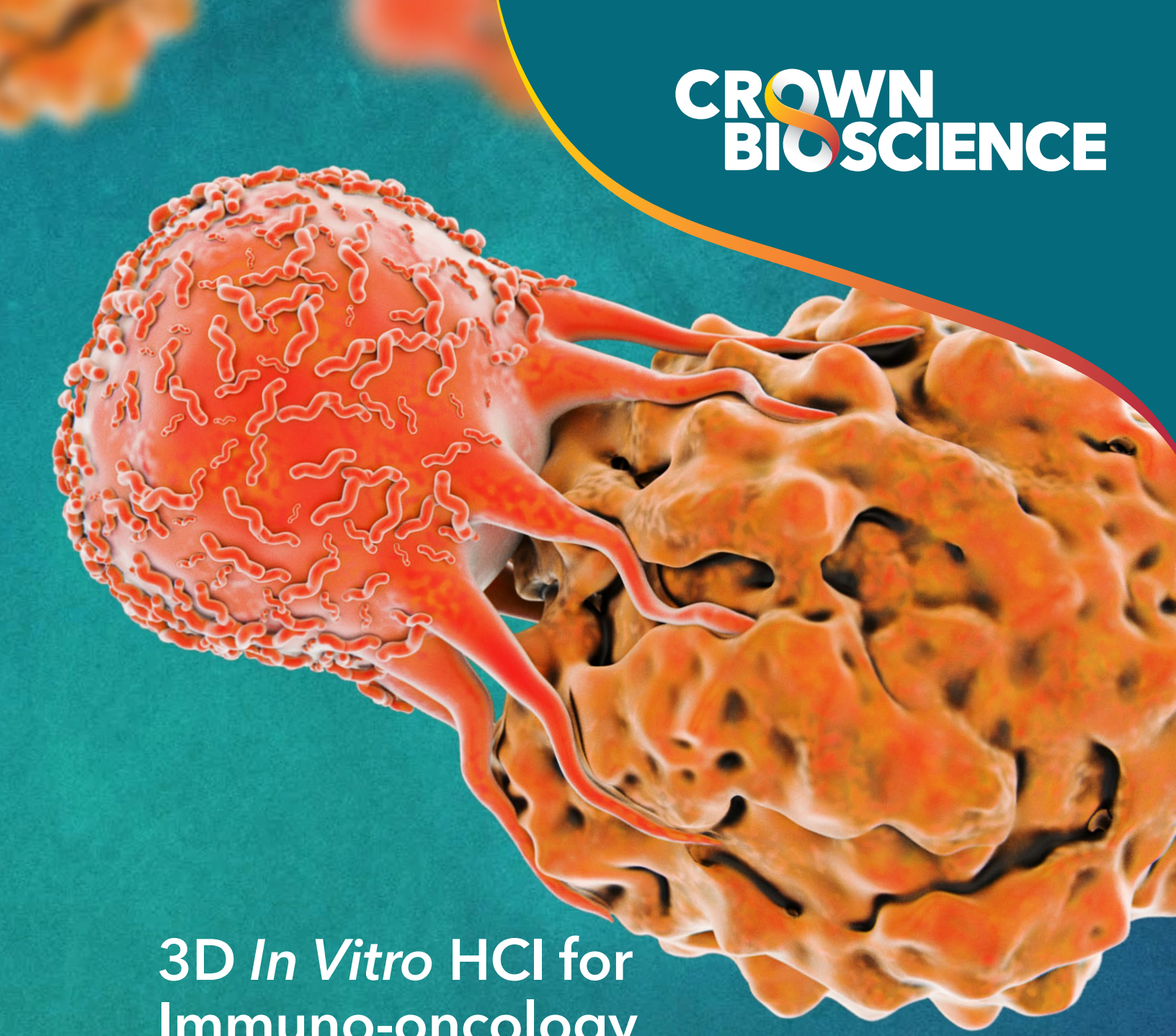
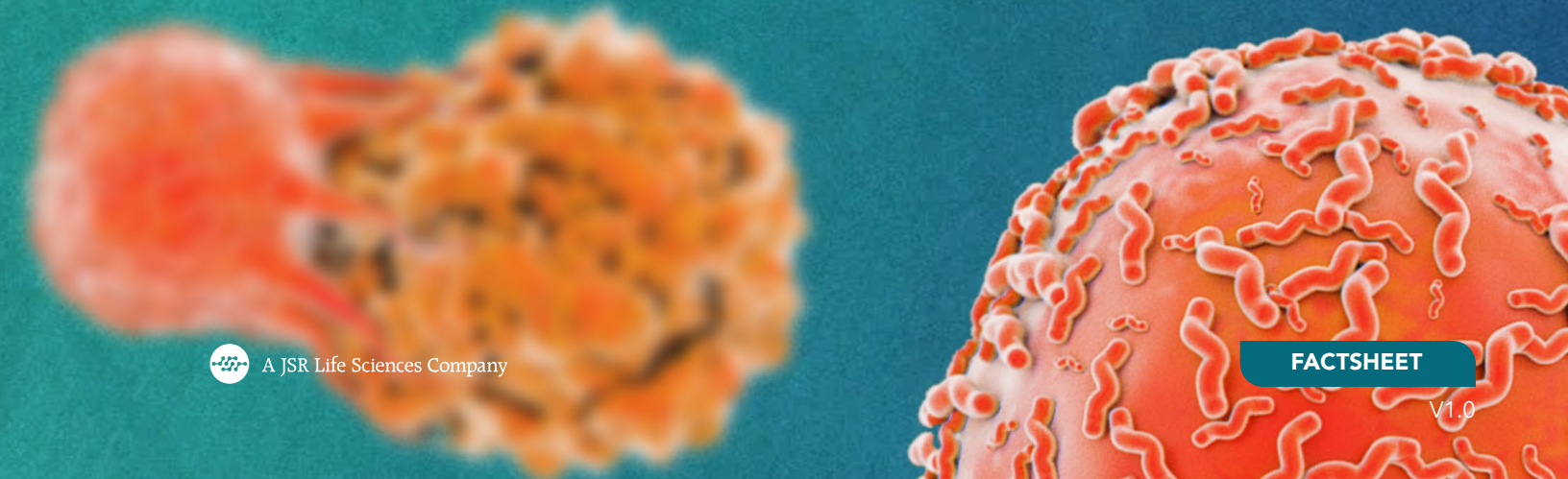
CROWN BIOSCIENCE



