ICT01, AN ANTI-BTN3A MAB, AND NL-201, AN ALPHA-INDEPENDENT IL-2/IL-15 AGONIST, COMBINE TO ELICIT A POTENT ANTI-TUMOR RESPONSE BY SYNERGISTICALLY STIMULATING VG9VD2 T CELL ACTIVATION AND PROLIFERATION

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Background γ 9 δ 2 T-cells are attractive mediators of cancer immunotherapy due to their strong cytolytic and pro-inflammatory activities and the positive correlation between tumor infiltration and good prognosis [1,2]. ICT01, a novel anti-BTN3A mAb activating γ 9 δ 2 T-cells, is being evaluated in a Phase 1/2a clinical study (NCT04243499)[3,4]. Previous studies have shown that IL-2 (Proleukin®) promotes γ 9 δ 2 T-cells expansion following ICT01 stimulation, which may be clinically useful given that γ 9 δ 2 T-cells are normally <5% of total T-cells [5]. However, the severe toxicity of IL-2 has limited its widespread use. NL-201 is a de novo alpha-independent IL-2/IL-15 agonist that preferentially stimulates CD8 T and NK cell proliferation at low concentrations, enabling a potentially wider therapeutic index than IL-2, and is being evaluated in a Phase 1 clinical study (NCT04659629)[6,7]. Here, we explore the potential of ICT01 and NL-201 to synergistically stimulate the activation and proliferation of γ 9 δ 2 T-cells.

Methods Flow cytometry was used to assess IL-2R signaling (pSTAT5), and γ 9 δ 2 T-cell activation and expansion after in vitro culture of huPBMCs with ICT01, NL201 or the combination. Tumor cell killing activity was monitored upon co-culture of huPBMCs with tumor cell lines (Incucyte). In vivo pharmacology was performed in NCG mice engrafted with 20x10⁶ huPBMCs and treated with ICT01 (1 mg/kg IV)±NL-201 (1, 3 or 10 μ g/kg IV). Immune cells were phenotyped by flow cytometry in blood and organs collected at sacrifice (Day 16).

Results NL-201 is ~100X more potent than IL-2 in triggering IL-2R signaling in γ 9 δ 2 T-cells, without preferential activity on Tregs. NL-201 plus ICT01 induces synergistic expansion of γ 9 δ 2 T-cells, approaching ~50% of T-cells after 8 days versus ~10% with single agents. In addition, the combination of NL-201 and ICT01 promotes γ 9 δ 2 T-cell effector memory differentiation, in contrast to IL-2, which induces primarily central memory phenotype. Importantly, NL-201 enhances ICT01-mediated killing of cancer cells by γ 9 δ 2 T-cells. In mice, a dose-dependent expansion of peripheral γ 9 δ 2 T-cells from ~1–2% at baseline to up to 40% of T-cells was observed in the ICT01+NL-201 combination groups. Consistently, γ 9 δ 2 T-cell number and frequency increase in spleen and lungs of the ICT01+NL-201 treated animals as compared to controls. Expanded γ 9 δ 2 T-cells in the combination groups display an effector memory phenotype, confirming our in vitro results.

Conclusions These results demonstrate the ability of the ICT01+NL-201 combination to synergistically trigger γ 9 δ 2 T-cell activation, expansion and anti-tumor activity and support clinical evaluation of this combination as a novel therapeutic approach for cancer patients.

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Ethics Approval All procedures involving animals described in this study have been reviewed and approved by the local ethic committee (CELEAG) and the French Ministry of Research.

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