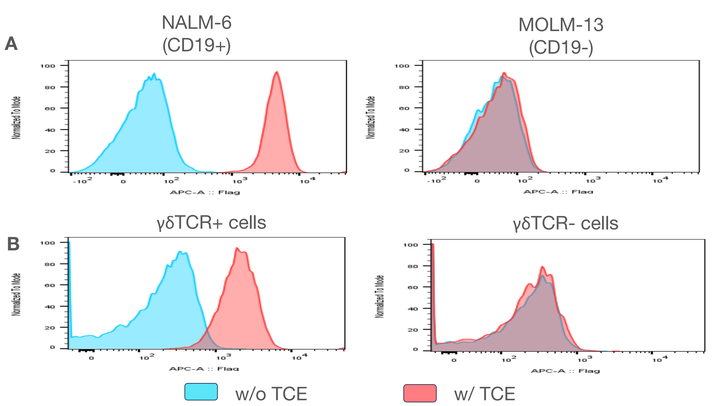

Fig 2. Specific binding of INB-619 to target cells

NALM-6 (left) or MOLM-13 (right) cells (A), and γδ T-enriched cells from zoledronate-expanded PBMCs (B) were incubated ± CD19TCE for 30 min, washed, and stained with anti-flag for flow analysis.

INB-619 enhances γδ T cytotoxicity against CD19+ cells

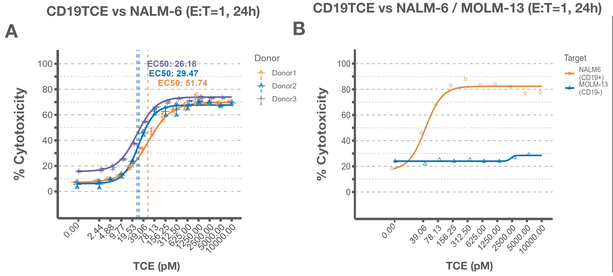

Fig 3. INB-619 induces strong and target-specific γδ T cytotoxicity

CD19TCE induces dose-dependent γδ T cell cytotoxicity against NALM-6 (A) and specific cytotoxicity against NALM-6 or MOLM-13 cells (B). EC50 values define cytotoxic efficacy. Experiments were performed with multiple donors (N = 3), and donor variability is reflected in the differences in EC50.

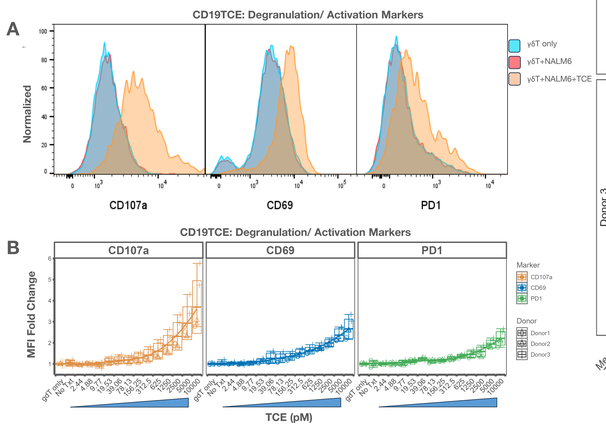

Fig 4. INB-619 promotes γδ T activation and degranulation

γδ T cell degranulation and activation markers after co-culture with NALM-6 cells (E:T=1; 24h) ± CD19TCE (A) and the upregulation of these markers in a TCE dose-dependent manner after treatment (N=3) (B).

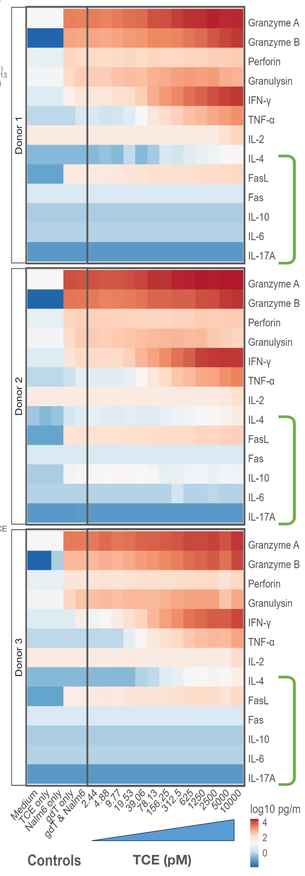

Fig 5. INB-619 induces cytokine release in γδ T and NALM-6 co-culture

Cytokine release was measured after 24h of co-culture of γδ T cells and NALM-6 (E:T=1; N=3).