3D *In Vitro* HCI for Immuno-oncology

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



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FACTSHEET

V1.0

Clinically relevant models and functional assays are needed to evaluate the immunomodulatory effects of drugs on tumor-immune system interplay. Utilizing OcellO technology, Crown Bioscience'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.

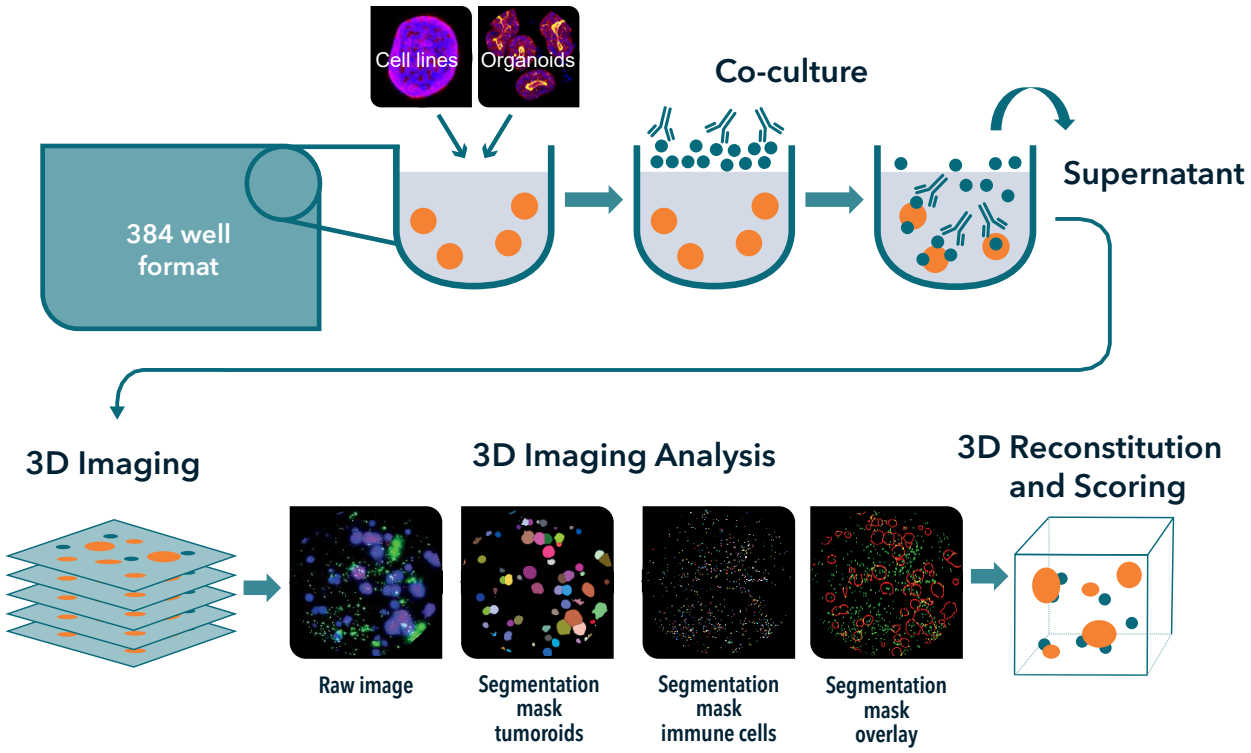
Introduction

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



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

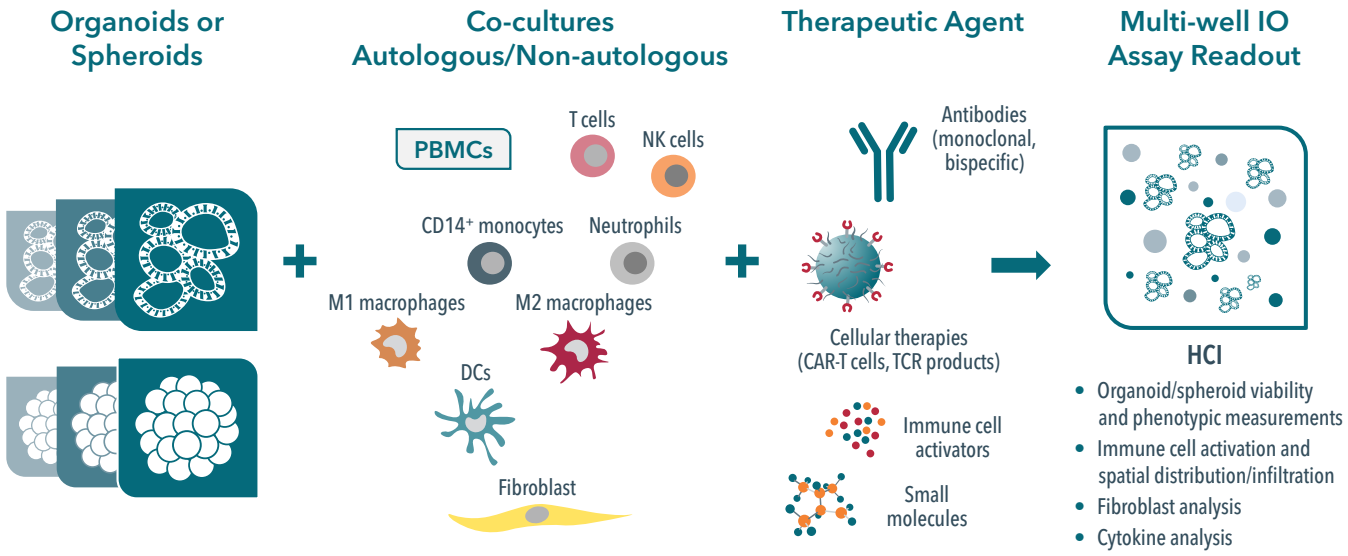
