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Enhancement of anti-tumor immunity by ICT01: a novel γ9δ2 T cell-activating antibody targeting Butyrophilin-3A (BTN3A)

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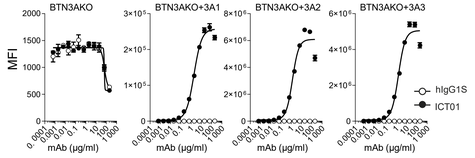
Background:

γδ T-cells are innate-like lymphocytes described as potent killers of cancer cells whose infiltration into tumors is associated with a positive prognosis[1,2]. γ9δ2 T-cells are the major γδ T cell sub-population in peripheral blood in humans and non-human primates. During infection or tumorigenesis, phosphoantigens accumulate in the cell bind to Butyrophilin-3A1 (BTN3A1) leading to a conformational change and subsequent activation of γ9δ2 T-cells[3] as shown by the production of IFNγ and TNFα, cytolysis of target cells, and interplay with other immune cells. γ9δ2 T-cells are regarded as an interesting target in cancer immunotherapy.

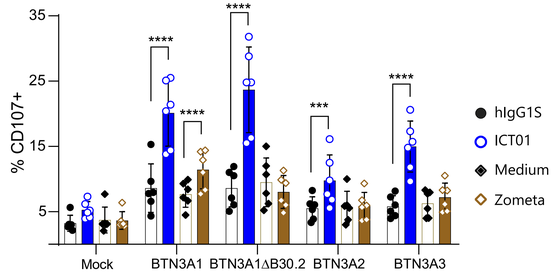
ImCheck Therapeutics is developing ICT01, a humanized Fc-silenced IgG1 anti-BTN3A, that activates γ9δ2 T-cells for the treatment of patients with solid or hematologic tumors.

1- ICT01 Binds to BTN3A1, 3A2, and 3A3 with High Avidity and Specificity & Triggers γ9δ2 T-cells Activation



A. ICT01 binds to BTN3A1, BTN3A2 and BTN3A3 with similar avidity.

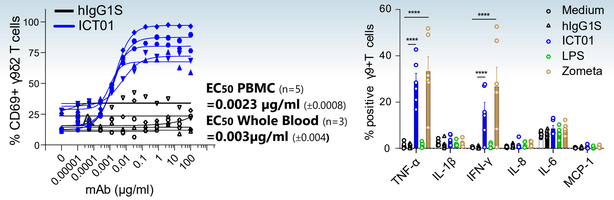
Hek293T BTN3A-KO cells or transiently transfected with BTN3A1, BTN3A2 or BTN3A3 isoforms were stained with increasing concentrations of ICT01 or isotype control (hIgG1S). Binding avidity was evaluated by flow cytometry.



B. ICT01 activates γ9δ2 T cells through 3A1, 3A2 and 3A3 isoforms binding on target cells and does not require pAgs, which only activate via 3A1

Hek293T BTN3A-KO cells transiently transfected with BTN3A1WT or lacking intracellular B30.2 domain (ΔB30.2), BTN3A2WT or BTN3A3WT were treated with ICT01 or isotype control (hIgG1S) (1µg/ml 2 hours) or Zometa (50µM over-night) and co-cultured with *in vitro* expanded γ9δ2 T cells (E:T ratio 1:1). After 4 hours, % of CD107ab positive γδ T cells was monitored by flow cytometry.

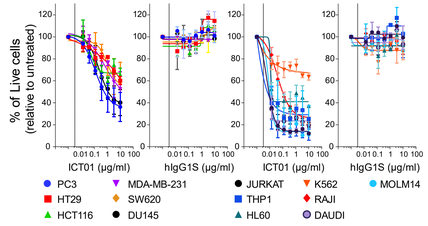
2- ICT01 Triggers γ9δ2 T Cell Activation and IFNγ & TNFα Production in human PBMC *in vitro*



A. Fresh PBMCs of healthy donors (n = 5) were incubated with increasing concentrations of ICT01 or isotype control (IgG1S) for 2 days. Frequency of activated CD69+Vγ9+T cells (identified as CD14-CD19-CD3+Vγ9TCR+) was assessed by Flow Cytometry. EC50 of γ9δ2 T cell activation upon ICT01 stimulation in PBMC and whole blood are indicated as mean ± SEM.

