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ICT01, an anti-BTN3A mAb that activates Vγ9Vδ2 T cells, plus interleukin-2: a potent and promising combination for cancer immunotherapy

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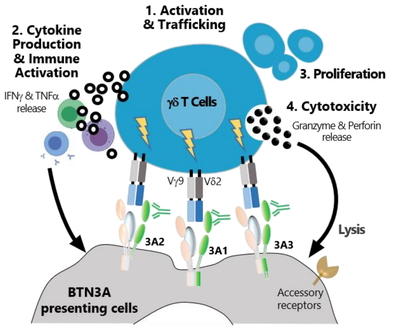
Background:

γδ T-cells are attractive targets for cancer immunotherapy given their strong cytolytic and pro-inflammatory cytokine secretion activities, and the association between tumor infiltration and positive prognosis[1,2]. ImCheck Therapeutics is developing ICT01, an anti-human butyrophilin-3A (BTN3A/CD277) mAb specifically activating γ9δ2 T-cells in a phosphoantigen (pAg)-independent manner. ICT01 is currently in a Phase 1/2a study in solid and hematologic tumors (NCT04243499).

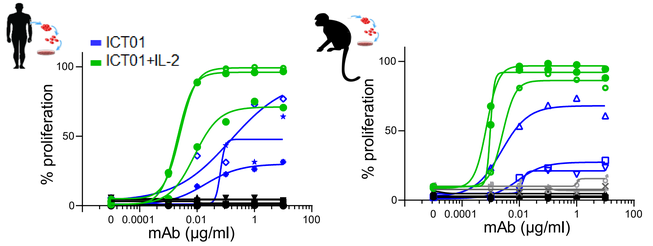
IL-2 has been shown to expand γ9δ2 T-cells *in vitro* and in non-human primates in the presence of pAgs[3,4,5]. Considering the MOA of ICT01 (**Figure below**), our hypothesis was that the combination of ICT01 plus IL-2 would induce selective, proliferative effects on γ9δ2 T-cells as an approach to potentiate γ9δ2 T cell-mediated cancer immunotherapy.

ICT01 binding → BTN3A active conformation (stress mimicking mechanism)



1. In vivo γ9δ2 T cell activation with trafficking in <30 min
2. Cytokines lead to activation of other immune cells, incl αβ T Cells, APCs, & B cells
3. Proliferation under appropriate conditions (e.g., presence of IL-2, IL-15)
4. Cytotoxic attack byγ9δ2 T cells requires 2nd signal from stressed/malignant/infected cells

1. IL-2 Significantly Enhances ICT01-Mediated γ9δ2 T Cell Proliferation in Human and Cynomolgus PBMCs *in vitro*

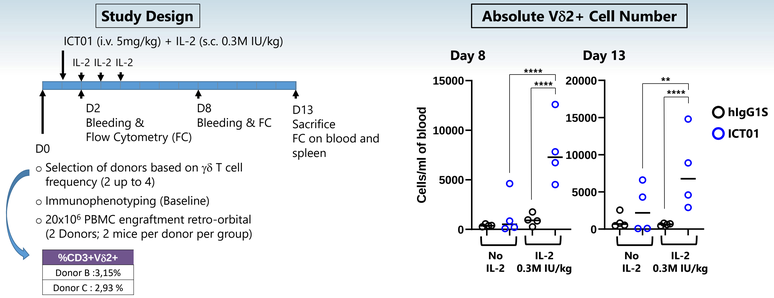


RBC lysed leucocytes from human and cynomolgus monkey (n=3) were stained with CTV and cultured in vitro for 5 days in presence of increasing concentration of ICT01 w/o IL-2 (20IU/ml). % Proliferation was analyzed by Flow Cytometry based on CTV dilution.

RESULTS:

1. IL-2 significantly enhanced ICT01-mediated γ9δ2 T-cell proliferation with almost 100% of proliferating γ9δ2 T-cell compartment in the combination group versus ~30% with ICT01 alone.
2. The effects of ICT01+IL-2 combination on γ9δ2 T cell expansion appeared similar in humans and cynomolgus monkeys.

