Correlation of Baseline Circulating Vγ9Vδ2 T Cells Counts and Pharmacodynamic Activity of ICT01 in Cancer Patients: Preliminary Results from EVICTION and a Novel Patient Enrichment Strategy

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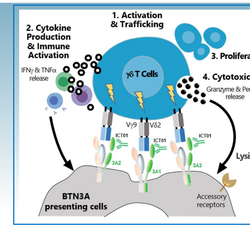
Introduction: ICT01 is a novel anti-BTN3A immunotherapeutic mAb for activating Vγ9Vδ2 cells that is currently being evaluated in EVICTION an ongoing Phase 1/2a clinical trial in patients with solid or hematologic cancers (NCT04243499).

EVICTION is a FIH, 2-part, open-label, multicenter (EU and US), basket trial assessing safety and activity of ICT01 monotherapy and ICT01 in combination with pembrolizumab in patients with advanced-stage, relapsed/refractory cancer.

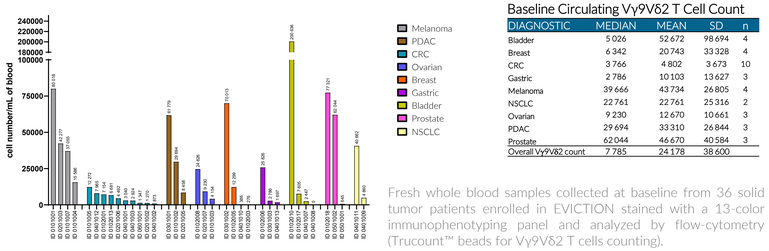
The MOA of ICT01 is to indirectly activate Vγ9Vδ2 T cells that secrete inflammatory cytokines (e.g., INF-γ, TNF–-α), migrate into tumors to coordinate antitumor immune responses, and kill cancer cells. Therefore, we wanted to determine if the baseline number of Vγ9Vδ2 effector cells would be useful as a potential selection criterion for patients more likely to respond to ICT01.

Methods: In Part 1 (Dose Escalation), patients received IV ICT01 as a 30-minute infusion every 3 weeks with doses escalating from 20 µg to 200 mg based on a favorable safety profile (i.e., No DLTs). Blood samples were collected at multiple timepoints for PK (Chimera Biotec, Germany) target occupancy (PrecisionForMedicine, Germany), immunophenotyping and cytokine analyses (PrecisionForMedicine, Germany), . Tumor biopsies were collected at baseline and on day 28 (7 days after the 2nd dose of ICT01) and assessed by multiplex IHC coupled with digital pathology and NanoString for BTN3A and immune cells infiltration and activation (HalioDX/Veracyte, France).



ICT01 MOA
Binds to all three BTN3A isoforms triggering: 1) vδ2 T-cell activation and trafficking from the circulation within 30 minutes, 2) Production of IFNγ and TNFα leading to immune cell activation, 3) Proliferation in the presence of cytokines, and 4) Cytotoxic attack of malignant cells that express a 2nd necessary signal.

1- Baseline Circulating Vγ9Vδ2 T Cell Counts are Variable Between and Within Each Group of Solid Tumor Patients



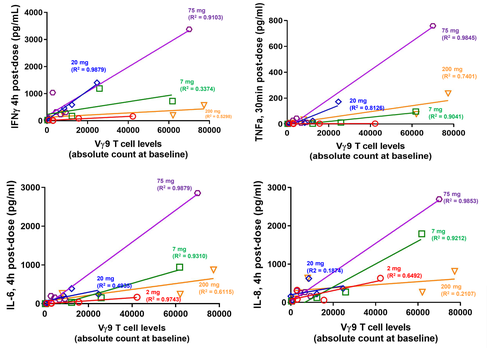
Baseline Circulating Vγ9Vδ2 T Cell Count

DIAGNOSTIC	MEDIAN	MEAN	SD	n
Bladder	5 026	52 672	98 694	4
Breast	6 342	20 743	33 328	4
CRC	3 766	4 802	3 673	10
Gastric	2 786	10 103	13 627	3
Melanoma	39 666	43 734	26 805	4
NSCLC	22 761	22 761	25 316	2
Ovarian	9 230	12 670	10 661	3
PDAC	29 694	33 310	26 844	3
Prostate	62 044	46 670	40 584	3
Overall Vγ9Vδ2 count	7 785	24 178	38 600	

Fresh whole blood samples collected at baseline from 36 solid tumor patients enrolled in EVICTION stained with a 13-color immunophenotyping panel and analyzed by flow-cytometry (Trucount™ beads for Vγ9Vδ2 T cells counting).

