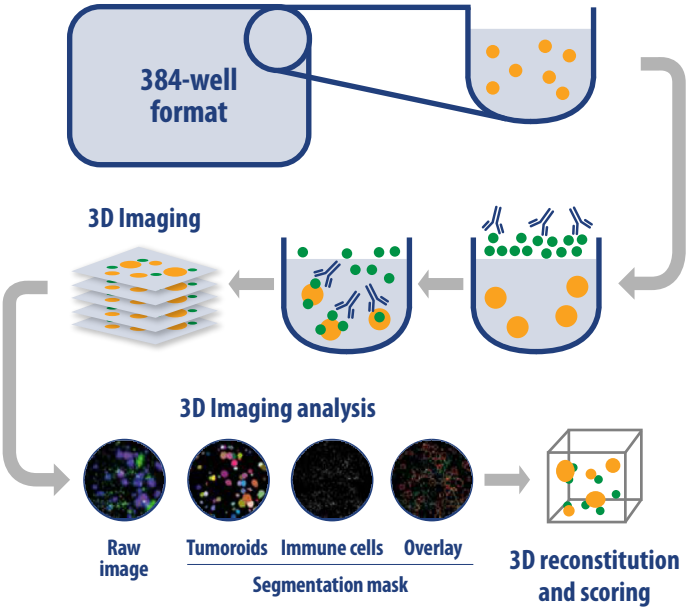
3D *In Vitro* HCI for Immuno-Oncology

Efficient testing of treatment effects in assays yielding functional data on tumor-immune system interplay

Clinically relevant models and functional assays are needed to evaluate the immunomodulatory effects of drugs on tumor-immune system interplay. Utilizing OcellO technology, CrownBio's unique high content imaging (HCI) platform combines 3D models including organoids and spheroids with high content screening, enabling efficient analysis of immuno-oncology (I/O) therapeutic effects in a physiologically relevant environment.

3D co-cultures are optimized for study requirements. Tumor cells are grown embedded in a 3D extracellular matrix protein-rich hydrogel; immune cells are added together with test compounds and co-cultures are maintained for 1-4 days.

Immune cells are stained separately to allow for distinction from cancer cells. After "optical sectioning", 3D image stacks are reconstituted. Robust, high-throughput (384-well) screening is done using image-based measurements of selected features including T cell invasiveness, total tumor volume, shape and size of tumoroids.



Key advantages:

- Automated analysis and robust quantification of activity of immune cells
- Functional readouts: immune cell priming, active migration, and infiltration into tumoroids, tumor cell killing, and myeloid polarization
- Physiologically relevant 3D microenvironment
- Visualization of immune cell interaction with the tumor
- HLA-matched cell types

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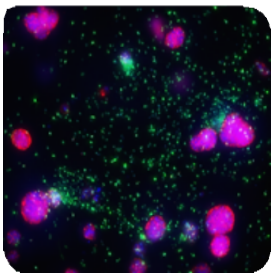
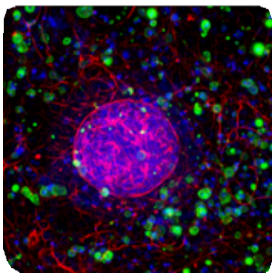
A suite of scalable assays with flexible design

- Assays can be customized by replacing any of the cellular players
- This allows for testing of a diverse range of immunotherapies that focus on different immune compartments and target diverse cancer indications

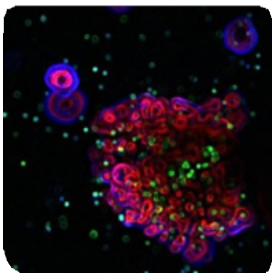
Cancer cell line spheroids + PBMCs



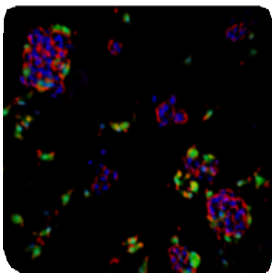
***Ex vivo* PDX + CD8+ T cells**



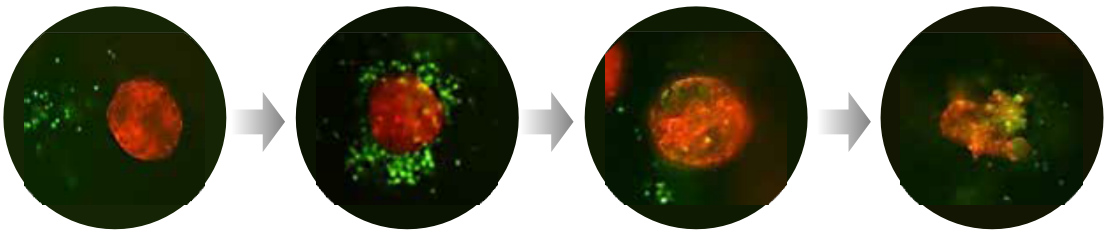
CRC organoids + engineered T cells



Spheroids + myeloid cells



Visualize steps of tumor cell killing by activated T cells over time



migration **aggregation** **infiltration** **killing**

Take full advantage of image-based compound testing services using co-cultures of cancer and immune cells

These services test the ability of compounds to potentiate infiltration of T cells into the tumor and enhance their cytotoxic activity. Diverse cultured tumor tissues, including tumoroids derived from established tumor cell lines, colorectal cancer organoids and PDX material, can be used in these assays. Tumoroids are co-cultured with partially HLA-matched PBMCs from healthy donors, purified T cell populations (e.g., CD8+, CD3+), engineered T cells, CAR-T cells or myeloid cells differentiated *in vitro* (e.g., DCs, M1 and M2 macrophages).