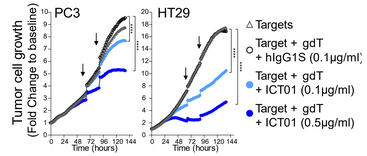
B. Fresh PBMCs of healthy donors (n = 6) were incubated with ICT01 or isotype control (hIgG1S) (10µg/ml), LPS (0.01µg/ml) or Zometa (10µM) for 1 day. Percentages of Vγ9+T cells positive for indicated cytokines were evaluated by flow cytometry. Data are mean ± SEM. P value calculated using 2way ANOVA and Holm-Sidak's multiple comparisons test. ****p<0.001.

3- ICT01-Activated γ9δ2 T Cells Selectively Kill Malignant Cells with No Effects on BTN3A-Expressing Healthy Cells



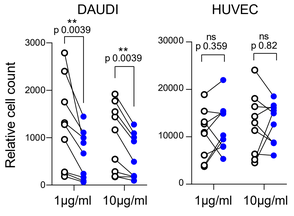
A. Dose dependant killing of multiple cell lines.

Indicated tumor cell lines were co-cultured with *in vitro* expanded γ9δ2 T cells (E:T ratio 1:1) in presence of increasing concentration of ICT01 or isotype control (hIgG1S) with rhIL-2 (20 IU/ml). After 24 hours, the ATP level in the culture was monitored using CellTiter Glo reagent generating a luminescent signal proportional to the amount of living cells. The figure shows the % of live cells calculated for each target cell lines as percentage of luminescent signal in untreated condition.



B. Sustained serial tumor cell Killing by ICT01-activated γ9δ2 T cells in human PBMC.

Indicated fluorescently labelled tumor cell lines were incubated with PBMCs of healthy donors (n=4) at 10:1 E:T ratio in the presence of ICT01 or isotype control (hIgG1S). Tumor cell growth was assessed by live imaging using Incucyte instrument. Arrows indicate fresh target cells added every 24hrs in the co-culture.

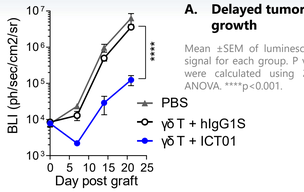


C. Normal BTN3A-expressing cells are not targeted by ICT01-activated γ9δ2 T Cells

PBMC from 9 healthy donors were incubated overnight with PKH26-stained HUVEC and CTV stained Daudi cells at an E:T ratio of 100:1, and ICT01 at 1 or 10µg/ml (blue circle) or isotype control (hIgG1S; black open circles) used at the same concentrations. The relative numbers of CTV+ CD19+ Daudi cells (left panel) or PKH26+ HUVEC cells (right panel) were quantified by flow cytometry using counting beads. Results are expressed as relative cell numbers by dividing the number of target cells by the number of counting beads. P value calculated using Wilcoxon test: **, p<0.01.

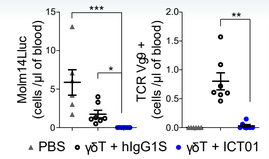
4- ICT01 Delays Tumor Growth and Prolongs Survival in Mouse Hematologic Tumor Models

NSG mice engrafted with the human AML BTN3A+ tumor cell line (MOLM-14) and treated weekly by 3x10⁶ adoptively transferred human γ9δ2 T cells and IL-15/IL15-Rα complexes and bi-weekly with ICT01 or isotype control (10mg/kg)



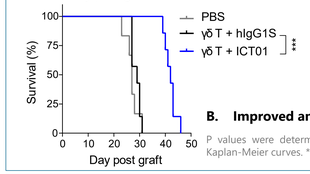
A. Delayed tumor growth

Mean ±SEM of luminescence signal for each group. P value were calculated using 2way ANOVA. ****p<0.001.



C. Decreased circulating tumor cells & γ9δ2 T cells tissue margination

Flow cytometry analysis of circulating MOLM-14 and γ9δ2 T cells at Day 19 post-tumor cell graft. P values calculated using 1way ANOVA. *p<0.05, **p<0.01, ***p<0.005.



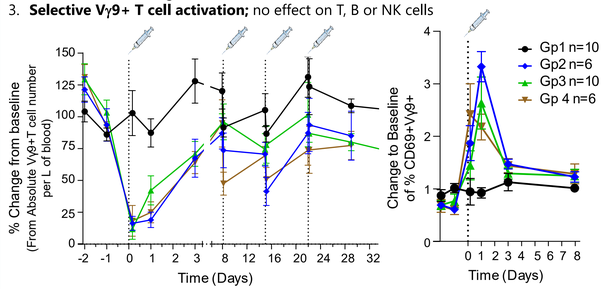
B. Improved animal survival

P values were determined using the log-rank test based on Kaplan-Meier curves. ***p<0.005.

