Tumour organoid and T cell co-cultures to evaluate CAR-T cell cytotoxicity

Poster #908

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Introduction

Recent advances in cancer immunotherapy had a positive impact on the life expectancy of patients for a large range of clinical indications and the number of patients eligible for cancer immunotherapy is expected to expand steadily with the development of new therapies and combination strategies. However, promising therapeutic developments face hurdles in translating preclinical findings into therapy since conventional two-dimensional (2D) cancer models hold low clinical predictive value. HUB has developed an innovative alternative, building on the finding that adult stem cells proliferate and organise into three-dimensional organotypic structures when they are embedded into extracellular matrix. Patient-derived organoids – or HUB Organoids® - are generated from normal and malignant tissues and stored as biobanks with high quality and reproducibility. HUB Organoids recapitulate complex characteristics of the original parental tissue, including molecular heterogeneity, and morphological and functional traits. Organoid can be used to test reactivity of engineered T cells as demonstrated by recently published work(1).

Since cancer progression and responses to immunotherapy are determined by immune cell interactions in the tumor microenvironment, we developed an assay in which CRC tumor organoids were co-cultured with T cells modified with chimeric antigen receptors (CAR-T cells) to assess their cytotoxic potential. CAR-T cell therapy has been shown to be efficacious against many haematological malignancies, however its therapeutic application to treat solid cancers remains challenging(2). Our organoid and T cell co-cultures offers a good platform to study the response of tumor organoids to CAR-T cells and will greatly contribute to our understanding of the critical factors that determine a successful CAR-T cell therapy.

Methods

- Target antigen expression in CRC PDOs was characterized by flow cytometry (**Figure 2**)
- Expression of checkpoint molecules such as PD-L1 was evaluated in CRC-PDOs by flow cytometry (**Figure 3**)
- Tumor reactivity of CAR-T cells was evaluated in co-cultures with CRC-PDOs. PDO killing and T cell activation was detected via activation of caspase 3/7 apoptotic signal and further confirmed with IFNγ secretion by ELISA (**Figure 4**)

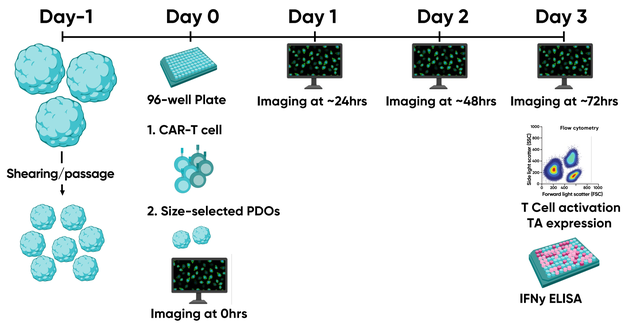

Figure 1. Schematic overview of CRC PDO and T cells co-culture assay to assess CAR-T activity. Workflow describing the different data collection timepoints and readouts used in PDO and T cell co-culture assays to evaluate CAR-T cell activity.

Results

We tested the cytotoxicity of CAR-T cells which target a specific target antigen on human CRC organoids. Six HUB CRC organoid models were selected based on differential expression levels of the target antigen which was confirmed by flow cytometry analysis. To evaluate CAR-T cell mediated organoid killing, caspase 3/7 signal (readout for apoptosis) was monitored at various timepoints and IFNγ secretion was measured by ELISA to evaluate T cell activation. Staurosporine-treated organoids, organoids alone, and nonspecific (NS) CAR-T cells co-cultured with organoids were all included as separate controls in all assays. CRC-organoid killing is consistent with antigen expression levels in organoids, indicating the specificity of the tested CAR-T cells. Nevertheless, some CRC-organoid models were shown to be less susceptible to CAR-T cell mediated killing, and further evaluation revealed upregulation of check-point molecules such as PD-L1 in these models, implying potential resistance of these organoid models to specific cell killing.

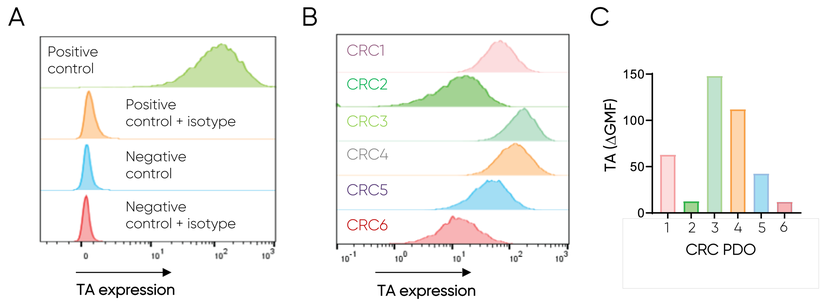

Figure 2. Flowcytometry characterization of target antigen expression in 6 CRC PDO models. **A** Histograms showing target antigen (TA) expression in Positive and Negative controls (cell lines positive or negative for TA). Isotype antibody was used as a negative control for the staining. **B.** Histograms showing TA expression in six CRC PDOs **C.** Bar graphs quantifying ΔGMF of positive cells for TA in six CRC PDOs. ΔGMF was calculated based on the geometric mean fluorescence intensity (MFI) difference between of samples and isotype control