Make use of the most relevant patient-derived models with Crown Bioscience OrganoidBase™

- More than 500 organoid models from 15 different cancer indications, capturing the diversity of the patient population
- Screen multiple indications to find the most suitable one for your cancer-agnostic compounds
- Or evaluate the patient-to-patient variability among a panel of your target indication
- Test off-target effect using our matched healthy-tumor pairs or study resistance with our metastatic models

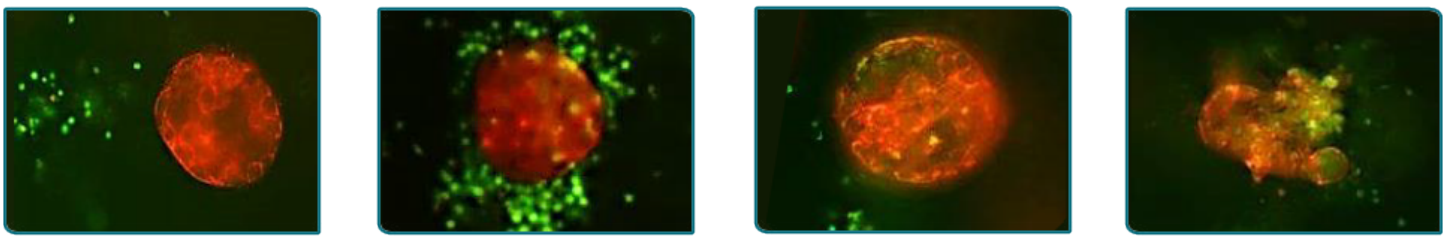
Take full advantage of image-based compound testing services using co-cultures of cancer and immune cells

