

A patient-derived lung-on-chip model to evaluate on-target/off-tumour toxicity of the therapeutic FOLR1-targeting T-Cell Bispecific antibody

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AlveoliX
In-vitro models inspired by nature

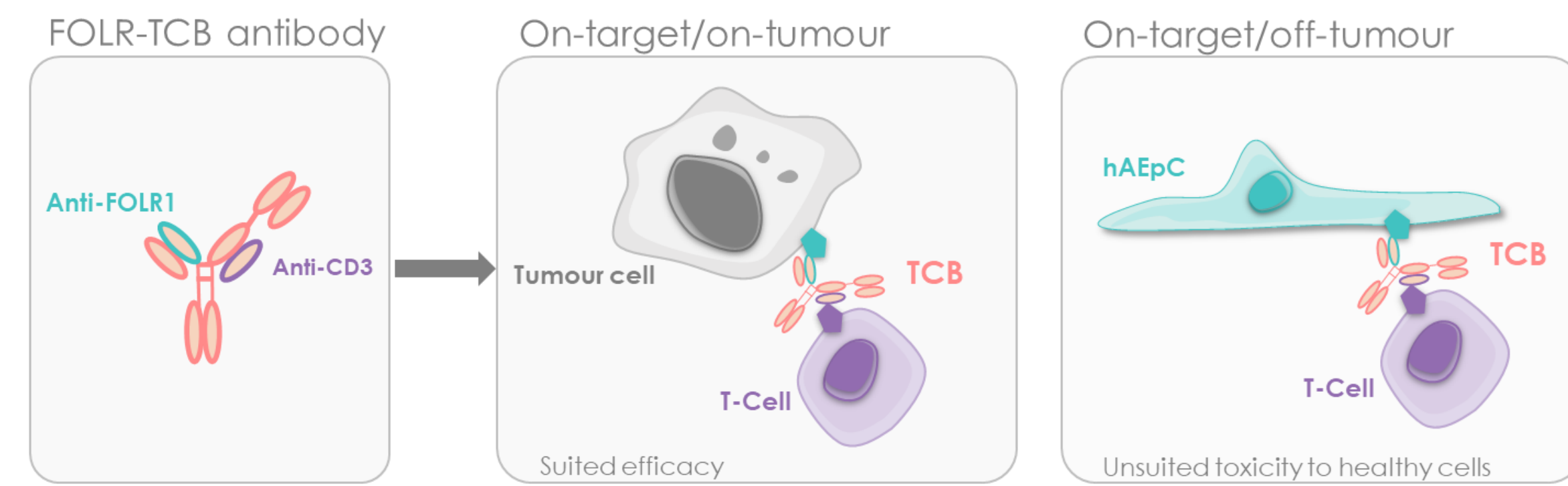


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Introduction

Folate-receptor 1 (FOLR1) is overexpressed in many types of cancer (ovarian, lung, breast) [1], making it a **suitable target** for targeted-tumour immunotherapies. FOLR1/CD3 T-cell bispecific antibodies (FOLR1-TCB) are engineered to recognize both FOLR1 and the T-cell receptor CD3, enhancing tumour targeting by T-cells, recognition and effective killing.

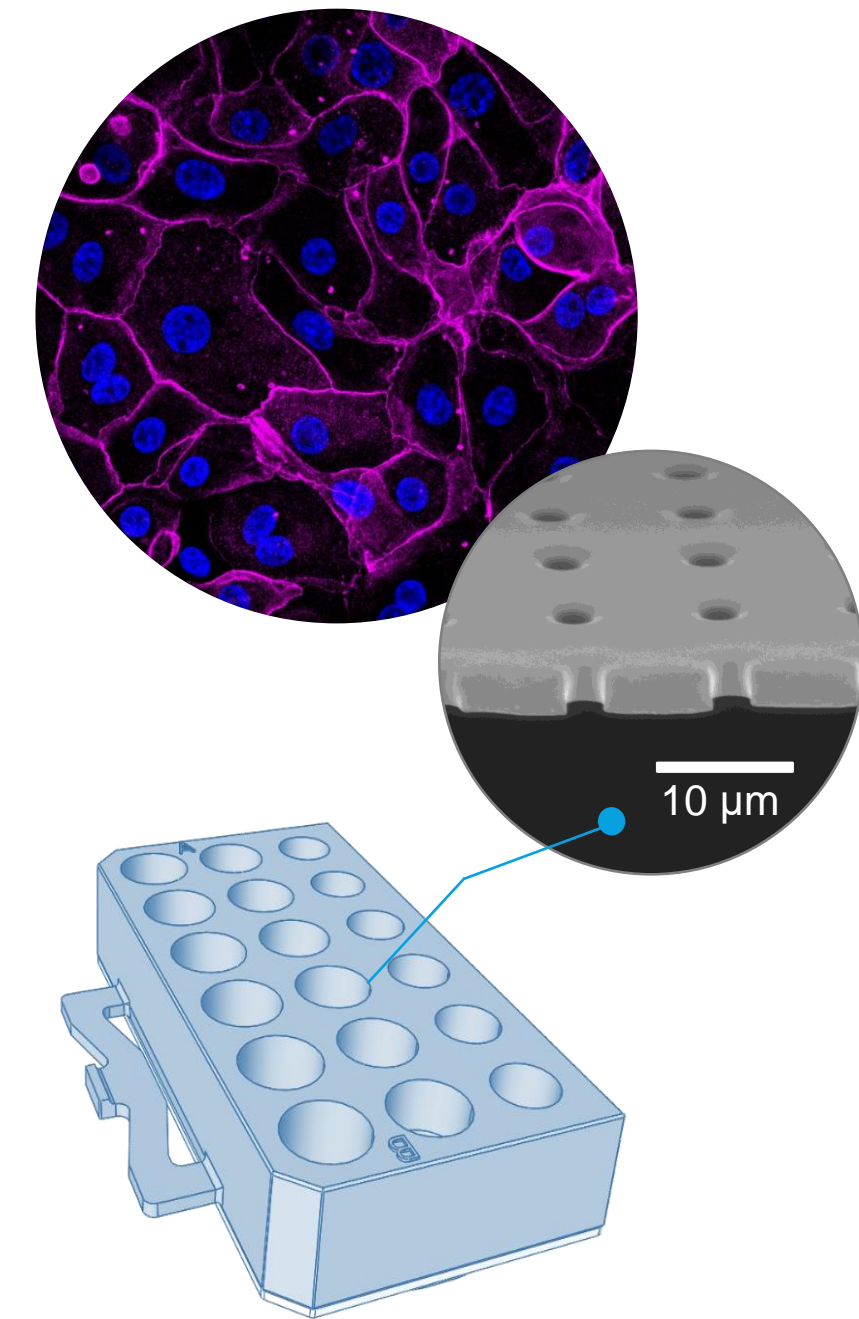
Because FOLR1 is also expressed in normal epithelial cells in the lung or the kidney [2], albeit at lower levels, such therapeutic antibodies present the risk of an **on-target/off-tumour toxicity** [3].



