ICT01, an anti-BTN3A monoclonal antibody, and NL-201, an alpha-independent IL-2/IL-15 agonist, combine to elicit a potent anti-tumor response by synergistically stimulating Vγ9Vδ2 T cell activation and proliferation

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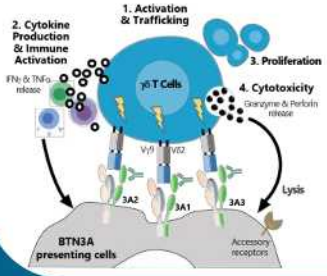
Abstract # 563

1 ICT01 is a first-in-class anti BTN3A mAb that selectively activates Vγ9Vδ2 T cells

ICT01 binding → BTN3A active conformation (stress mimicking mechanism)

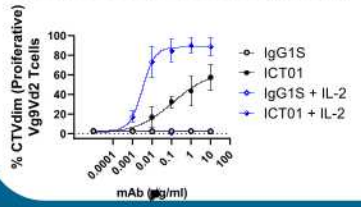


Targeting Vγ9Vδ2 T Cells via BTN3A

1. Vγ9Vδ2 T cells are part of the first line of defense against cancer, bridging the innate and adaptive immune response
2. **ICT01 MOA**: 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. ICT01 overcomes 2 key limitations of prior efforts to activate Vγ9Vδ2 T cells: intracellular phosphoantigen-dependence and BTN3A1 restriction
4. ICT01, is being evaluated in a Phase 1/2a clinical study in MonTx and in combination with Pembrolizumab (NCT04243499)

2 rhIL-2 enhances ICT01-mediated Vγ9Vδ2 T cell proliferation in human PBMCs

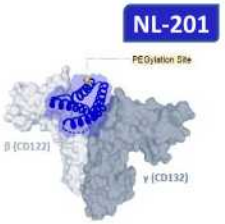
% Proliferation (CTV dilution) of Vγ9Vδ2 T cells analyzed by Flow Cytometry after 5 Days of culture of Hu-PBMC (n=3). IL-2 used at 20 IU/mL



Results: rhIL-2 enhanced ICT01-mediated Vγ9Vδ2 T cell proliferation with almost 100% of proliferating Vγ9Vδ2 T cells in the combination group at doses of ICT01 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 cancer patients

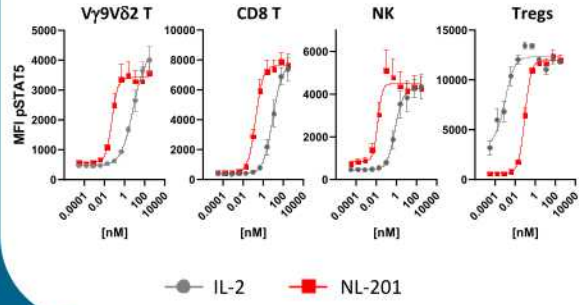
3 NL-201 was designed to overcome the limitations of IL-2 immunotherapy



1. Aldesleukin (rhIL-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, aldesleukin increases the number of Tregs by 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 aldesleukin**
4. NL-201 dimerizes the β and γ signaling subunits of the 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 (NCT04659629)

4 NL-201 is more potent than rhIL-2 to activate Vγ9Vδ2 T cells, CD8 T cells, and NK cells, while less potent on Tregs

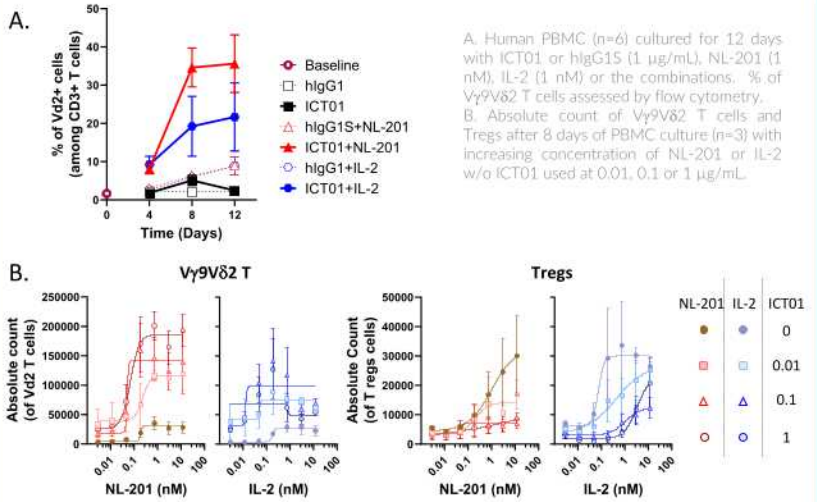
Flow cytometry assessment of P-stat5 20 min post-stimulation with IL-2 (Proleukin) or NL-201