Results:
1. Colorectal cancer patients (n=10) had the lowest baseline Vγ9Vδ2 T cells, which may be disease- and/or prior treatment-related
2. Other cancers had a wider range of Vγ9Vδ2 T cell counts, but smaller sample sizes that need to be expanded.
3. No correlation with sex, age, hematology labs, baseline absolute counts or frequency of granulocytes, monocytes, B cells, T cells, CD8, CD4 and NK cells. Prior therapy was too heterogeneous given the different tumor types enrolled.

2- Elevated Serum Cytokine Levels post-ICT01 Dosing are Related to Baseline Circulating Vγ9Vδ2 T Cell Count

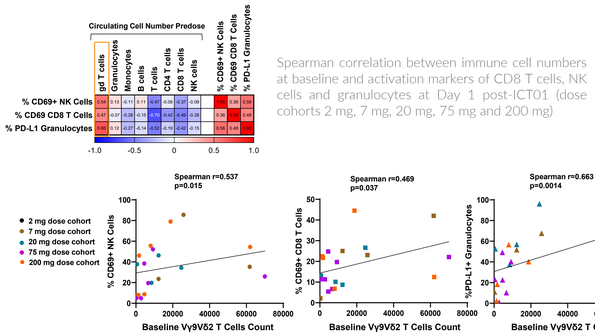


Serum samples collected at baseline, 30min, 4h, 24h, 7 days and 21 days post-ICT01 dosing and tested for 10 cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, TNF-α) and 9 chemokines (Eotaxin, Eotaxin-3, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, TARC) using commercial kits (V-plex, MesoScaleDiagnostics).

Across 2mg, 7 mg, 20 mg, 75 mg and 200 mg ICT01 dose cohorts, maximum levels were observed at 30 min post-dose for TNF-α and at 4 hours post-dose for IFN-γ, IL-6, IL-8, CXCL10, CCL2, CCL21, CCL26, CCL13 and CCL17. Low to very low levels of other tested cytokines and chemokines were observed.

Results:
1. Trend for correlation between Vγ9Vδ2 T cell baseline levels and peak levels of IFN-γ, TNF-α, IL-6 and IL-8 (figures above) and CXCL10, CCL2, CCL21, CCL26, CCL13 and CCL17 (data not shown) across 2 mg dose cohort to 200 mg dose cohort.
2. Trend for ICT01 dose-response from 2 to 75 mg, lower levels observed at 200 mg that may be due to rapid migration of Vγ9Vδ2 T cells out of the circulation before cytokines can be released.

3- Activation Markers on NK cells, CD8 T cells and Granulocytes are also Related to Circulating Vγ9Vδ2 T Cell Counts

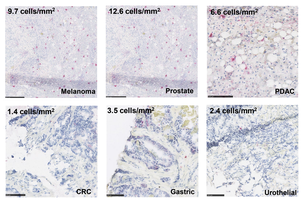


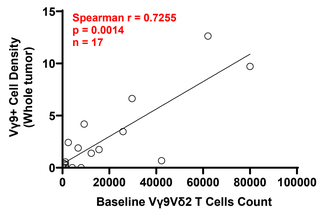
Spearman correlation between immune cell numbers at baseline and activation markers of CD8 T cells, NK cells and granulocytes at Day 1 post-ICT01 (dose cohorts 2 mg, 7 mg, 20 mg, 75 mg and 200 mg)

Fresh whole blood samples collected at baseline from solid tumor patients enrolled in EVICTION stained with a 13-color immunophenotyping panel and analyzed by flow-cytometry. CD69 and PD-L1 activation markers assessed in Vγ9Vδ2, granulocytes, monocytes, B cells, CD4 T cells, CD8 T cells, and NK cells.

Results:
1. ICT01 doses ≥7 mg induce rapid margination from circulation of Vγ9Vδ2 T cells, CD8 T cells and NK cells (ref 1), with increase of CD69 expression in NK cells and CD8 T cells and increase of PD-L1 expression on granulocytes.
2. Trend for correlation between baseline circulating Vγ9Vδ2 T cells and activated circulating CD8 T cells, NK cells and granulocytes.