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 spheroids derived from established tumor cell lines, patient-derived organoids (PDOs) and patient-derived xenograft organoids (PDXOs), can be used in these assays.

The chosen models are co-cultured with partially HLA-matched peripheral blood mononucleated cells (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).



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



migration → aggregation → infiltration → killing

Immune cells Spheroid

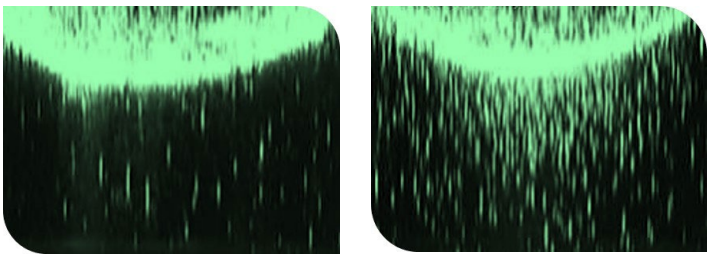
Functional Endpoints: T cell migration

Immune cells are stained with a cell tracker and added to the hydrogel with the spheroids/organoids present. Immune cells migrate to the lower parts of the hydrogel where the tumor cells are located. Migration can be enhanced by the treatment. Image analysis is performed to score the number of immune cells in different z-positions.

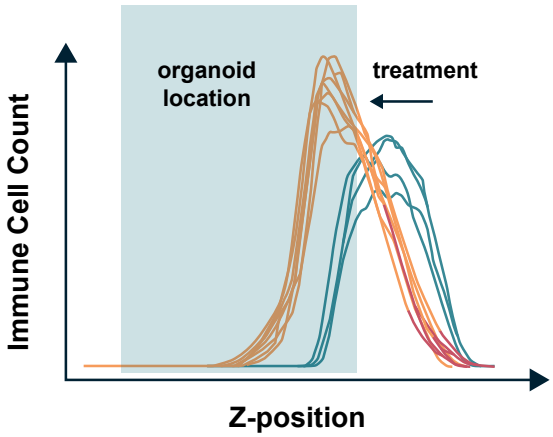
Functional Endpoints: T cell infiltration and killing

Spheroids expressing defined TAA are co-cultured with CD3+ T cells. Two different T cell engagers (bi-specific antibodies targeting CD3 on immune cells and TAA on tumor cells) are added in different doses. Image analysis is performed to measure dose-dependent increase in T cell infiltration after 4 days of co-culture and tumor killing after 7 days of co-culture.

