In vitro cell based cytotoxicity and T cell activation assays to assess safety and efficacy of engineered T cell therapies

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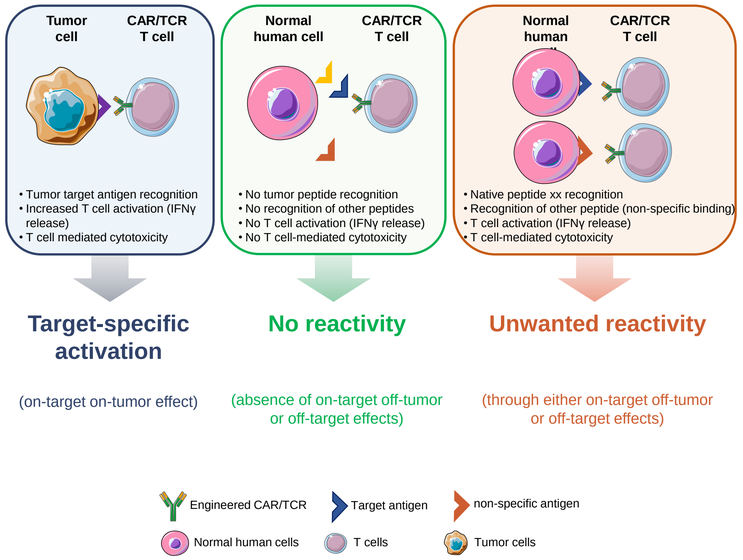
1 INTRODUCTION

Chimeric antigen receptor (CAR) and T cell receptor (TCR) engineered T cells are part of a big wave of immunotherapies showing great promise in cancer clinical trials. With the first T cell based therapies targeting hematological malignances now approved, their next challenge is solid tumors. Solid tumors create an additional challenge due to the lack of tumor specific target antigens, posing significant safety risks, i.e. on-target on-tumor, on-target off-tumor and off-target toxicities. Toxic effects previously reported vary from mild-severe cytokine release syndrome (CRS) to neurotoxicity and death.

We have developed *in vitro* assays utilizing human cells from healthy tissue and/or differentiated iPCS-derived cells to assess on-target off-tumor and/or off-target cytotoxicity for engineered T cell therapies. The presence of CAR-T cell mediated cytotoxicity was measured through co-culture with healthy human cells to assess unwanted CAR-T reactivity as well as a high target antigen expressing control cancer cell line to confirm CAR-T functionality. The human cell type was selected based on its potential safety risk by establishing low level protein expression of the target antigen. Readouts included IFNγ production determined by MesoScale Discovery platform as a measure of T cell activation and Hoechst/PI staining of target cells by flow cytometry.



2 CAR-T CELLS PRODUCTION

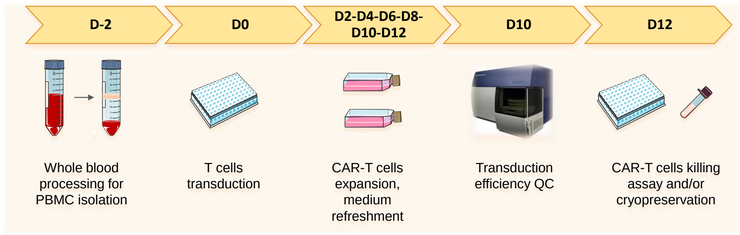


Fig. 1. Schematic overview of CAR-T cells production. PBMCs were freshly isolated from human buffy coats (D-2) and cultured for 2 days with IL-2 and CD3/CD8 beads to enrich T cell population. Two days later T cells were transduced (D0) using a second generation lentivirus containing a 4-1BB co-stimulatory domain and a 2A-EGFRt tag for transduction detection. Transduced T cells were cultured and expanded until day 12 (D12). A fraction of CAR-T cells was collected to assess transduction efficiency QC (quality check) by flow cytometry at day 12 (D12), while the rest of CAR-T cells was pooled and, either used for *in vitro* T cells-mediated killing assay, or cryopreserved.