To reliably predict the safety of this clinical candidate, it is necessary to employ relevant human models, that trustfully rebuild the interactions within the key players: (1) human alveolar epithelial cells expressing the target protein, (2) T-cells and (3) a microenvironment simulating the *in-vivo* situation.

Methods

With the aim of establishing a patient-derived lung-on-chip (^{AX}LOC) model with human alveolar epithelial primary cells (^{AX}hAEPc), preliminary proof of concept experiments were performed with immortalized human alveolar epithelial cells (^{AX}iAEC), which capture some important physiological features of ^{AX}hAEPc. ^{AX}iAEC were treated for 72h with the indicated concentration of FOLR1-TCB together with Peripheral Blood Mononuclear Cells (PBMC) on standard 96-well plates or on the AlveoliX LOC [4].



- Standard cell culture systems
- AlveoliX Lung-On-Chip
- Primary-derived cell line
- Patient-derived primary cells

Proof of concept

Safety study

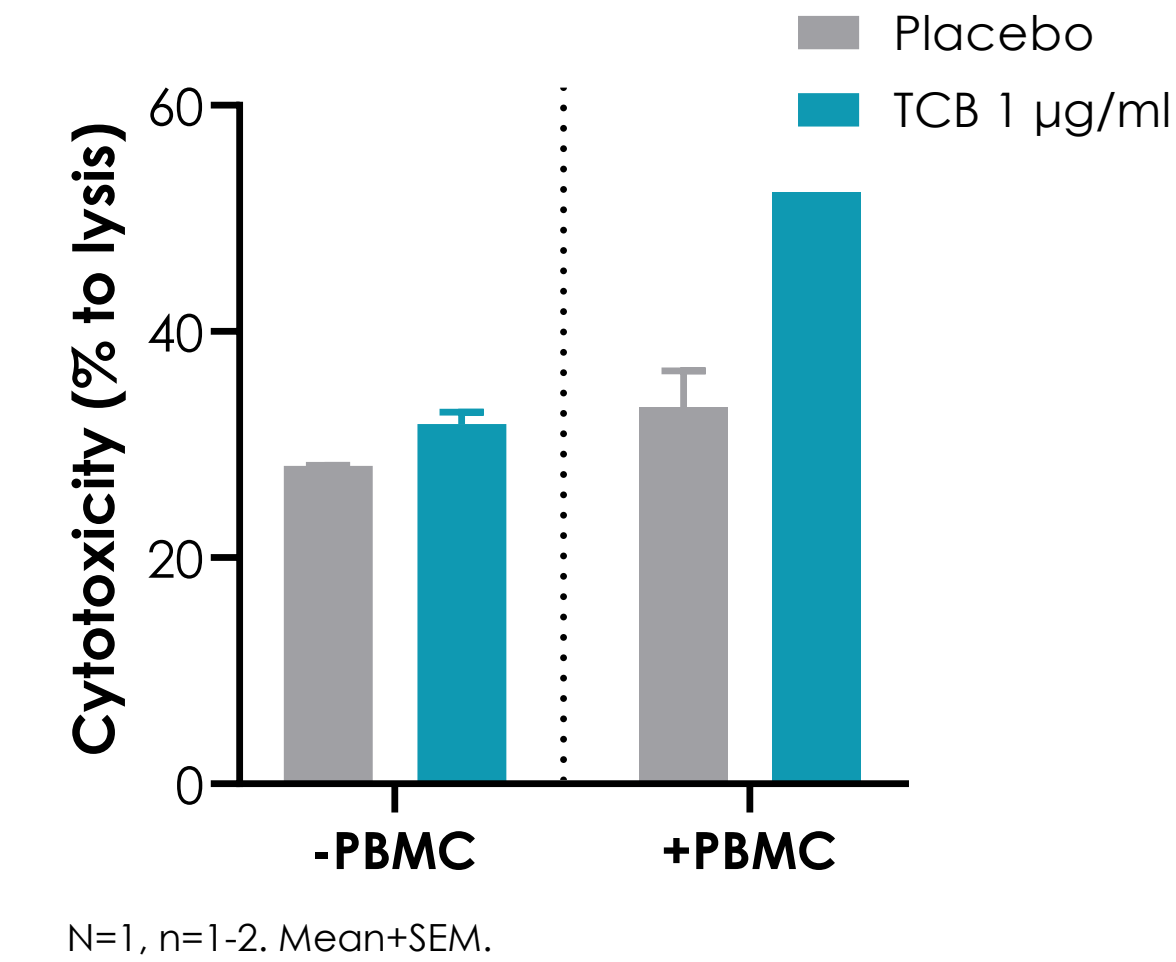
For the safety study, ^{AX}hAEPc derived from human donors, were seeded on the thin membrane of the ^{AX}LOC. Cells were treated for 72h with Placebo or FOLR1-TCB at the indicated concentrations, with PBMC in direct contact with the epithelium. To evaluate treatment effects, barrier integrity (trans-barrier electrical resistance (TER) measurements) and cytotoxicity (membrane damage - LDH, apoptosis - Caspase 3/7 - CellEvent) were assessed over time. Inflammation (multiplex ELISA), immune cell recruitment (CellTrace) and activation (flow cytometry) were also analysed.

