Autologous Organoid-T cell co-culture platform as a powerful personalized model for Immuno-Oncology therapy

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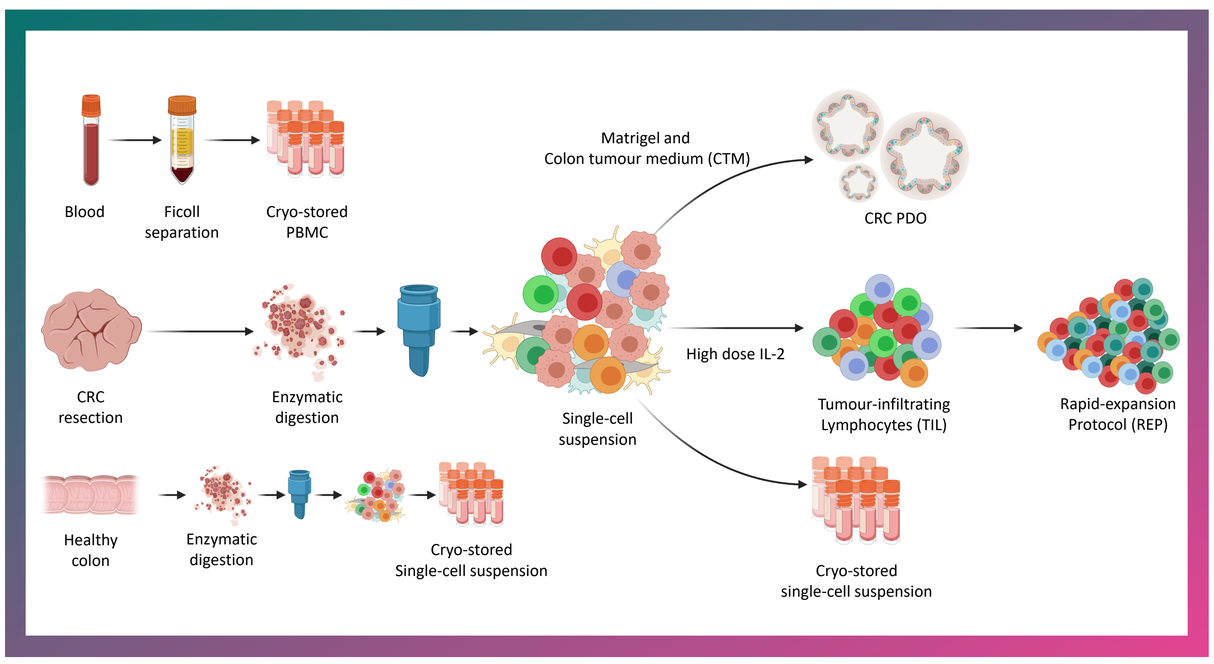
Introduction

Immune-oncology (IO) is a fast developing and effective treatment strategy to combat cancer. IO modulators such as checkpoint inhibitors, and bi-specific antibodies are being developed increasingly by biotech and pharmaceutical industry. Platforms that reliably model the tumour–immune-cell interaction will greatly contribute to our understanding of the critical factors that determine a successful IO therapy for solid tumours. Hubrecht Organoid Technology (HUB) offers protocols to generate "living" biobanks of patient-derived tumour and normal 3D organoid cultures and their autologous immune cells from different epithelial organs, including but not limited to, gastrointestinal tract and lung. This technology allows for the in vitro expansion of Patient-Derived Organoids (PDO) from healthy- and tumour tissues as three-dimensional primary cell cultures which retain the histological and mutational features of the original tumour tissue. HUB offers a co-culture system based on colorectal cancer (CRC) PDO and their paired immune cells such as tumour infiltrating lymphocyte (TIL).

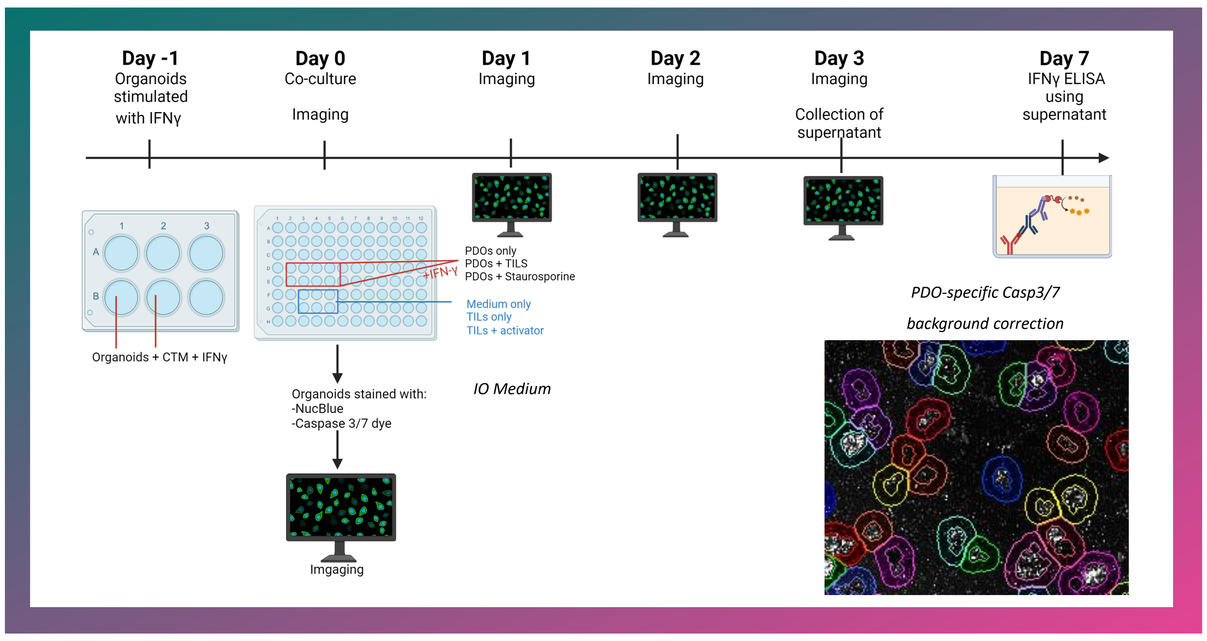
Figure 1. Biobanking of CRC-PDO and autologous TIL