3 CAR-T CELLS FUNCTIONALITY ASSESSMENT

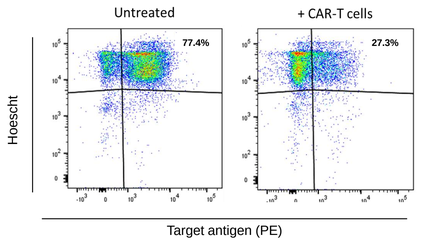


Fig 2. Targeted depletion of target antigen-expressing cells following co-culture with CAR-T cells. CAR-T cells were co-cultured with cells expressing the target antigen. After 48h cells were stained with a cell viability dye (Hoescht, y-axis) and an antibody specific for the target antigen (x-axis). Stained cells were analyzed by flow cytometry. CAR-T cells strongly depleted target antigen expressing cells (upper right quadrant, 27.3%) as compared to untreated cells (without CAR-T cells; upper right quadrant, 77.4%).

Table 1. Example of cell types utilized by CRL within various T cell therapy cytotoxicity *in vitro* assays.

Tissue/Organ	Cell type	Source
Heart	Cardiomyocytes	iPSC-derived or primary cells
Kidney	Glomerular epithelial cells	Primary cells
Lung	Bronchial epithelial cells	Primary cells
Liver	Hepatocytes	iPSC-derived or primary cells
CNS	Neurons	iPSC-derived
CNS	Astrocytes	iPSC-derived or primary cells
Vasculature	Vascular endothelium cells	Primary
Bone marrow	Bone marrow mononuclear cells	Primary
Blood	Whole blood	Primary cells
Tonsils	Tonsils	Primary cells

4 CAR-T CELLS MEDIATED KILLING

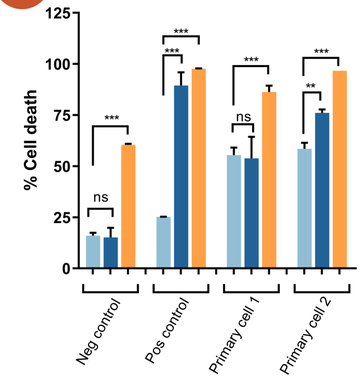


Fig 3. Quantification of CAR-T cell mediated cytotoxicity. CAR-T cells were co-cultured with control cells; expressing high levels of the target antigen (Pos control) or lacking the expression of the target antigen (Neg control) and with two at risk primary cell types. After 48h cells were collected and stained with viability/cell death dyes (Hoescht/PI) and analyzed by flow cytometry. CAR-T cells showed good functionality by exhibiting a strong capacity to kill target antigen-expressing cells (pos control), while no unwanted CAR-T cell reactivity in the Neg control condition was observed. Primary cell type 1 showed a clear absence of response from the CAR-T cells, where a ~20% increase in killing was observed against Primary cell 2 indicating a potential safety risk. The Toxicity control confirmed the functionality of the assay by measuring strong cell cytotoxicity in both conditions.

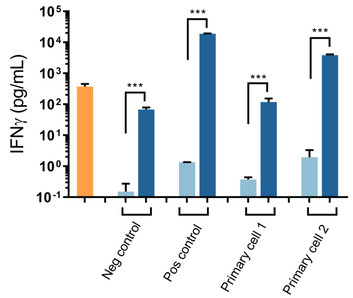


Fig 4. IFNγ production as a measure of CAR-T cells functionality. CAR-T cells were co-cultured with control cells; expressing high levels of the target antigen (Pos control) or lacking the expression of the target antigen (Neg control) and with two at risk primary cell types. After 48h CAR-T cell activation was assessed by measuring IFNγ release using a MesoScale Discovery platform. Increased expression of IFNγ was observed for all CAR-T culture conditions compared to untreated cells, with the Neg control and Primary cell 1 showing similar levels to the CAR-T only condition, indicating background expression. For the Pos control and Primary cell 2 an increased IFNγ expression above background was observed, indicating CAR-T cell specific activation.

5 CONCLUSION & PERSPECTIVES

Safety risks associated with cell based IO-therapies is the biggest challenge for the success of these therapies, performing a thorough safety screen on healthy primary human tissues is therefore crucial. Our study generated high quality data of the CAR/TCR cell, confirming functionality by showing consistent T cell activation and killing against a positive control cell line. Moreover, we were able establish a clear absence of activity against human cells thus providing insight into the safety of the CAR/TCR therapy.

The developed safety assays provide a robust and rapid platform to assess on-target off-tumor and off-target effects within immuno-oncology therapies, either TCR or CAR T cells, in both early stage development or late stage testing of the therapeutic product. Through inclusion of a wide range of human primary cells, both high risk tissues and major organs at risk of off-target toxicity, a clear safety profile can be generate *in vitro* for these novel T cell therapies.This study demonstrates the added value of *in vitro* cell-based assays for the translation of experimental IO-therapies to the clinic.