Summary & Impact

- Immunotherapies such as checkpoint inhibitors, CAR-Ts and immune-targeting Ads have great promise for cancer treatment. Translational cell-based assays are required to optimize these approaches.

- Here we describe image-based, immune cell-killing assays of 3D tumour spheroids, geared for assessing the efficacy of novel immune-modulators.

- Human tumour cell lines expressing RFP were used to form spheroids in 96-well ULA plates. Immune cells were then added and activated to kill. Spheroid viability was assessed over time (up to 10 days) by measuring the loss of RFP fluorescence using IncuCyte live-cell analysis.

- This method is exemplified with a range of immune cell types (PBMCs, T-cells, NK-cells) and activation, including anti-CD3 & IL-2.

- In an ADCC format, Herceptin induced a concentration-dependent specific killing of Her-2 expressing tumours. Higher concentrations of Herceptin were required in 3D vs 2D ADCC assays. These data demonstrate how immune-cell killing and ADCC assays can be extended from traditional 2D mono-cultures to 3D spheroid assays, providing the potential for greater translational relevance. These assays will be highly valuable in the search for novel immunomodulators.

96-Well live-cell assays for immune cell killing of 3D tumour spheroids

M. Oliver1, K. Patel1, N. Holtz2, E. Endesley2, T. Dale1 & D. Trezise1
1Essen BioScience, Welwyn Garden City, AL7 3AX UK or 2Ann Arbor, MI, 48108, USA

96-Well 3D Immune Cell Killing Assay Workflow

- Blended phase and fluorescent images of A549 NucLight Red™ spheroids in the presence and absence of immune cells.
- A549 cells (2.5x10^6 cells/well) seeded with PBMCs activated with anti-CD3 (10 ng/ml) and anti-2 (10 ng/ml). Cytotoxicity was quantified based on the red fluorescent intensity.
- Data demonstrates an E:T ratio-dependent destruction of tumour spheroids by the activated T-cell population.
- Note that E:T ratio optimisation is required as non-targeted cell death was observed at E:T ratios >1:1.

Continuous Live-Cell Analysis: Methodology

- **Incucyte® S3 Live-Cell Analysis System**
  - A flexible assay platform that can host a standard tissue culture plate in the Incucyte® imaging chamber.
  - Utilizes Incucyte® Image Analysis Software, which can perform real-time FLU Intensity and Fluorescence mask analyses.

- **Incucyte® Software**
  - Fast, flexible, and portable control hub for continuous live-cell analysis featuring intuitive data capture, image processing, and manual control.

- **Incucyte® Reagents & Consumables**
  - A suite of non-protecting cell viability and reagent supports designed for stable imaging with the Incucyte® S3 Live Cell Imaging System.

Fluorescence as a Measure of Spheroid Cytotoxicity

- **Phase & fluorescence**

Herceptin Induced ADCC in HER2-Positive SKOV-3 cells

- HER2-positive SKOV-3 or HER2-negative A549 NucLight Red™ spheroids (2.5x10^6 wells) were seeded with PBMCs (6.25x10^6/ml) and treated with Herceptin (mbb targeting HER2 receptors).
- Herceptin >10 µg/ml-1 induced concentration-dependent inhibition of SKOV-3 spheroid growth.
- Herceptin-induced cytotoxicity was measured in SKOV-3 but not A549 spheroids.

Herceptin-mediated ADCC in HER2-positive SKOV-3 cells

- A similar assay was conducted in a 2D culture model. SKOV-3 cells (1.6x10^5) were seeded overnight prior to the addition of PBMCs (8x10^5) and subsequent treatment with Herceptin.

- SKOV-3 tumour spheroids appear to exhibit “300x lower Herceptin sensitivity in comparison to 2D.

- Note the apparent 34% inhibition of the 3D spheroid at the lowest test concentration (0.08 µg/ml). This may suggest that a biphasic concentration-response curve may exist, where the outermost cells behave as in the 2D model, whereas the spheroid centre has lower sensitivity.

- Further experimentation is required to further understand the differential effects of Herceptin in 2D vs 3D models.