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Comparative analysis of anti-HER2 antibody-drug conjugates in tumor spheroid models

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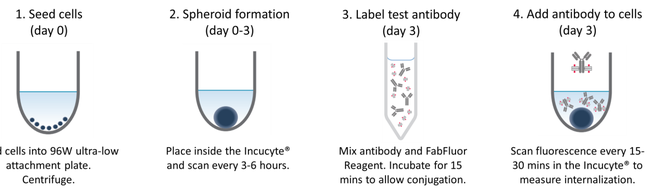
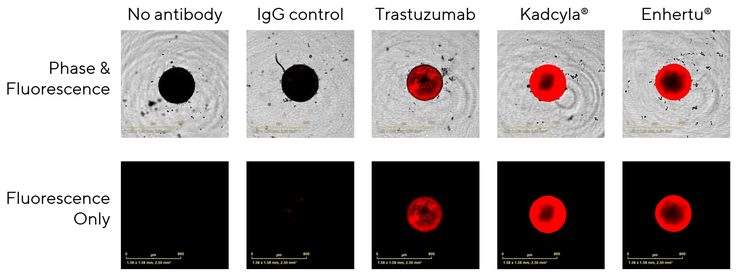
Introduction

Robust techniques are needed to screen the anti-tumor activity of antibody-drug conjugates (ADCs) *in vitro*. Traditional monolayer models are cost-effective and scalable but lack many features of the tumor microenvironment, such as complex cell-cell and cell-extracellular matrix interactions. Tumor spheroids afford a more translational model for quantification of ADCs, with more realistic drug effects and a closer relationship to the *in vivo* scenario. These data showcase temporal measurements of ADC internalization and cytotoxicity performed using the Incucyte® Live-Cell Analysis System. At assay endpoint, spheroids were dissociated for analysis using the iQue® High-Throughput Screening (HTS) Cytometry Platform to yield further information on spheroid health and composition. This combined workflow was able to distinguish ADC mechanisms of action using single and multi-spheroid models.

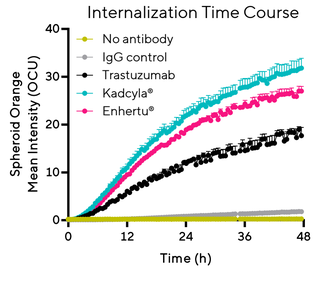
1. Internalization

Assay workflow



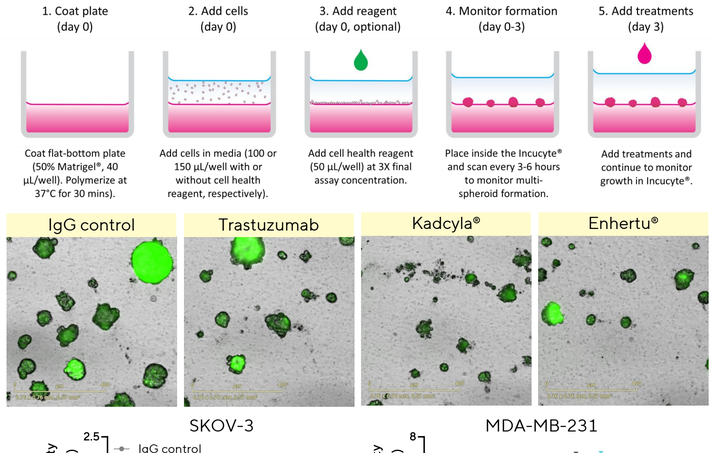


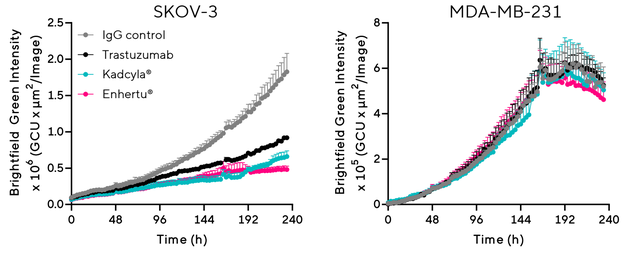
- 4 K/well BT474 cells were seeded into ultra-low attachment (ULA) plates for 72 hours to promote single spheroid formation.
- Antibodies were labeled with Incucyte® Human Fabfluor-pH Orange Antibody Labeling Dye then added to spheroids (n=3).
- Upon internalization into cells, the dye enters the acidic endosomal and lysosomal pathways, increasing its fluorescence. The fluorescence readout correlates with the level of antibody internalization that has occurred.
- Phase and fluorescence images (10X) were captured every 15 minutes using the Incucyte® System.
- Internalization of ADCs (Kadcyla® and Enhertu®) was greater than internalization of Trastuzumab.