Results

Proof of concept with primary-derived cell line

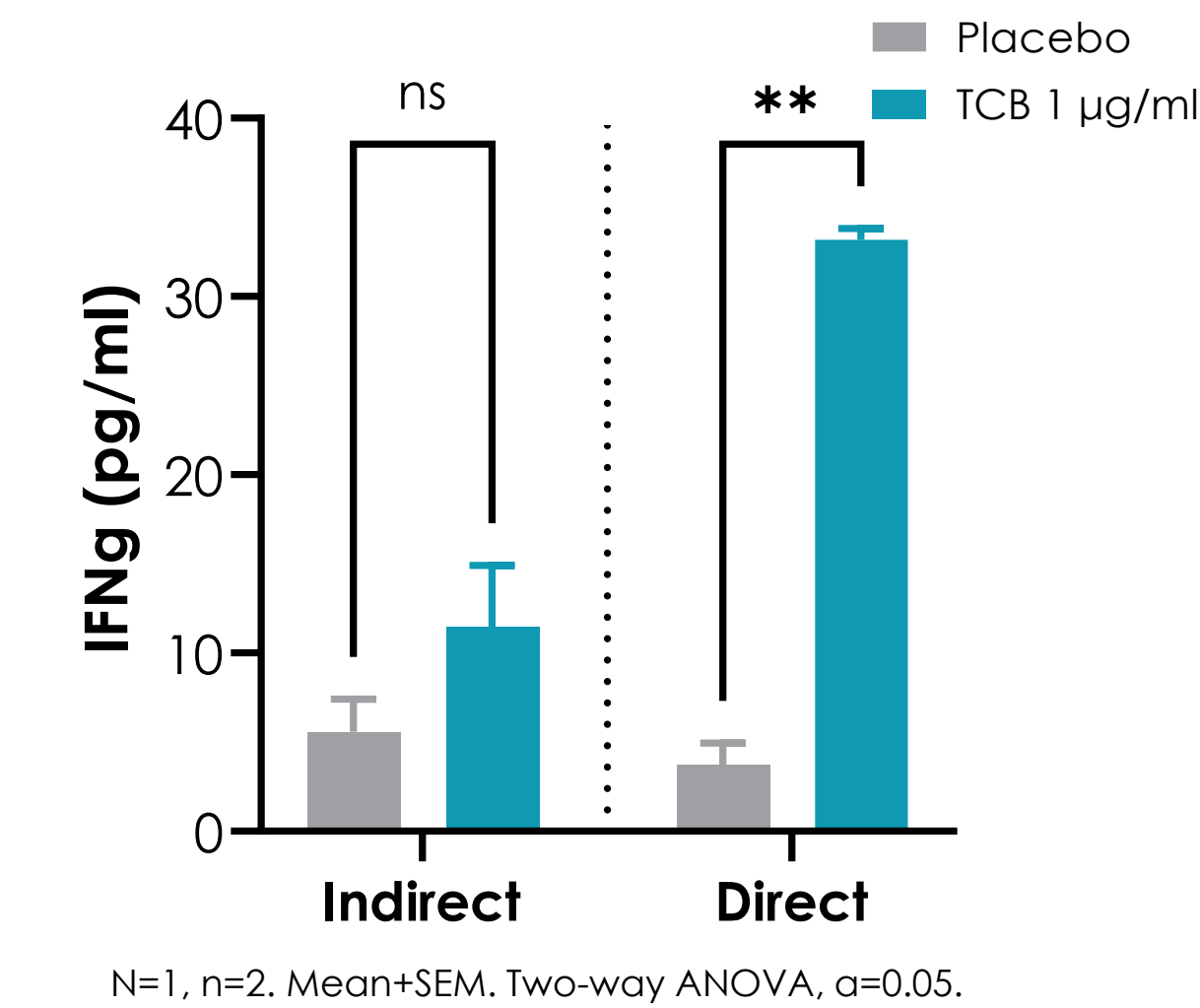
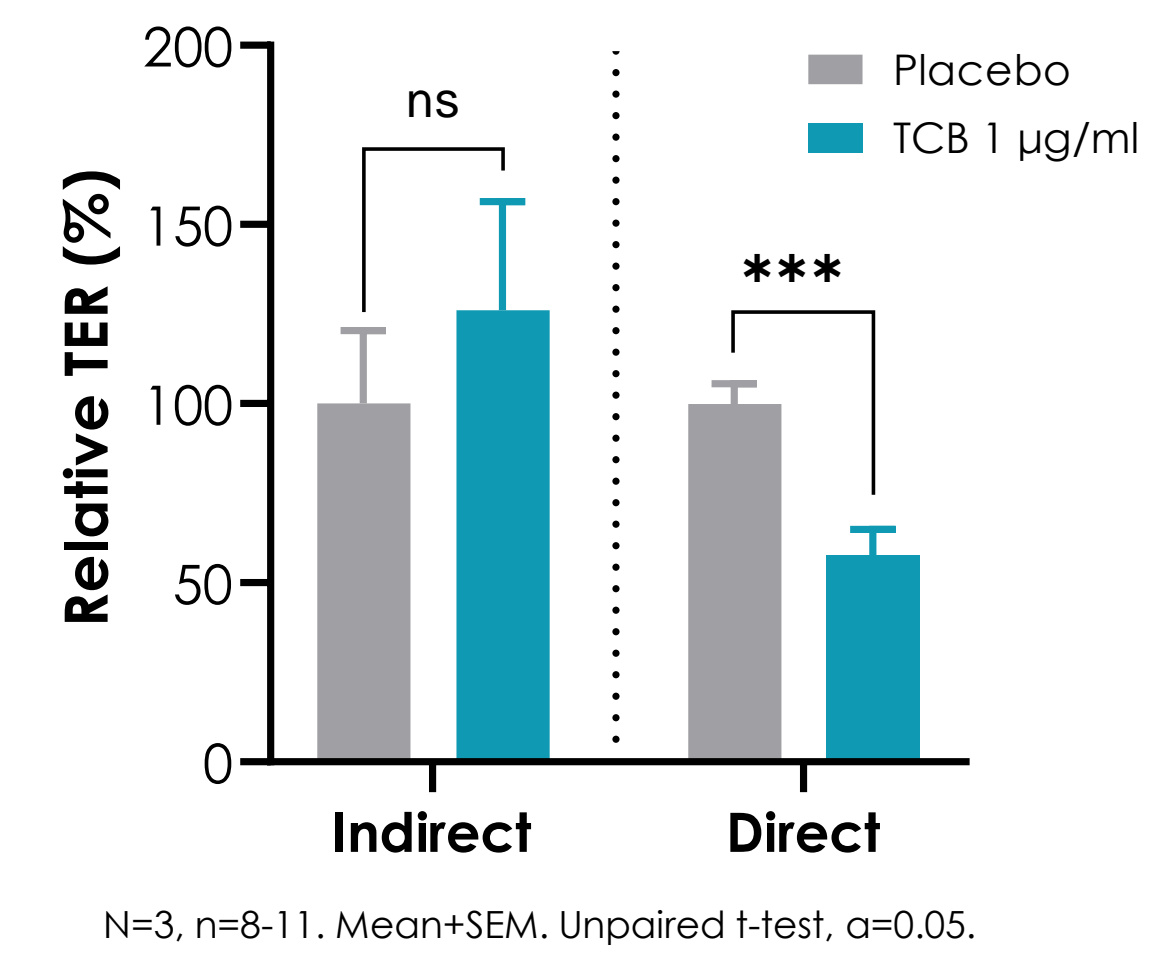
Immune component