Methods

- Co-isolation of colon PDO and their paired TIL from the resected tissue were performed using mechanical and enzymatic digestion adopted from Dudley *et al.* procedure. TIL master cell bank (MCB) was generated by expansion of TIL 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. Tumor reactivity of TIL was evaluated in co-culture with paired 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 reactivity of TIL was enriched via repeated exposure to paired CRC PDO and subsequently CD137+ CD154+ activated TIL were selected and underwent a rapid expansion protocol (REP). Clonality of TIL were evaluated by TCRvβ analysis (**Figure 4**).

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



Results

Tumour PDO were further characterized for expression of immune regulatory receptors such as PD-L1, CD80 and CD86. We developed a robust protocol for simultaneously co-isolation and expansion of tumour PDO and their paired TIL with efficiency of 75%. Moreover, HUB developed a protocol to enrich tumour reactive TIL by repeated tumour PDO exposure. Enrichment of tumour reactive T cells led to skewed T cell receptor (TCR) repertoire and improved tumour organoid killing in co-culture with paired tumour PDO which was detected by several readouts such as image based apoptotic signal, expression of T cell activation markers and secretion of proinflammatory cytokine.

Figure 3. CRC PDO-TIL co-culture assay

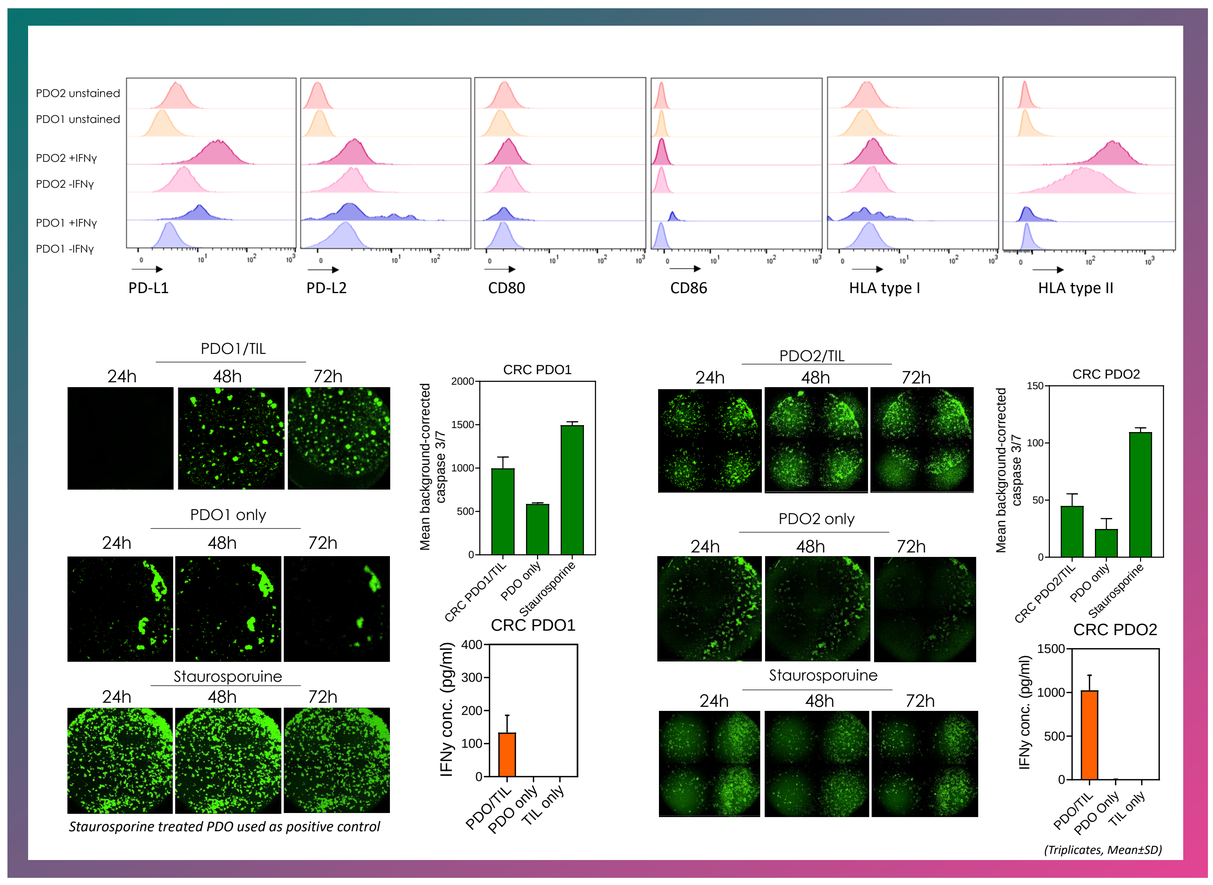
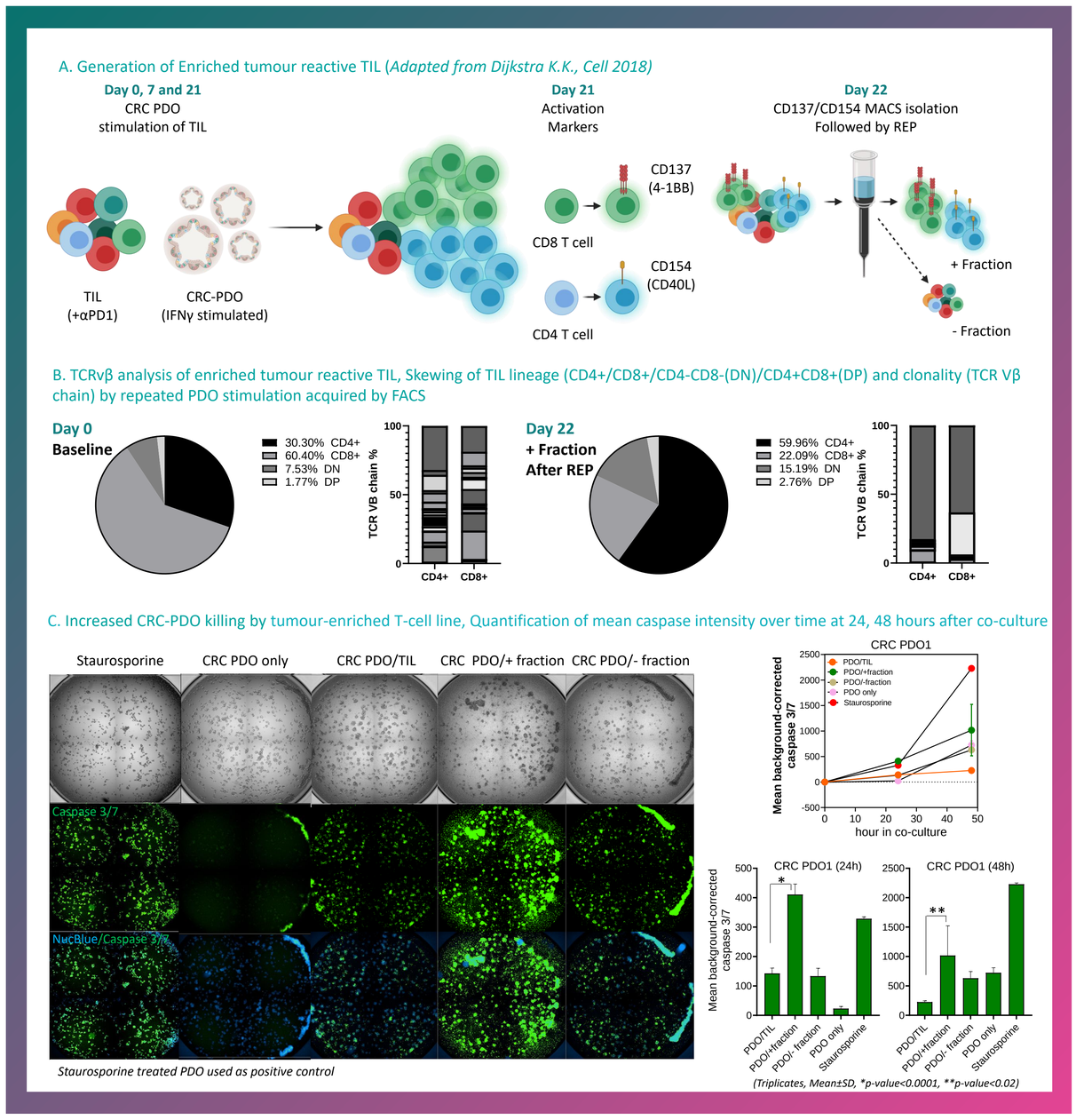


Figure 4. Generation, characterization and anti-tumour reactivity of enriched CRC PDO-reactive TIL



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.

Conclusion

- 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

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