Autologous organoids and T cell co-cultures as a powerful personalized platform for immunotherapy development

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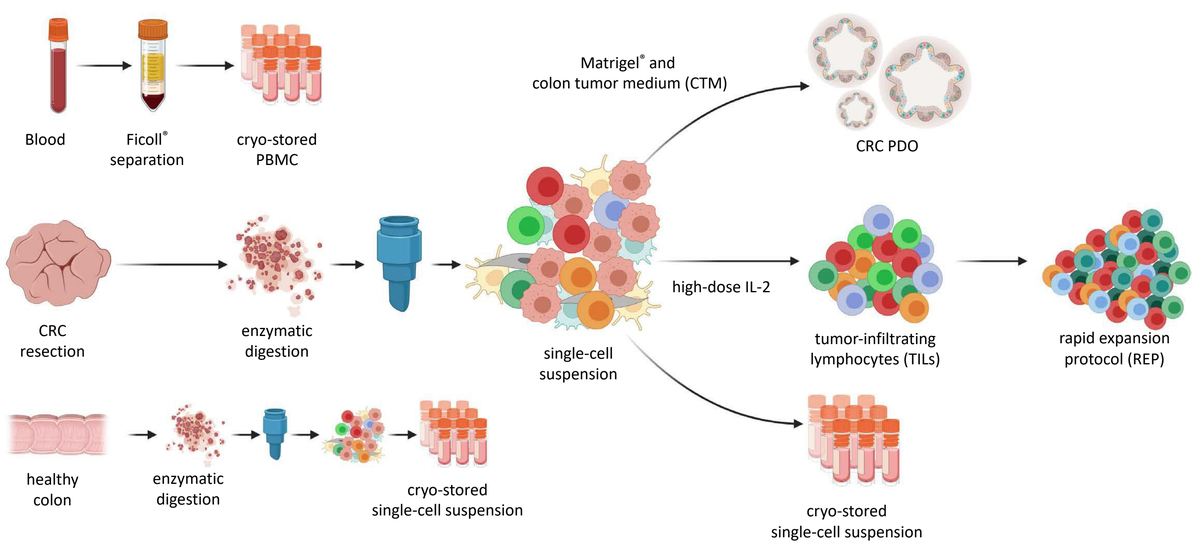
Introduction

Immunotherapy is a fast developing and effective treatment strategy to combat cancer. New immuno-oncology (IO) modulators such as checkpoint inhibitors, and bispecific antibodies are increasingly populating the drug development pipelines, however, preclinical platforms that reliably model the tumor and immune cell interaction are still lacking. HUB Organoid Technology allows the development and in vitro expansion of Patient-Derived Organoids (PDO) from normal and tumor patient tissues as three-dimensional primary cell cultures which retain the histological and mutational features of the original tumor tissue. Here we describe the development of "living" biobanks of patient-derived tumor and normal organoids matched with autologous immune cells from different epithelial organs, including but not limited to, the gastrointestinal tract and lungs. Additionally, we show the establishment of a co-culture platform based on colorectal cancer (CRC) PDO and their paired immune cells such as tumor infiltrating lymphocyte (TIL).

Figure 1. Biobanking of CRC-PDO and autologous TILs



Methods

- ✓ Colon organoid development and isolation of paired TILs from patient resections were performed using mechanical and enzymatic digestion adapted from Dudley *et al.*[1] A TIL master cell bank (MCB) was generated by expansion of TILs in presence of high dose IL-2. (Figure 1).
- ✓ PDO were further characterized in terms of expression of immune check-point molecules by flow cytometry. TILs tumor reactivity was evaluated in co-cultures with matched CRC-PDO. PDO killing and T cell activation was detected via activation of Caspase 3/7 apoptotic signal in image analysis and further confirmed with IFNγ secretion by ELISA (Figure 2 and 3).
- ✓ Tumor reactive TILs were enriched via repeated exposure to paired CRC PDO and subsequently CD137+ CD154+ activated TILs were selected and underwent a rapid expansion protocol (REP). TILs clonality was evaluated by TCRvβ analysis (Figure 4).

Results

Tumor PDO were further characterized for expression of immune regulatory receptors such as PD-L1, CD80 and CD86. We developed a robust protocol for simultaneous co-isolation and expansion of tumor PDOs and their paired TILs with efficiency of 75%. Moreover, we developed a protocol to enrich tumor reactive TILs by repeated exposure to tumor PDOs. Enrichment of tumor reactive T cells led to skewed T cell receptor (TCR) repertoire and improved tumor organoid killing in co-cultures with paired tumor PDOs which was detected by several readouts such as imaging-based apoptotic signals, expression of T cell activation markers, and secretion of proinflammatory cytokines.