FOLR1-TCB causes cytotoxicity only in presence of immune cells, confirming the specificity of its mode of action. It is therefore crucial to consider the immune component in the model.



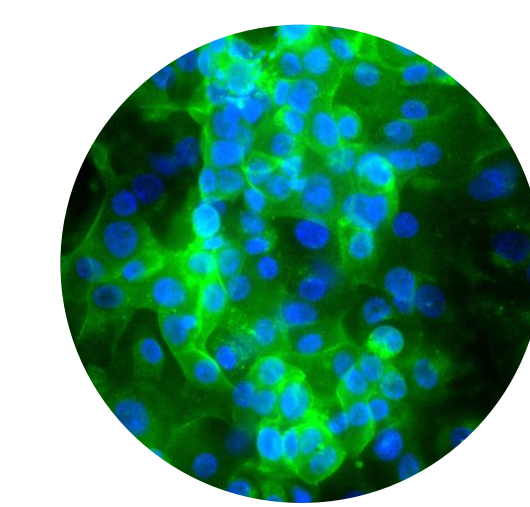
Cell-cell interaction

The effect of direct contact between immune and target cells was investigated on ^{AX}iAEC on the LOC. As it could be expected, direct contact is required in order to observe an effect of FOLR1-TCB, as showed by the drop in TER and the cytokine increase (IFN γ) only in the corresponding group.



Safety assessment of FOLR1-TCB on a patient-derived lung-on-chip

Target detection



FOLR1 IF staining of hAEPc on-chip. N=6 donors (repr. picture). EVOS M700 40x, Fiji.

FOLR1 protein expression was detected by immunofluorescence on hAEPc. Alveolar epithelial cells from six different donors were stained after culture on the ^{AX}LOC.