4- Baseline Vγ9+ Cell Density in Tumors is Related to Baseline Circulating Vγ9Vδ2 T Cell Count

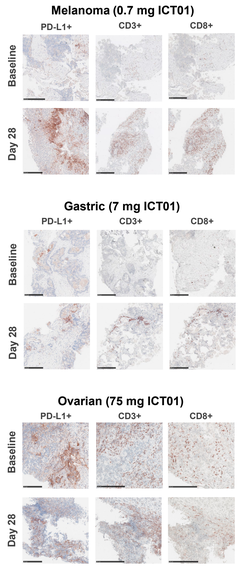


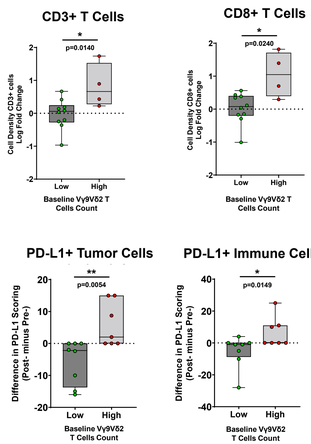


Fresh whole blood samples collected at baseline from 17 solid tumor patients stained with a 13-color immunophenotyping panel and analyzed by flow-cytometry (Trucount™ beads for Vγ9Vδ2 T cell counting).
Vγ9+ cell density (number of cells per mm²) quantified in baseline tumor biopsy using Digital Pathology (fresh frozen tissue, automated cell counts per tumor area, whole tumoral section, HalioDx/Veracyte, Luminy, France). Representative stainings on left panel.

Results:
1. Low baseline Vγ9+ T cells density in tumors from most patients.
2. Trend for correlation between baseline circulating Vγ9Vδ2 T cells and Vγ9+ T cell density in baseline tumor biopsy.

5- Patients with High Baseline Vγ9Vδ2 T Cell Counts Display Higher Tumor Immune Cell Infiltration and PD-L1 expression post ICT01





% of PD-L1+ tumor and immune cells assessed by a trained pathologist on both baseline and post-treatment tumor biopsies (FFPE section). CD3+ and CD8+ cell densities quantified on the same material using Digital Pathology (FFPE section, automated cell counts per tumor area, HalioDx/Veracyte, Luminy, France). Representative stainings for three patients on left panel.

For CD3 and CD8, 14 patients from groups A1-A6 with available biopsy pairs separated into two groups according to baseline Vγ9Vδ2 T cells level (cut-off 20,000 cells/mL) and Log10(FoldChange) in cell density (post- over pre-treatment) were plotted.

For PD-L1, 15 patients from groups A1-A5 with available biopsy pairs separated into two groups according to baseline Vγ9Vδ2 T cells level (cut-off 5,000 cells/mL), and difference in PD-L1 scoring in post- vs. pre- ICT01 treatment biopsies were plotted.

Results:
1. Increased immune infiltration (CD3+ and CD8+ T cells) post-ICT01 dosing in patients with high baseline Vγ9Vδ2 T cells counts.
2. Immune activation (PD-L1-positive cells) post-ICT01 trended higher in patients with high baseline Vγ9Vδ2 T cells counts.

6- Conclusions and Clinical Perspectives

These preliminary results suggest that baseline peripheral Vγ9Vδ2 T cell counts are related to the pharmacodynamic activities of ICT01 in cancer patients. Further analysis of that cell compartment at baseline and throughout ICT01 treatment is currently under investigation through in depth phenotyping by flow cytometry and *ex vivo* functional assays.

A patient enrichment strategy based on baseline Vγ9Vδ2 T cell counts will be evaluated in the expansion arms of EVICTION, where a minimum baseline count will be one of the eligibility criteria.

1- Coordinated Activation of Antitumor Responses of vδ2 and CD8 T Cells by Targeting BTN3A with ICT01 in Patients with Solid Tumors: Aurélien Marabelle, Martin Wermke, Christiane Jungels, Johann de Bono, Norbert Vey, Elena Garralda, Steven Le Gouill, Patricia LoRusso, Aude De Gassart, Patrick Brune, Emmanuel Valentin, Marina Iché, Daniel Olive, Paul A. Frohna. ESMO 2021 - EVICTION Trial Abstract 958O (ID4530)