Figure 2. Schematic overview of CRC PDO-TIL co-culture assay

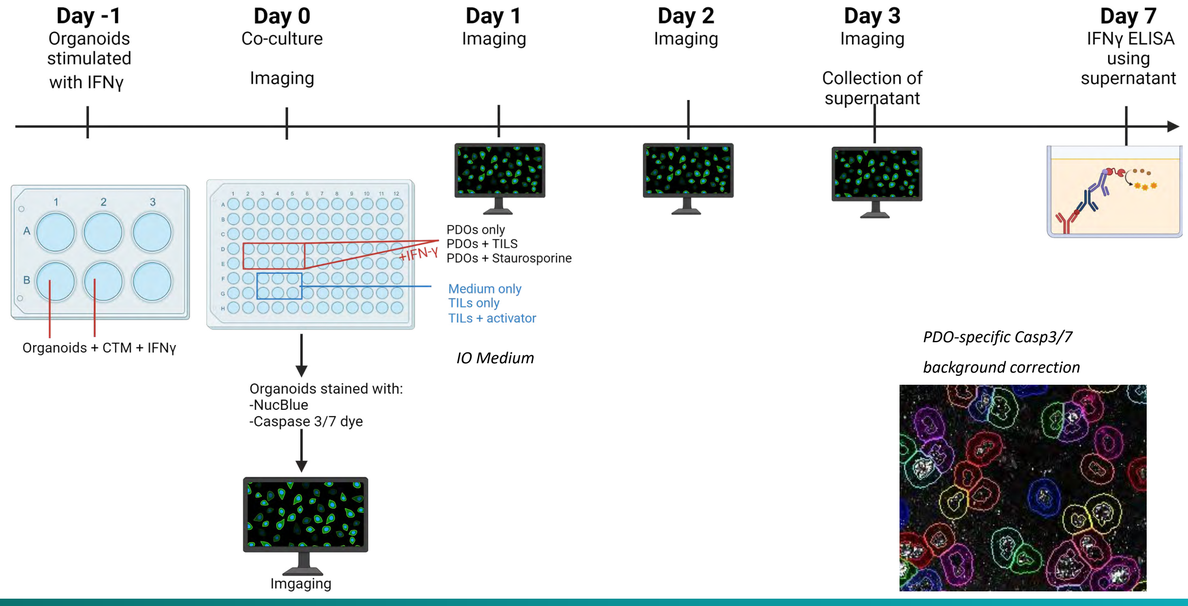


Figure 3. CRC PDO-TIL co-culture assay

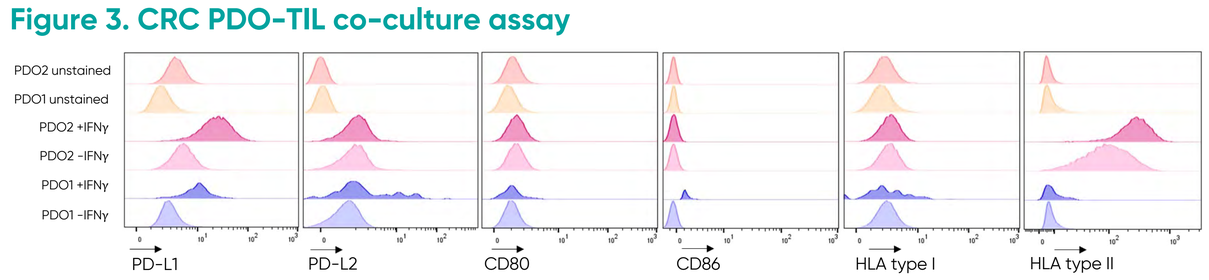
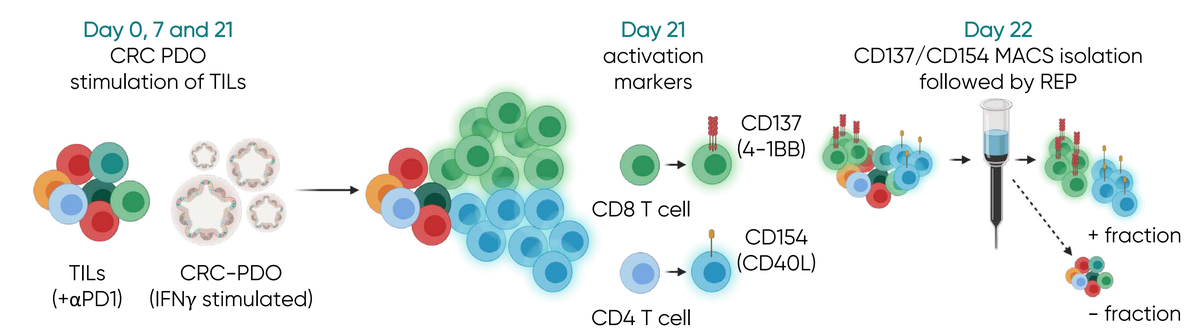
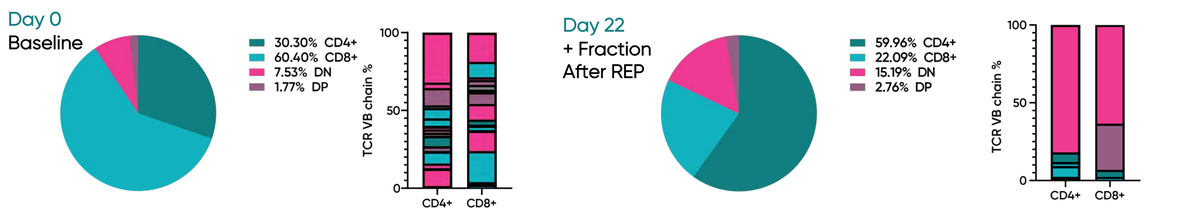