Recruitment and activation of immune cells

Immune cell recruitment by the epithelial layer was monitored by live imaging using stained PBMCs (CellTrace). The quantification revealed a significant increase in adherent PBMCs in the presence of FOLR1-TCB, proving the specific and effective mechanism of action of this engineered TCB.

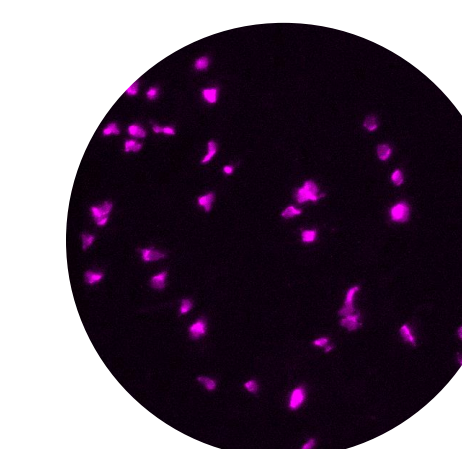


Image processing

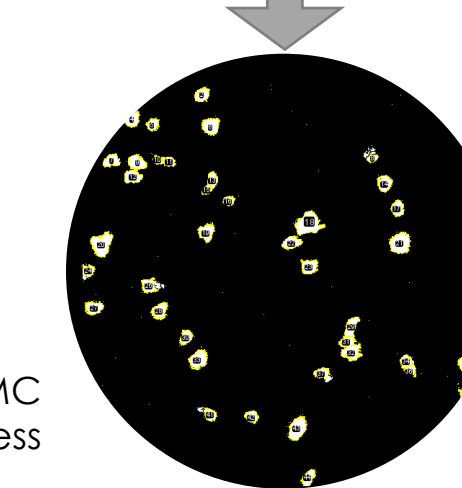
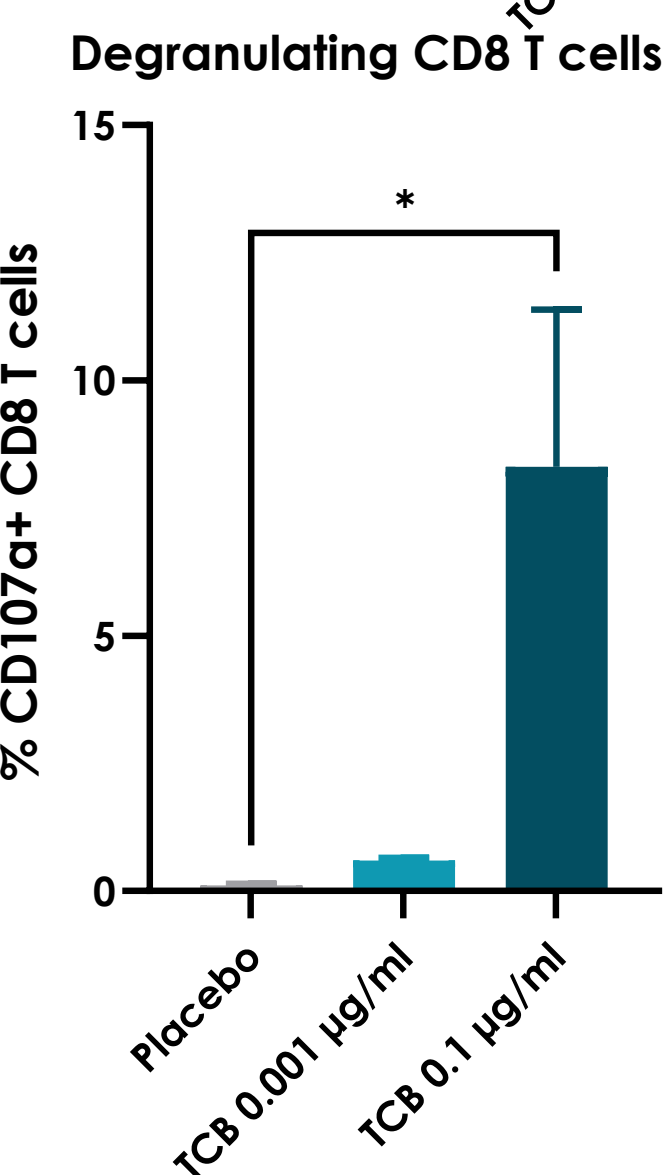
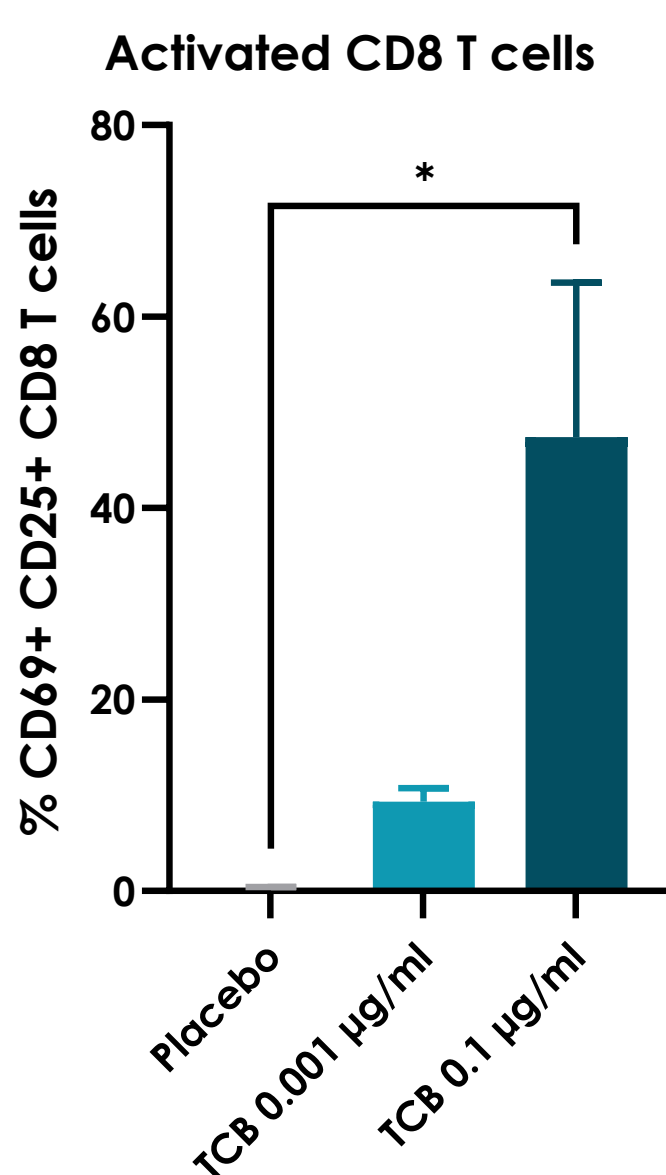
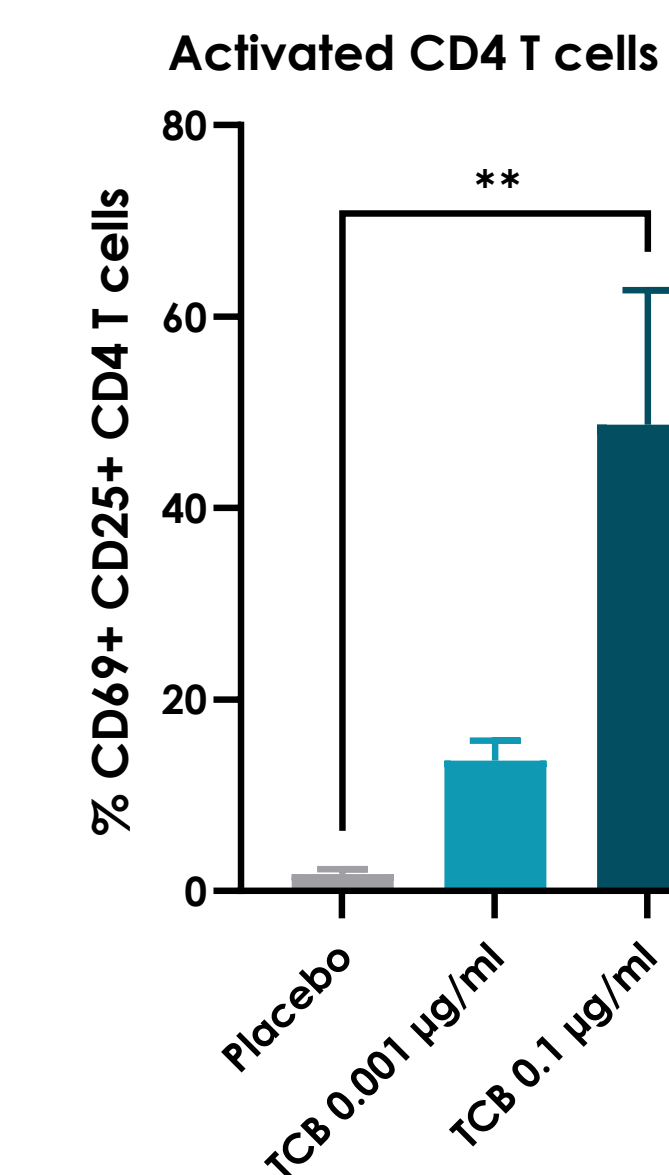
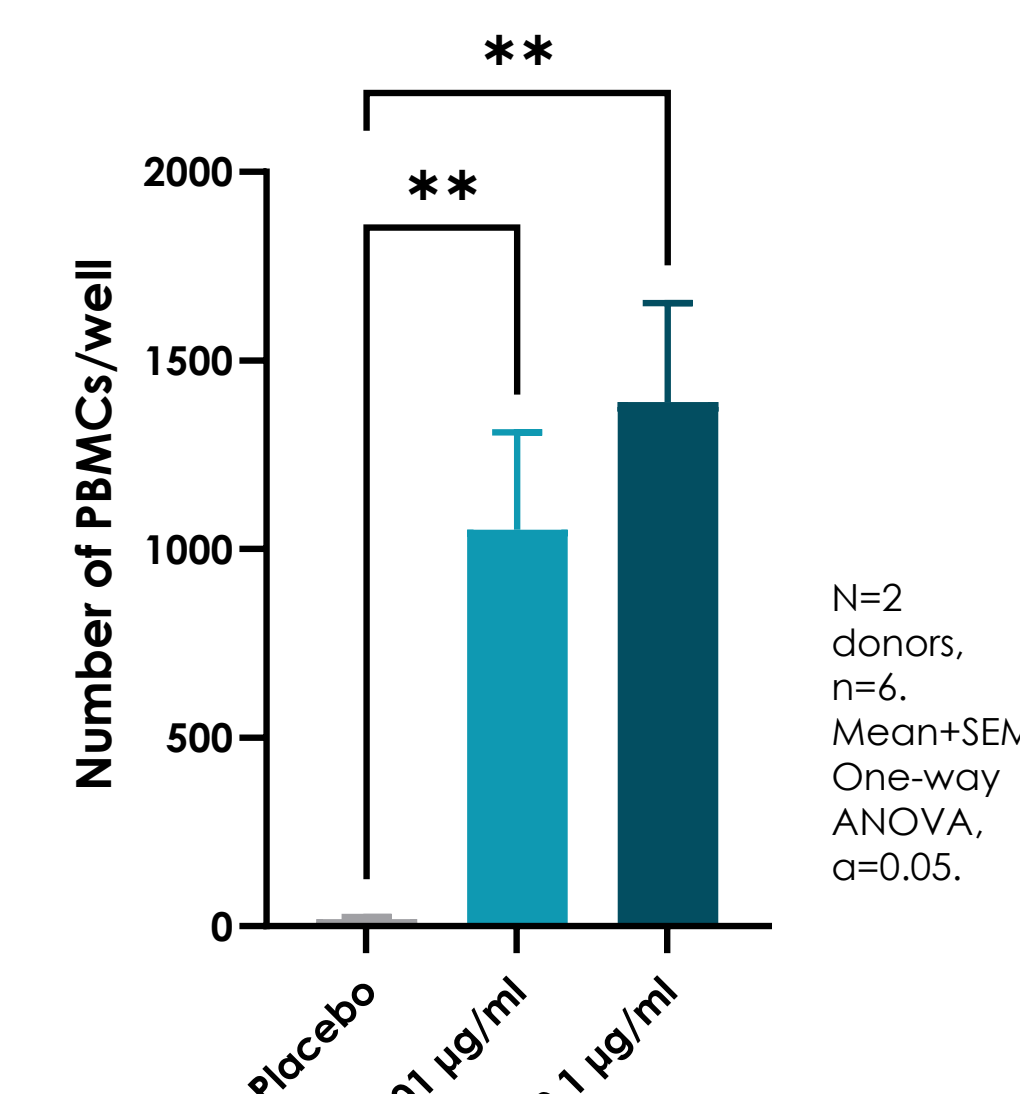


