Organoid co-cultures with autologous T cells to assess toxicity and efficacy of bispecific antibodies

Poster #2924

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Introduction

Recent advances in cancer immunotherapy positively impacted the life expectancy of patients for an extensive range of clinical indications. With new treatment strategies and druggable targets being identified at an increasing pace, the number of patients eligible for cancer immunotherapy is expected to expand steadily. However, promising therapeutic developments face hurdles in translating preclinical findings into therapy since conventional 2D cancer models hold low clinical predictive value. HUB developed an innovative alternative, building on the discovery that adult stem cells proliferate and organize into three-dimensional organotypic structures when embedded into an extracellular matrix. Patient-derived organoids (PDOs) 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.

Since cancer progression and responses to immunotherapy are governed by immune cell interactions in the tumor microenvironment, we developed an assay in which non-small cell lung cancer (NSCLC) tumor organoids are co-cultured with autologous T cells to evaluate the activity of a bispecific antibody (BsAb), to assess its cytotoxic potential. Using our co-culture assays, we show T cell-mediated killing of tumor organoids expressing the antigen targeted by the BsAb. PDO killing correlated with target antigen expression and BsAb concentration. Our organoid and T cell co-cultures offer an excellent platform to study the response of tumor organoids to T cell BsAb (T-BsAb) and will significantly contribute to our understanding of the critical factors determining successful immunotherapies.

Methods

- Organoid and T cell co-cultures were established to assess activity of a test T-BsAb, an image-based readout was validated for organoid killing, and T cell activation measured via quantification of IFN-γ secretion by ELISA (**Figure 1**)
- Expression of Target Antigen (TA) for the T-BsAb was confirmed by immunohistochemistry which was performed on parental normal and tumor tissue as well as on tissue-derived normal and tumor PDOs (**Figure 2.**)
- Tumor reactivity induced by the T-BsAb was evaluated in T cells co-cultures with paired NSCLC PDO and normal PDO. PDO killing was detected via image-based analysis of caspase 3/7 apoptotic signal in and further confirmed by ELISA measurement of IFN-γ secretion from T cells (**Figure 3**).

Results

We tested the cytotoxicity and efficacy of a T-BsAb targeting a tumor-specific antigen on human NSCLC organoids. Three HUB NSCLC organoid models were selected based on target antigen expression that was confirmed by histochemistry and flow analysis (data not shown). Results presented only corresponds to one of the three PDO models. To evaluate T-BsAb mediated organoid killing, caspase 3/7 expression was monitored at various timepoints and IFNγ secretion was measured by ELISA. For control, CD3/CD28 trans-activator beads and a nonspecific T-BsAb were included in the assay. T-BsAb-dependent organoid killing correlates with antigen expression levels, indicating the specificity of the tested T-BsAb.

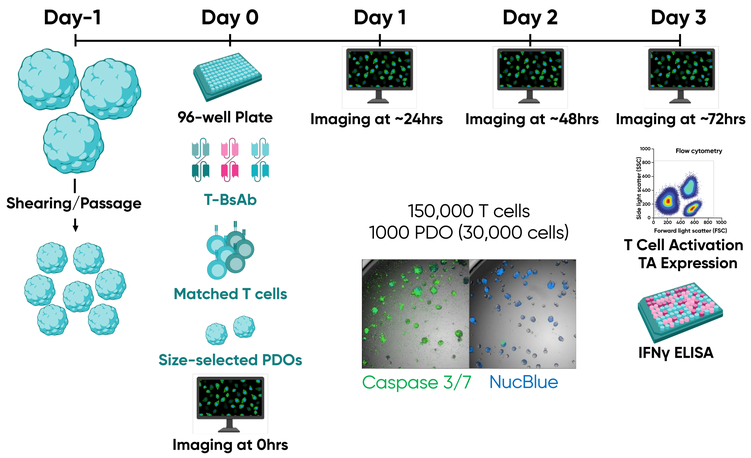

Figure 1. NSCLC PDO and TIL co-culture to assess T-BsAb activity. Schematic workflow describing different data collection timepoints and readouts used to evaluate T-BsAb activity using PDO and TILS co-cultures.