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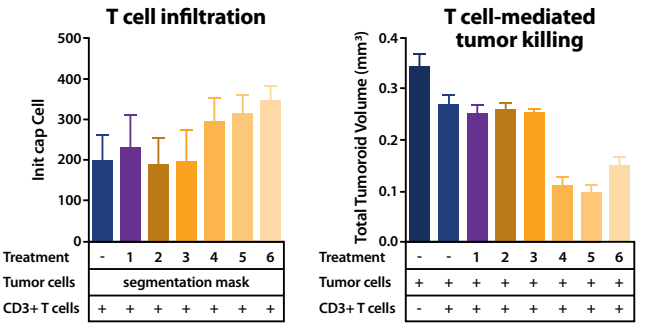
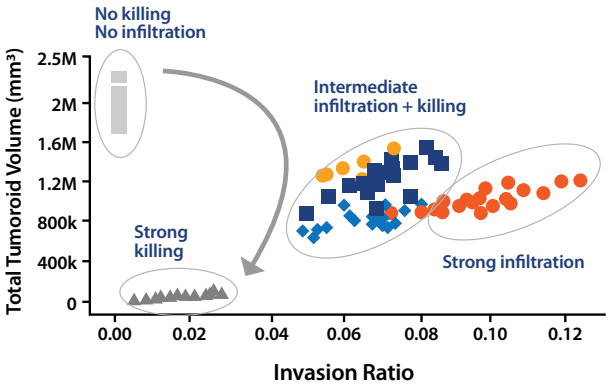
Functional Endpoints: T Cell Infiltration and Tumor Killing

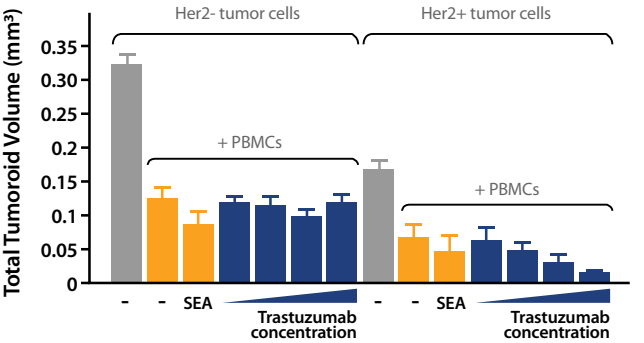
Breast cancer cells were seeded in 3D to form tumoroids. CD3+ T cells isolated from PBMCs were added with immunomodulators. Immune cells and cancer cells were stained separately and imaged. Automated image analysis measured infiltration of immune cells into tumoroids and tumor cell killing treatment effects were quantified.





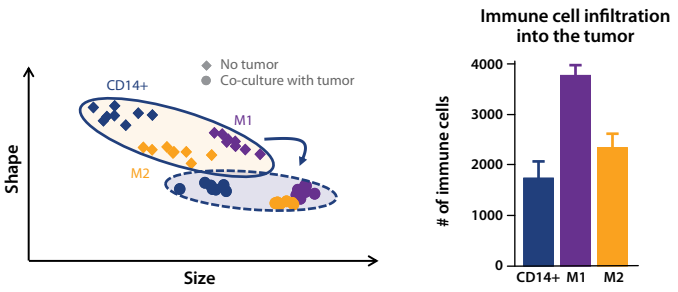
Determination of Intermediate Stages of Immune Cell Activation

Differentially pre-treated PBMCs were co-cultured with SKBR-3 tumoroids. Bi-parametric analysis of tumoroids size versus T cell invasiveness enables a better understanding of a drug immunomodulatory profiles and how they impact tumor infiltration and/or killing.



Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC)

Her2- and Her2+ breast cancer cell lines were seeded in 3D to form tumoroids. PBMCs were added and cultured further in the presence of trastuzumab. Cultures were fixed, stained and imaged. Image-based analysis was applied to measure immune cell-mediated killing. Enhanced tumor killing induced by trastuzumab was quantified.



Co-Culture of Myeloid Cells with Tumoroids

Co-culture of tumoroids with the myeloid subsets leads to a change in their phenotypic profile. CD14+ monocytes show characteristics associated with macrophage differentiation, while M1 and M2 macrophages start to overlap their phenotypic profiles. M1 macrophages are the most efficient in infiltrating tumoroids.

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