Figure 4. Generation and characterization of CRC PDO-reactive TIL

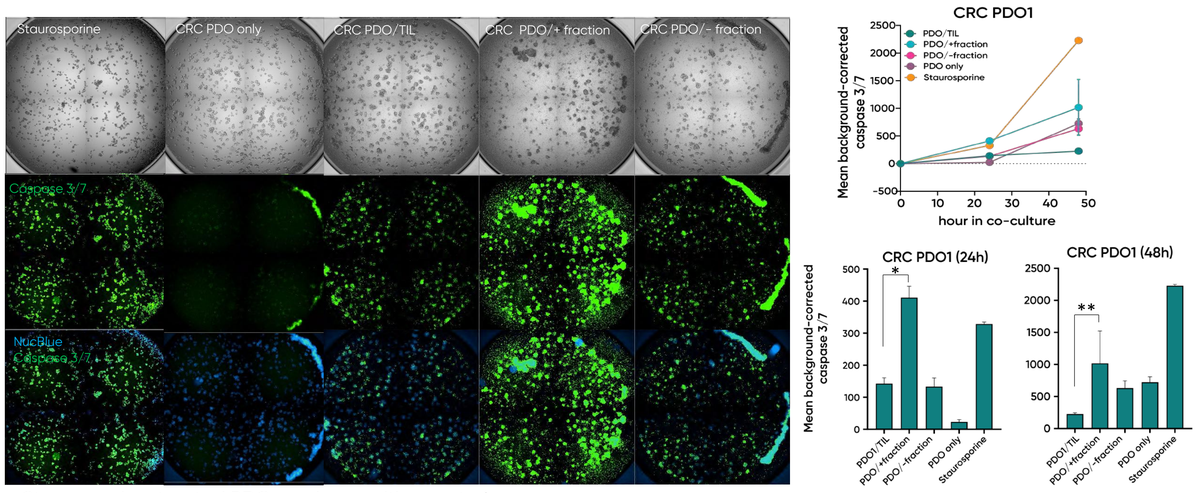
A. Culture enrichment of tumor reactive TILs (adapted from Dijkstra K.K. et al, Cell 2018)



B. FACS analysis of enriched tumor reactive TILs (TCRvβ), skewing of TIL lineage (CD4+/CD8+/CD4-CD8-(DN)/CD4+CD8+(DP) and clonality (TCR Vβ chain) by repeated PDO stimulation acquired by



C. Increased CRC-PDO killing by tumor-enriched T cells, quantification of mean caspase intensity over time at 24 and 48 hours after co-culture



Staurosporine-treated PDO used as positive control

*(triplicates, mean±SD, *p-value<0.0001, **p-value<0.02)*

Summary

- ✓ CRC organoids can be generated with paired immune cells
- ✓ PDO-TIL co-cultures provide a screening platform for:
  - T-cell immunomodulators
  - αCD3-bispecific antibodies
  - Cancer vaccines

References

1. Dudley *et al*; Generation of Tumor-Infiltrating Lymphocyte Cultures for Use in Adoptive Transfer Therapy for Melanoma Patient; *J Immunother.* 2003; 26(4): 332–342.
2. Dijkstra *et al*; Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. *Cell.* 2018 Sep 6;174(6):1586-1598.e12. doi: 10.1016/j.cell.2018.07.009. Epub 2018 Aug 9.

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