5- ICT01 in Cynomolgus Monkeys: Good Safety, Predicted PK, and Specific Activation of γ9δ2 T Cells

Group Number	Group Description	Dose Level (mg/kg) Weekly-4weeks	Animals/Group		Necropsy After	
			Males	Females	4 Weeks	10 Weeks
1	Control	0	5	5	3M / 3F	2M / 2F
2	Low	5	3	3	3M / 3F	- / -
3	Middle	25	5	5	3M / 3F	2M / 2F
4	High	100	3	3	3M / 3F	- / -

1. Pharmacokinetics: **t1/2 approx. 9 days in cynos**, scales to ~21 days in humans
2. **Full target occupancy** achieved on peripheral blood BTN3A-expressing T and B cells within 1hr after dosing for all doses tested
3. **Selective Vγ9+ T cell activation;** no effect on T, B or NK cells



Flow cytometry analysis of circulating immune cells in blood samples. Figure showed the relative to baseline (2 predose occasions) Vγ9+ T cell number and % of CD69+ Vγ9+ T cell for each group at indicated sampling time (Mean ±SEM for each group).

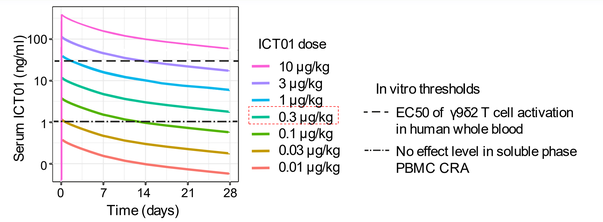
4. **No abnormal clinical observations** (Weight, temperature, etc…)
5. **No cytokine release syndrome**: only mild increase in cytokines (Il-1, IL-2, IL-6, TNFα, IFNγ) after first dose normalizing within 24 hours; no recurrence with subsequent doses
6. Histopath of 1 of 6 animals in 100 mg/kg group showed inflammatory immune infiltrate primarily along GI tract; no associated clinical observations

NOAEL of 25 mg/kg/week

6- FIH Clinical Trial: Starting Dose Rationale

Predicted Serum ICT01 Concentrations in Humans Following a 30-minute IV ICT01 Infusion:

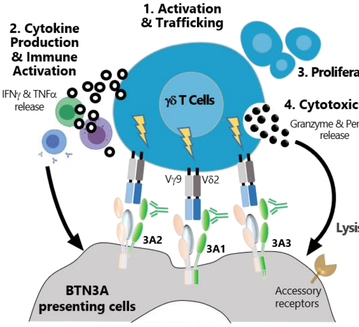
Relationship to In Vitro Concentration Thresholds



The proposed starting dose for Cohort 1 in patients with solid tumors has been based on the EC50 for the intended pharmacodynamic effect of γ9δ2 T cell activation in a whole human blood assay (0.03 µg/mL).

The monkey PK model was scaled to humans and used to simulate clinical doses to maintain first dose Cmax below the EC50 of γ9δ2 T cell activation. A dose of 0.3 µg/kg (flat dose of about 20 µg for a 70 kg adult) is predicted to maintain first dose Cmax below about 10 ng/mL, and would be expected to result in some γ9δ2 T cell activation, which is considered acceptable in view of the lack of adverse effects associated with pharmacodynamically active in vivo doses of 0.1 to 25 mg/kg in monkeys.

Conclusions & Clinical Development Plans



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 by γ9δ2 T cells requires 2nd signal from stressed/malignant/infected cells

ICT01 is currently being evaluated in EVICTION: a first-in-human, dose escalation and cohort expansion study in patients with advanced stage solid and haematological malignancies that have failed approved therapeutic options (NCT04243499). The study is being conducted at multiple clinical study sites in the EU and the US.

References:

1. Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nature Medicine*. 2015;21(8):938–945.
2. Tosolini M, Pont F, Poupot M, et al. Assessment of tumor-infiltrating TCRVγ9Vδ2 γδ lymphocyte abundance by deconvolution of human cancers microarrays. *OncoImmunology*. 2017;6(3):e1284723.
3. Harly C, Guillaume Y, Nedellec S, et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human γδ T-cell subset. *Blood*. 2012;120(11):2269–2279