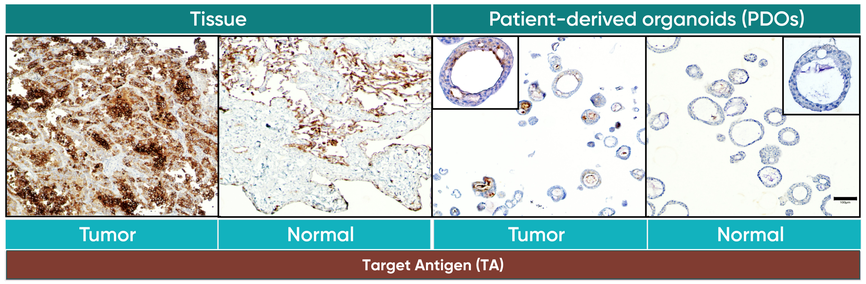

Figure 2. Expression of T-BsAb target antigen in tumor PDOs and original tissue. Representative histological sections of lung tissue (left panels) and PDOs (right panels) stained with an antibody that recognizes TA. Magnified images of single PDOs are also provided. Scale bar equals to 100 µm.

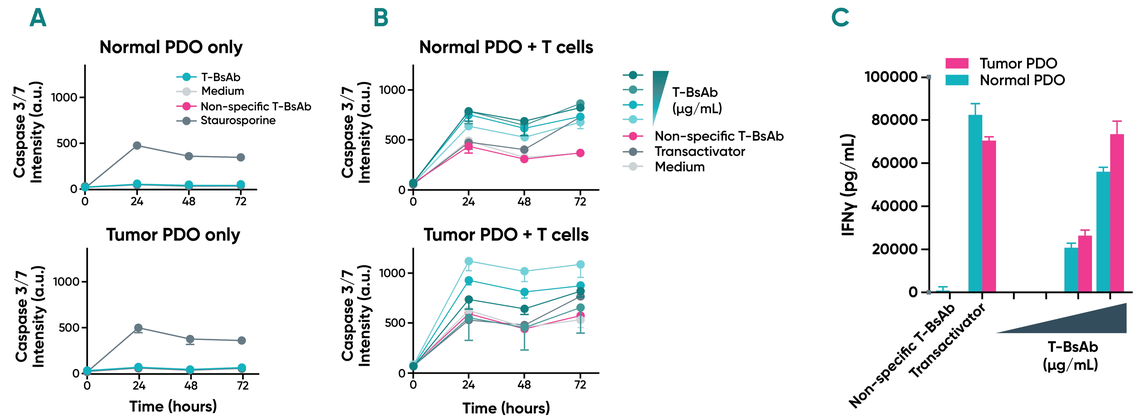

Figure 6. Tumor cytotoxicity induced by T-BsAb in a matched paired tumor-normal NSCLC derived PDO model. A. Time course quantification of caspase 3/7 fluorescent signal in tumor (right panel) and normal (left panel) PDOs when exposed to different control conditions. For organoid killing, staurosporine is a positive control while medium is a negative control. In absence of T cells, T-BsAb (TA and nonspecific) did not induce any caspase signal. B. Time course quantification of caspase 3/7 fluorescent signal in tumour (left panel) and normal (right panel) PDOs when exposed to different concentrations of TCB. Transactivator was included as positive control for T cell activation. C. IFN-γ levels secreted by T cells in co-culture with PDOs and the presence of nonspecific-TCB and different concentrations of TA T-BsAb. Transactivator was included as positive control for inducing IFN-γ secretion.

Summary

- HUB Organoids represent intra- and inter-tumor heterogeneity and recapitulate patient response.
- Expression of targeted antigens can be measured in HUB Organoids
- Co-culture assays have been established with NSCLC HUB Organoids and are able to detect cytotoxicity of T cell bispecific antibodies.

Conclusion

HUB Organoid Technology allows adult stem cells to proliferate and organise into three-dimensional organotypic structures, representing original tissue genetics and phenotype. Patient-derived organoids are generated from normal and malignant tissues and stored as biobanks. Using our organoid-immune cell co-culture system, we assess the toxicity and efficacy of bispecific antibodies targeting tumor antigens. Readouts include image-based analysis, flow cytometry, and cytokine release as measurements of T cell activation and cytotoxicity against autologous tumour and normal organoids.

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