INB-619 depletes B cells from PBMCs of SLE patients

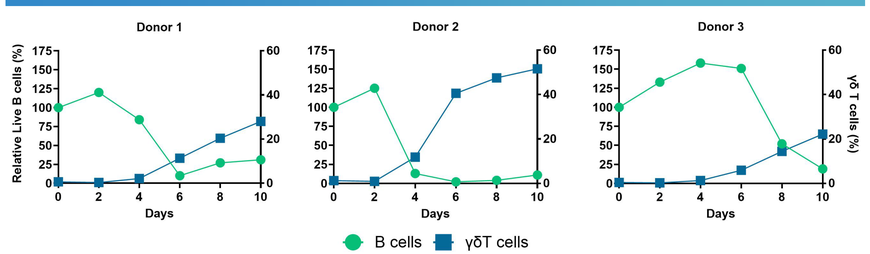

Fig 6. SLE-B cells depletion and γδ T cell expansion with INB-619

Three SLE donors with active disease manifestation were chosen for the study. The timelines of γδ T cell expansion (blue squares) and depletion of B cells (green circles) cross in all three SLE donors studied, indicating that the expansion of T cells is critical for the depletion of autoimmune B cells. 5nM INB-619 was used with all three donors.

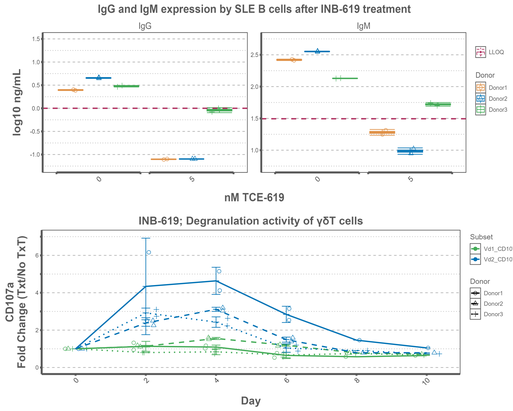

Fig 7. Loss of secreted antibodies upon INB-619 treatment

Depletion of B cells in all three SLE donors was further demonstrated by loss of secreted IgG1 (left) and IgM (right) in an ELISA test performed with culture supernatants from Day 8 with no media exchanges.

Fig 8. Degranulation of γδT cells with INB-619

Increases in γδT cell degranulation upon INB-619, measured by the surface expression of CD107A on both Vδ1+ T cells (green squares) and Vδ2+ T (blue squares), follow the trajectories of B cell depletion in all three donors.

INB-619 expands both Vδ1+ and Vδ2+ γδ T cells from PBMCs

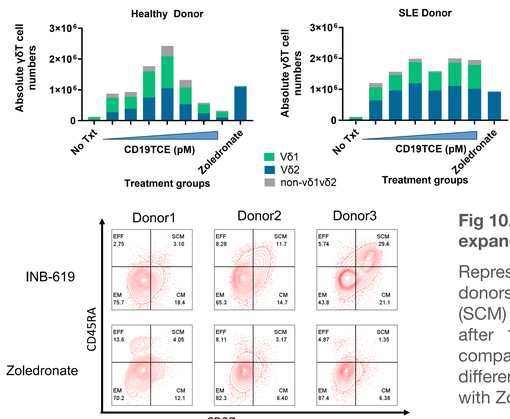

Fig 9. INB-619 promotes robust pan-γδ T cell expansion

Representative data from PBMCs from one healthy donor (left) and one SLE donor (right) treated with CD19TCE. γδ T cell expansion was analyzed on day 10. IL-2 only (No Txt) and Zoledronate + IL-2 serve as controls.

Fig 10. Phenotypic characterization of INB-619 expanded γδ T cells from SLE patient PBMCs

Representative data from PBMCs of three SLE donors show an increase in memory stem cells (SCM) and central memory cells (CM) populations after 10 days of expansion with INB-619. In comparison, the same donors showed differentiated T cell populations when expanded with Zoledronate.

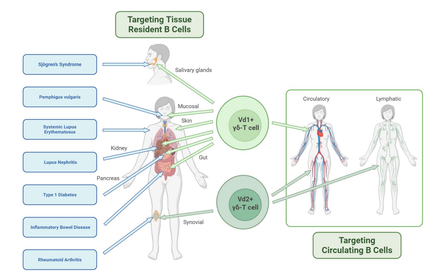

Fig 11. INB-619, pan-γδ TCE targeting may have advantages in autoimmune diseases

Expansion and binding to both Vδ1+ and Vδ2+ T cells allow the targeting of both circulating and tissue resident B cells which may result in deeper B cell depletion. γδ T cells home to and are resident to multiple tissues associated with autoimmune disease. Vδ2+ T cells have the added feature of phagocytosis and antigen presentation.

Conclusions

- INB-619 enhances specific and dose-dependent anti-tumor activity of γδ T cells at low picomolar EC50 levels, including activation, cytotoxicity, and cytokine release, against CD19+ ALL target cells and normal B cells.
- INB-619 exhibited a favorable cytokine release profile, with no detectable or minimal release of cytokines associated with CRS and tumor promotion, such as IL-4, IL-6, IL-10, and IL-17a.
- INB-619 induces a deep depletion of B cells in PBMCs from SLE donors which is also demonstrated by the loss of IgG1 and IgM. A robust expansion of both Vδ1+ and Vδ2+ γδ T cells from PBMCs of both healthy or SLE donors was shown, offering a powerful and integrated approach for B cell–mediated autoimmune indications.
- The expanded γδ T cells exhibit favorable phenotypic profiles, suggesting that INB-619 may also serve as a novel method for in vitro expansion of γδ T cells for therapeutic use.