Illustration of PBMC counting process



Harvested PBMC were analysed by flow cytometry. The presence of FOLR1-TCB led to an increased activation of CD4 and CD8 T cells, especially at the highest concentration. The marker 107a+ revealed the presence of degranulating CD8 T cells, eventually involved in the process of killing target cells.

Conclusions

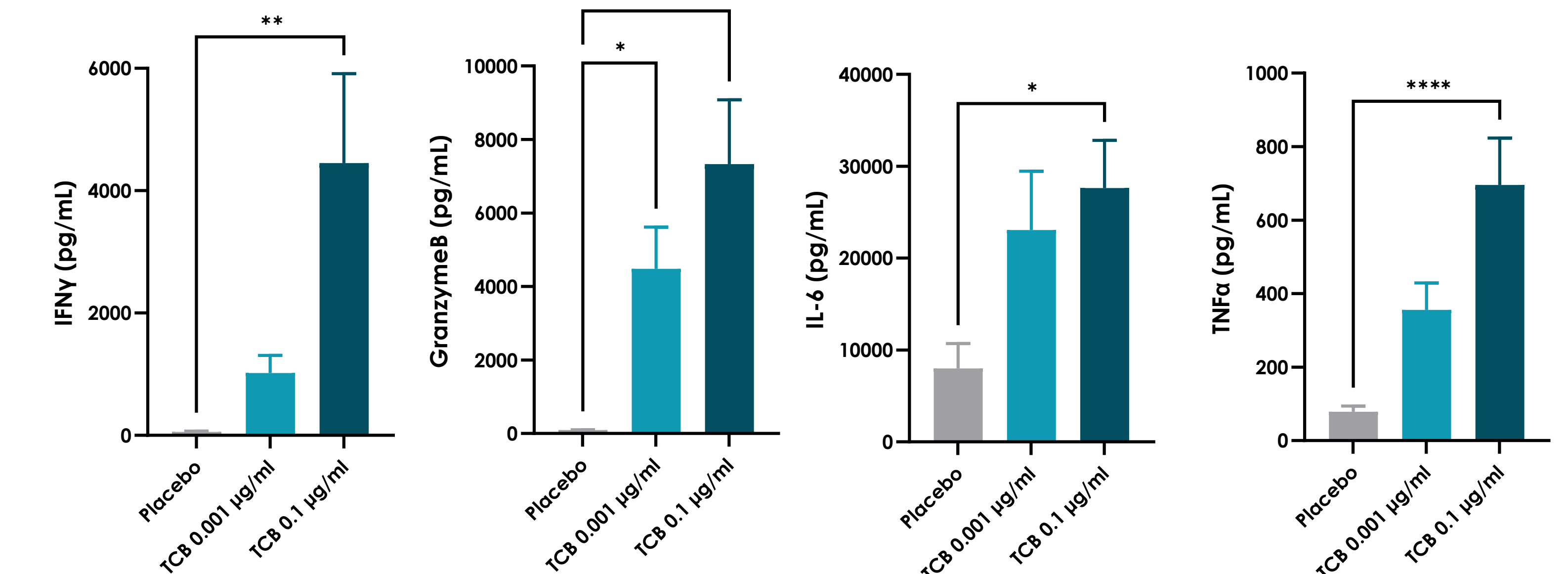
- Our model provides a physiologically relevant reconstruction of the human *in-vivo* situation culturing **patient-derived cells** in a **soft microenvironment** and easily allowing **cell-cell interactions**.
- We developed a reliable platform suitable for **safety assessment** of clinical candidates, easy to **multiplex** with several read-outs.
- Our findings confirm a **possible on-target off-tumor alveolar toxicity** of FOLR1-targeting T cell-engaging therapies and propose a novel *in-vitro* model to **study the mechanisms involved** and to **select safer molecules**.