Treatment-induced immune cell migration

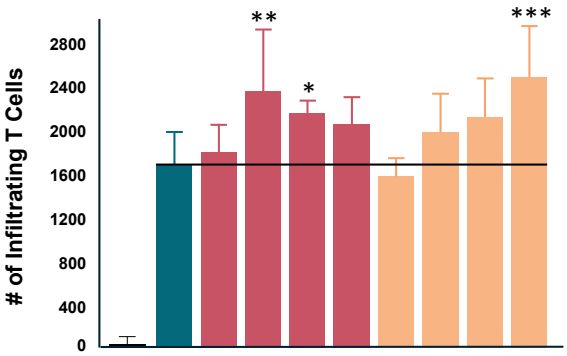


Treatment-induced immune cell migration toward organoids



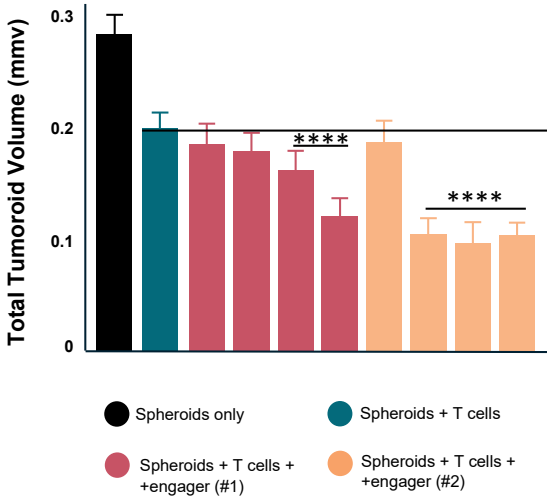
T cell infiltration

Early Time Point



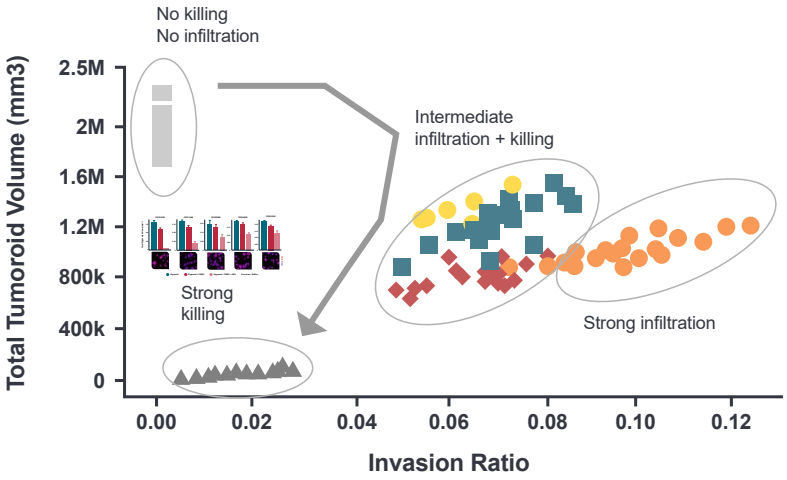
Spheroids killing

Later Time Point



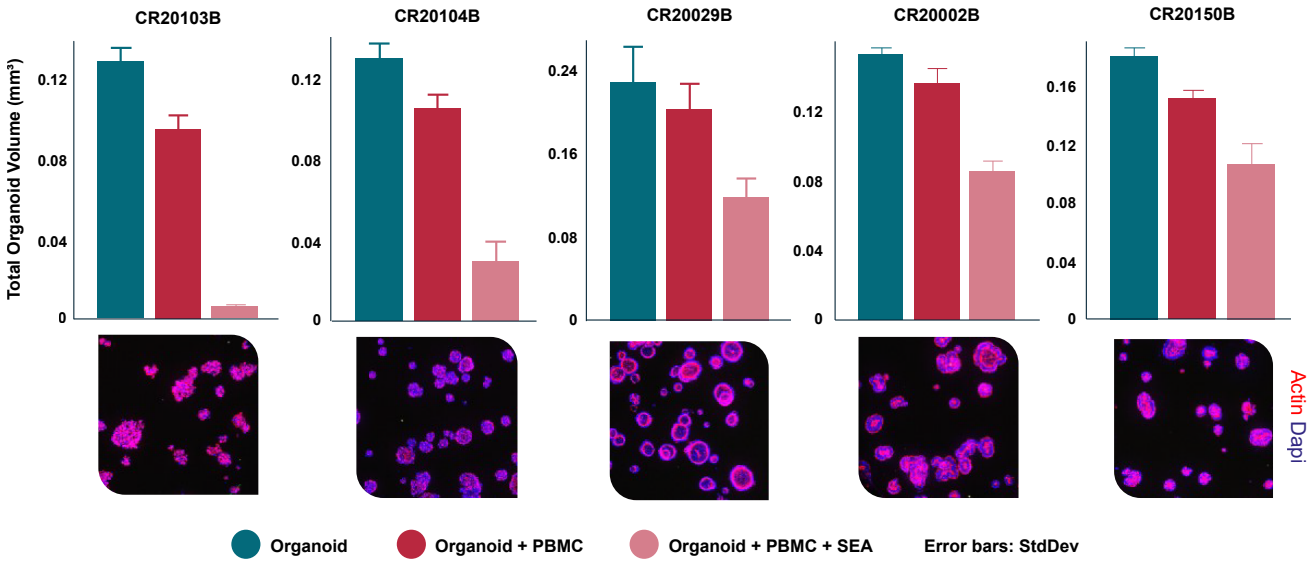
Determination of intermediate stages of immune cell activation

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



Representation of patient-to-patient variability

Five CRC organoid models with different morphological characteristics were co-cultured with naïve PBMCs from the same donor and showed a different sensitivity to immune cell-mediated killing. While all models showed a mild killing upon addition of naïve PBMCs, stimulation with SEA could lead to killing of the organoids from 30% up to 90%.



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