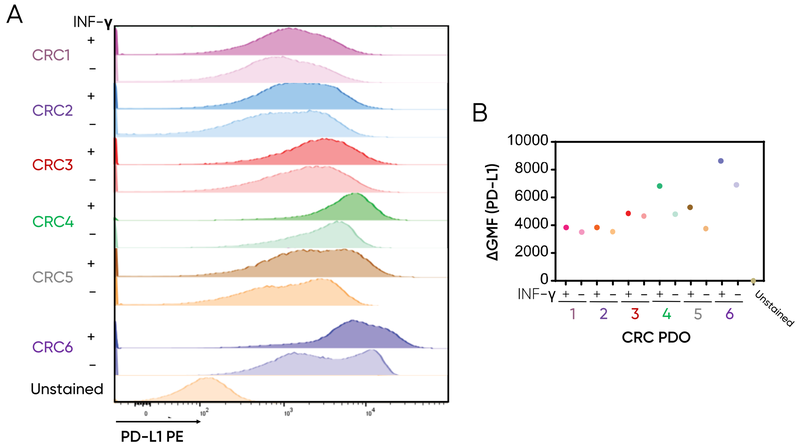

Figure 3. Flow cytometry characterization of PD-L1 in 6 CRC PDO models. **A.** Histograms showing PD-L1 expression in 6 CRC PDOs with and without IFN-γ stimulation. Quantification of **B.** ΔGMF and of PD-L1 positive cells on 6 CRC PDO models. ΔGMF was calculated based on the geometric mean fluorescence intensity (MFI) difference between of samples and unstained control.

Summary and conclusions

HUB Organoids and T cell co-cultures offers a clinically relevant platform to study the response of tumor organoids to CAR-T cells, contributing to our understanding of critical factors determining patient response in the clinic. We tested CAR-T cells cytotoxicity against tumor organoids and uncovered a possible mechanism for lack of response in some models. Our platform represents an innovative and patient-relevant approach to prioritize lead candidates for clinical application.

- HUB Organoids represent intra- and inter-tumor heterogeneity and recapitulate patient response
- Expression of immune check-point molecules can be measured in PDOs
- Co-culture assays have been established with CRC-PDOs and can be used to detect cytotoxicity of CAR-T cells.

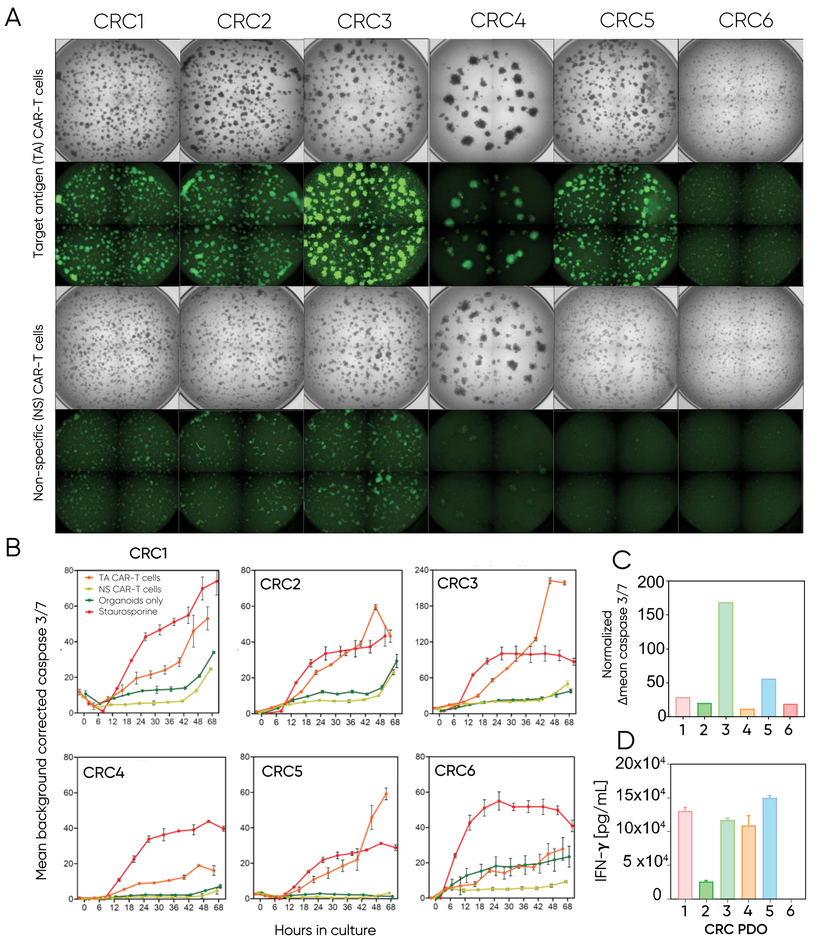

Figure 4. Anti-tumor cytotoxicity of CAR T cells against 6 CRC models. **A.** Representative bright field and Caspase 3/7 fluorescence images at 68 hours showing various levels of organoid killing (green signal) in co-culture with TA-CAR T cells (top panels) and NS-CAR T cells (lower panels) **B.** Time course quantification of caspase 3/7 fluorescent signals in TA-CAR T cells and NS-CAR T cell co-cultures. Staurosporine and only organoids were used as positive and negative technical controls, respectively. N=2, **C.** Δ Mean fluorescence intensity of caspase 3/7 signal at 68-hours. The signal from TA-CAR T cell co-cultures were normalized to the one from NS-CAR T cells. (Δ mean fluorescence intensity= MFI induced by TA-CAR T cells - MFI induced by NT-CAR T cells.) **D.** IFN-γ levels secreted by TA-CAR T cells after co-culture with different CRC PDO models.

References

1. Dekkers *et al*, Uncovering the mode of action of engineered T cells in patient cancer organoids; Nat Biotechnol (2022). https://doi.org/10.1038/s41587-022-01397-w
2. Aparicio *et al*, Cell Therapy for Colorectal Cancer: The Promise of Chimeric Antigen Receptor (CAR)-T Cells; Int. J. Mol. Sci. 2021, 22(21), 11781; https://doi.org/10.3390/ijms222111781

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