References

- [1] Scaranti et al (2020) *Nature Reviews Clinical Oncology*
- [2] Parker et al (2005) *Analytical Biochemistry*
- [3] Geiger et al (2020) *Nature Communications*
- [4] Sengupta et al (2022) *Frontiers*
- [5] Boda et al (2018) *Antiviral Research*

Inflammatory response

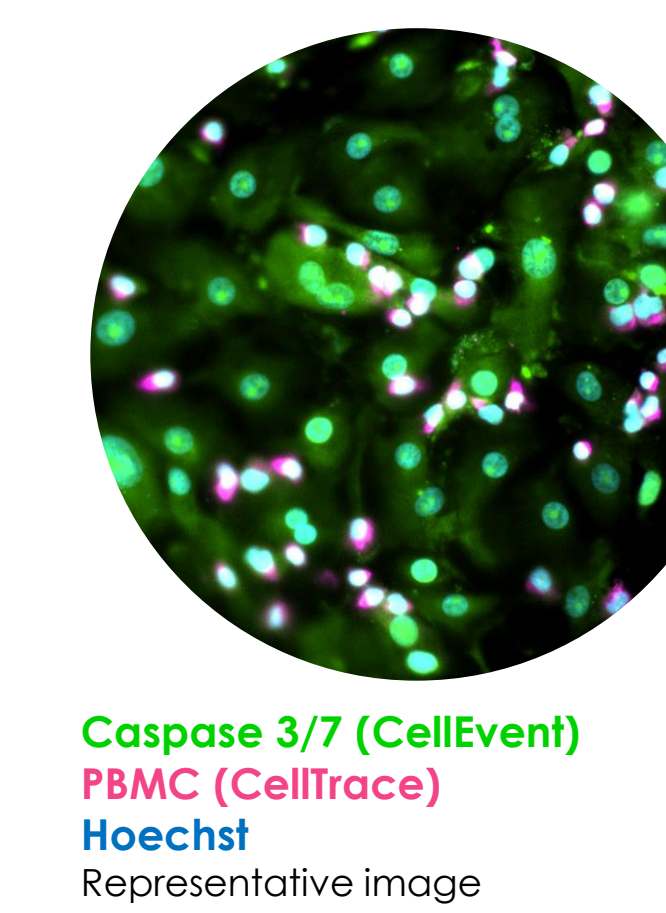
FOLR1-TCB treatment led to a significant and dose-dependent increase in the secretion of cytokines such as IFN γ , TNF α , IL-6 and GranzymeB, indicative of a pro-inflammatory response.



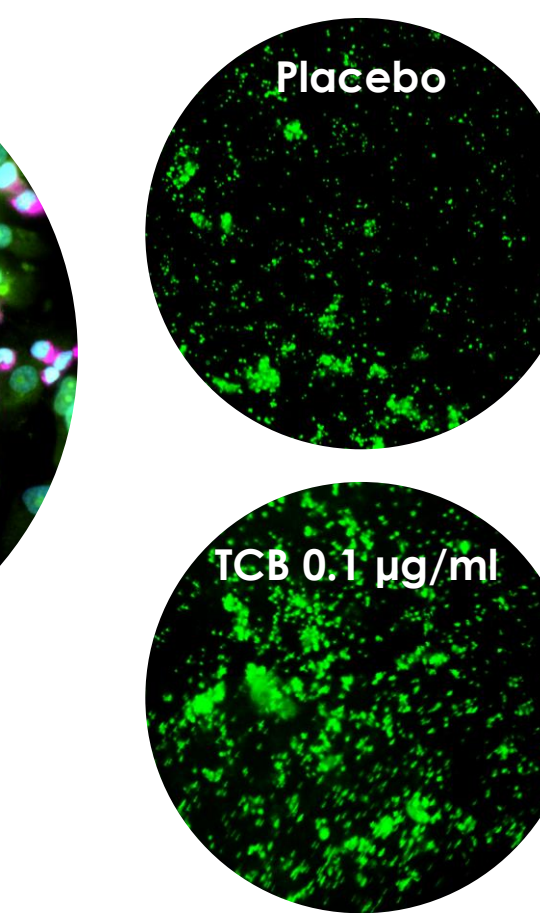
Cytotoxicity

As a measure for cytotoxicity, apoptosis was monitored by live imaging with a probe targeting Caspase 3/7 (CellEvent). After 72h of treatment with FOLR1-TCB, a trend towards increasing apoptosis was observed, in both tested concentrations. LDH release was also measured, as indicator of cell death and membrane damage. A trend towards increasing cytotoxicity was also observed, but only at the highest concentration.

Altogether, this suggests that within this timeframe, FOLR1-TCB triggers apoptosis and leads to membrane permeabilization and damage only at the highest dose, which may reflect late apoptotic events occurring earlier at highest concentrations.



Caspase 3/7 (CellEvent)
PBMC (CellTrace)
Hoechst
Representative image



Representative images of Caspase 3/7. EVOS M700 10x stitching.

