1. ICTO1 is a first-in-class anti-BTN3A mAb that selectively activates Vγ9Vδ2 T cells

Targeting Vγ9Vδ2 T Cells via BTN3A
- 1. Vγ9Vδ2 T cells are part of the first line of defense against cancer, bridging the innate and adaptive immune response.
- 2. ICTO1 MAb binds to all 3 BTN3A isoforms to induce an activated conformation that leads to activation of Vγ9Vδ2 T cells as shown in the figure.
- 3. ICTO1 overcomes 2 key limitations of prior efforts to activate Vγ9Vδ2 T cells: intracellular phosphatase superfamily dependence and BTN3A restriction.
- 4. ICTO1 is being evaluated in a Phase 1/2a clinical study in MMTV and in combination with Pembrolizumab.

2. rhlL-2 enhances ICTO1-mediated proliferation in human PBMCs

Results: rhlL-2 enhanced ICTO1-mediated Vγ9Vδ2 T cell proliferation with almost 100% of proliferating Vγ9Vδ2 T cells in the combination group at doses of ICTO1 that induced ~30% when used alone.

Significance: Promoting expansion of Vγ9Vδ2 T cells may be clinically useful given that Vγ9Vδ2 T cells are normally ~5% of total T cells in adults and cancer patients.

3. NL-201 was designed to overcome the limitations of IL-2 immunotherapy

- 1. Aledeleukin (rHL-2) is an approved immunotherapy for metastatic RCC and melanoma; however, severe toxicity has limited its widespread clinical use.
- 2. In addition to severe toxicity, aledeleukin increases the number of Trgcs binding to IL-2Rα (CD25), which may inhibit the antitumor immune response.
- 3. NL-201 is a de novo IL-2 and IL-15 agonist designed to overcome the limitations of aledeleukin.
- 4. NL-201 diminishes the βγ and sγ signaling subunits of IL-2 and IL-15 receptors without any binding interface for CD25, resulting in beneficial T and NK cell activation, with minimal impact on immunosuppressive regulatory T cells.
- 5. NL-201 is currently being evaluated in a Phase 1 clinical study.

4. NL-201 is more potent than rHL-2 to activate Vγ9Vδ2 T cells, CDB T cells, and NK cells, while less potent on Tregs.

Flow cytometry assessment of IL-2/15-pretreated NL-201

Results: NL-201 is ~100X more potent than IL-2 to trigger IL-2R signaling in Vγ9Vδ2 T cells. NL-201 is ~50X more potent than IL-2 to trigger IL-2R signaling in CDB T cells, and NK cells. NL-201 is ~100X less potent than IL-2 to trigger IL-2R signaling in Trgcs.

5. NL-201 plus ICTO1 induces synergistic expansion of Vγ9Vδ2 T cells in vitro

- 1. ICTO1 alone induces ~30% T cell expansion.
- 2. NL-201 alone induces ~50% T cell expansion.

Results: NL-201 + ICTO1 induces robust expansion of peripheral Vγ9Vδ2 T cells in Hu-PBMC-graded mice.

6. NL-201 enhances ICTO1-mediated killing of cancer cell lines by Vγ9Vδ2 T cells in vitro

- 1. NL-201 + ICTO1 enhances IC-10 and IG-93 killing of Vγ9Vδ2 T cells.

Tumor cell lines co-cultured with Hu-PBMC and ICTO1 or IG-93 +/- NL-201. Tumor cell growth induced by live imaging ( luciferase) over 5 days. For Hu-PBMC+IB + NL-201 and Hu-PBMC+IB + IG-93, mTumor was less than 0.05. P<0.001.

7. NL-201 plus ICTO1 induces a dose-dependent expansion of peripheral Vγ9Vδ2 T cells in Hu-PBMC-graded mice

- 1. ICTO1 alone induces ~30% T cell expansion.
- 2. NL-201 alone induces ~50% T cell expansion.

Results: NL-201 + ICTO1 induces robust expansion of peripheral Vγ9Vδ2 T cells that reach a mean of 22.34 and 40% of the total T cells in ICTO1 + NL-201 at 5, 3, and 10 μg/kg groups respectively.

8. Conclusions

- 1. ICTO1 plus NL-201 synergistically triggers Vγ9Vδ2 T cell activation, expansion and anti-tumor activity.
- 2. These data support clinical evaluation of this combination as a novel therapeutic approach for cancer patients.