Results:

1. NL-201 is ~100X more potent than IL-2 to trigger IL-2R signaling in Vγ9Vδ2 T cells
2. NL-201 is ~50X more potent than IL-2 to trigger IL-2R signaling in CD8 T cells, and NK cells
3. NL-201 is ~100X less potent than IL-2 to trigger IL-2R signaling in Tregs

5 NL-201 plus ICT01 induces synergistic expansion of Vγ9Vδ2 T cells in vitro

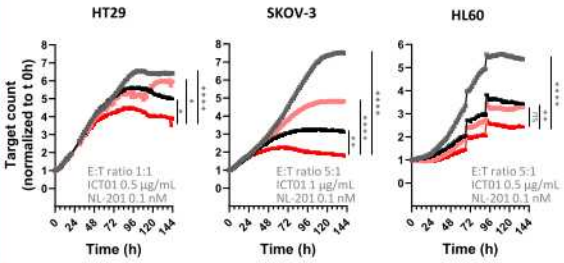


A. Human PBMC (n=6) cultured for 12 days with ICT01 or hIgG1S (1 µg/mL), NL-201 (1 nM), IL-2 (1 nM) or the combinations. % of Vγ9Vδ2 T cells assessed by flow cytometry. B. Absolute count of Vγ9Vδ2 T cells and Tregs after 8 days of PBMC culture (n=3) with increasing concentration of NL-201 or IL-2 w/o ICT01 used at 0.01, 0.1 or 1 µg/mL.

Results:

1. NL-201 plus ICT01 combination dose-dependently and synergistically induces expansion of Vγ9Vδ2 T cells, reaching ~35% of T cells in human PBMC after 8 days of treatment
2. Maximal Vγ9Vδ2 T cell expansion is achieved at doses of NL-201 that triggers only minor expansion of Tregs

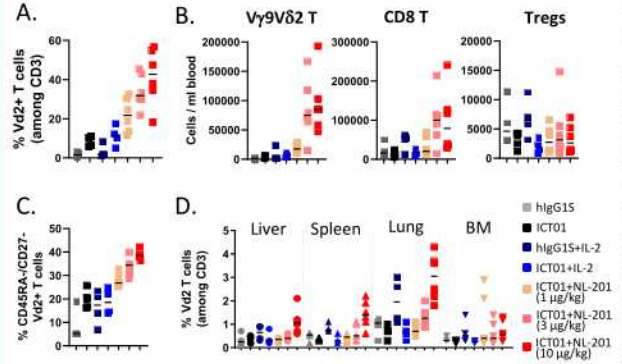
6 NL-201 enhances ICT01-mediated killing of cancer cell lines by Vγ9Vδ2 T cells in vitro



Tumor cell lines co-cultured with Hu-PBMC and ICT01 or hIgG1S +/- NL-201. Tumor cell growth assessed by live imaging (Incucyte) over 5 days. For HL60, fresh tumor cells were added in the co-culture after 3 and 4 days. One-way ANOVA and Holm-Sidak's multiple comparisons test: * P<0.05, **P<0.01, **** P<0.0001

Results: ICT01 plus NL-201 induces stronger killing of target cells as compared to either agent alone

7 NL-201 plus ICT01 induces a dose-dependent expansion of peripheral Vγ9Vδ2 T cells in Hu-PBMC engrafted mice



NCG mice engrafted with 20x10⁶ Hu-PBMCs (D0) and treated with ICT01 or hIgG1S (1 mg/kg IV at D1) alone or combined with IL-2 (0,3 M IU/kg IP at D1, D2, D3 and D4) or NL-201 (1, 3 or 10 µg/kg IV at D1). Blood samples were analyzed by flow cytometry at D8 for % of Vγ9Vδ2 T cells (A), absolute cell number (B) and Vγ9Vδ2 T cell differentiation status (C). Liver, Lung, Spleen and Bone Marrow (BM) were dissociated at sacrifice (D16) for flow cytometry analysis of % of Vγ9Vδ2 T cells (D).

Results:

1. ICT01+NL-201 induces a robust expansion of peripheral Vδ2+ T cells that reach a mean of 22, 34 and 42% of the total T cells in ICT01+NL-201 at 1, 3 and 10 µg/kg groups respectively
2. Vδ2+ T cells expanded with ICT01+NL-201 differentiate toward effector memory phenotype
3. ICT01+NL-201 combination sustain Vδ2 T cell survival in spleen and lung

8 Conclusions

1. ICT01 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