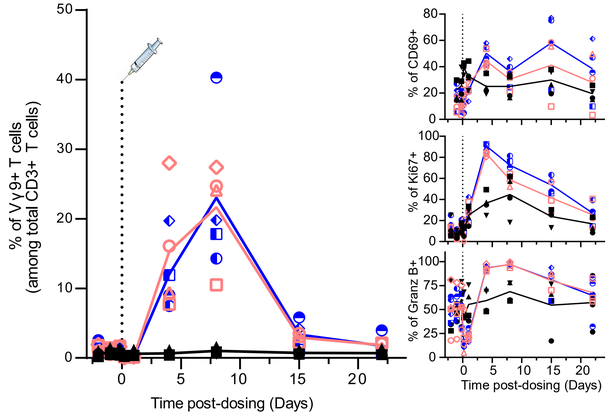
2. ICT01+IL-2 Induces Vγ9Vδ2 T Cell Expansion in NCG Mice Engrafted with Hu-PBMCs



RESULTS: Flow cytometry analysis of mice blood revealed a 5.5-fold increase in human γ9δ2 T-cell number in the combination groups compared to ICT01 or IL-2 alone treated animals, with γ9δ2 T-cell frequency reaching ~35% of the CD3+ T-cell compartment.

3. ICT01+ IL-2 Induces Significant Expansion & Long-term Activation of Vγ9Vδ2 T Cells in Cynomolgus Monkey

Group Number	Group Description	Dose Level ICT01 (mg/kg) IV	Dose Level IL-2 (million IU/animal once daily for 5 days per cycle) SC	Animals/ Group Males
1	Vehicle /IL-2	0	1	4 M
2	Low ICT01/ IL-2	1	1	4 M
3	High ICT01/ IL-2	5	1	4 M

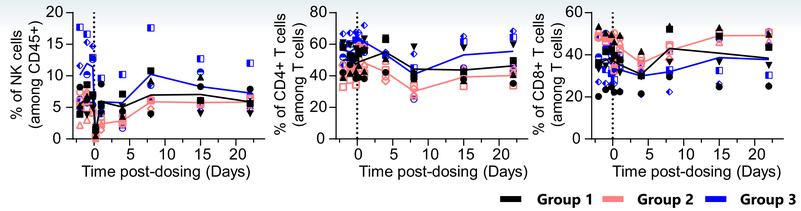


Cynomolgus blood samples collected at indicated times after first IL-2 or ICT01+IL-2 dosing, were stained with specific mAb cocktails to analyze the frequency an activation status of immune cell compartments by flow cytometry. Individual animals are represented with different symbols.

Group 1 (IL-2)
Group 2 (IL-2 + ICT01 1mg/kg)
Group 3 (IL-2 + ICT01 5 mg/kg)

RESULTS: Expansion and activation of peripheral γ9δ2 T-cells from ~1-2% at baseline to 10-30% of T cells on Day 8 in the combination treatment arms, which was not observed with IL-2 alone.

4. No Effect of ICT01 on NK, or CD4 and CD8 T Cells while IHC Shows γ9δ2 T Cells in Several Organs in ICT01+IL-2 Treated Cynos

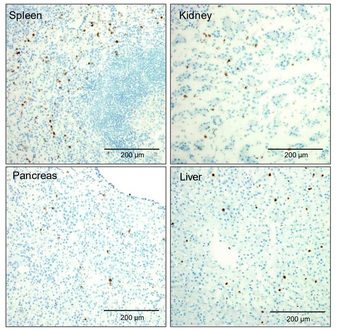


RESULTS:

1. No ICT01+IL-2 effect observed on other immune cell populations
2. Trend towards higher numbers of γ9δ2 T cells in several organs in ICT01+IL-2 treated monkeys, however, since these were healthy monkeys there was little expectation for a significant build up in any single organ/tissue.

ADDITIONAL OBSERVATIONS:

- No evidence for a systemic cytokine release syndrome at any time point.
- No evidence for cytotoxic effects on normal BTN3A expressing cells in blood or tissues.
- Adverse effects (AEs) with variable severity were observed (hypoactivity apathy, emesis, and reversible prolongation of activated partial thromboplastin time, and reversible changes in clinical chemistry (decreased albumin and total protein, increased creatinine and triglycerides).
- Most AEs were reversible and commonly associated with IL-2 alone but possibly more pronounced in the ICT01+IL-2 groups.
- **These toxicities were not reported in the 4-week GLP toxicity study with ICT01 alone at IV doses up to 100 mg/kg/week.**



Frozen tissue sections of a Group 2 animal immunostained with an anti Vγ9 specific mAb and DAB Map detection kit (Ventana reagents).

Conclusion and Clinical Perspectives

These results demonstrate that ICT01+IL-2 profoundly and selectively expands and activates γ9δ2 T cells, which is not observed with IL-2 alone.

The clinical combination of ICT01 plus a lymphoproliferative cytokine (e.g., IL-2) or a novel cytokine variant may be a powerful therapeutic approach for maximizing the immunotherapeutic potential of γ9δ2 T cells.

Currently, a new monkey study is ongoing to identify a safe and pharmacologically active combination of ICT01 + IL-2 or one of the novel cytokine variants.

References:

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