2. Multi-Spheroid Cytotoxicity

Assay workflow

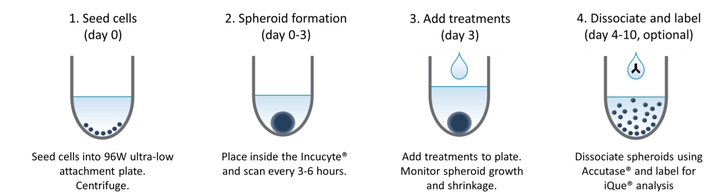




- Incucyte® Nuclight Green Lentivirus labeled SKOV-3 (high HER2) and MDA-MB-231 cells (low HER2) were seeded on a Matrigel® layer to promote multi-spheroid formation.
- Antibodies were added (2 µg/mL) and cells monitored using the Incucyte® via a 3-hour repeating scan schedule. Images are of SKOV-3 cells on day 9.
- ADCs induced high level death of SKOV-3 multi-spheroids (Kadcyla® (63.9%) and Enhertu® (73.7 %)) at assay endpoint compared to the IgG control.
- Trastuzumab induced considerable cell death, but at a reduced level compared to the ADCs (49.7%).

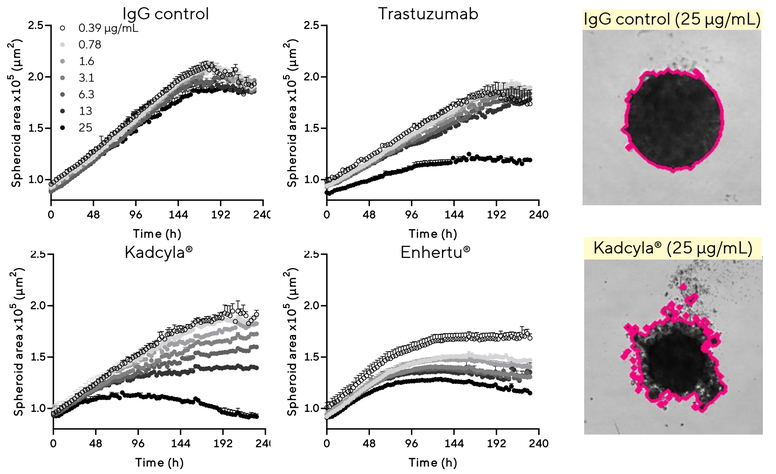
3. Single spheroid ADC cytotoxicity

Assay workflow



3. Single spheroid ADC cytotoxicity (cont.)

- Single spheroids formed from BT474 cells (1 K/well) were incubated with a range of concentrations of each antibody.
- Incucyte® images monitored spheroid area for 10 days (representative images from day 10 shown).
- Both ADCs induced a concentration dependent reduction in spheroid area over time, indicating cytotoxicity.
- Only the highest concentration of Trastuzumab was cytotoxic. Clearly this is not due to a cytotoxic payload and instead may be another mechanisms of action, such as inhibition of cell signaling in the PI3K-AKT pathway.



4. Bystander killing

- Single spheroids were formed from a 2:3 ratio of green labeled BT474 cells ($HER2_{high}$) and unlabeled MDA-MB-231 cells ($HER2_{low}$).
- Antibodies were added after 72 hours (5 µg/mL). Images were captured every 3 hours using the Incucyte® Live-Cell Analysis System. Spheroids were dissociated (day 8), labeled using iQue® Cell Membrane Integrity (R/Red) Dye and live cells were quantified using the iQue® HTS Platform.
- Only Enhertu® induced death of both cell types and an overall decrease in spheroid area due to its distinctive bystander killing action. This means that following release of the cytotoxic payload from lysed, HER2 expressing cells, it can diffuse into neighbouring cells, regardless of their HER2 expression, and induce their cytotoxicity.