Development of multi-spheroid co-culture 3D tumour assays using real-time live-cell analysis

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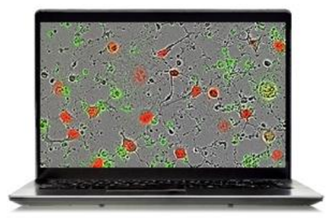
Summary & Impact

- Recapitulating the tumour microenvironment (TME) is widely acknowledged as being key to the development of more predictive 3D *in vitro* efficacy models.
- Here we describe a simple & robust 3D extracellular matrix (ECM)- based workflow for culturing tumour multi-spheroids (MS) with either stromal cells or immune cells.
- IncuCyte's S3 DF-Brightfield (DF-BF) image acquisition tool enables the ability to monitor & quantify changes in spheroid size & morphology (brightfield) as well as viability (fluorescence) using real-time live-cell analysis.
- These validation methods & data demonstrate the ability to kinetically visualise & quantify immune cell-mediated toxicity within tumour multi-spheroids as well as assess the impact of stromal cells on tumour resistance to chemotherapeutic agents.
- More advanced spheroid models, incorporating the ECM & additional cell types have the potential to provide more relevant translational models for the study of the tumor micro-environment on tumour biology.

IncuCyte® System for continuous live-cell analysis: Methodology

IncuCyte® S3 Live-Cell Analysis System

A fully automated phase contrast and two-color fluorescence imager that resides within a standard cell incubator for optimal cell viability. Designed to scan plates and flasks repeatedly over time.



IncuCyte® Software

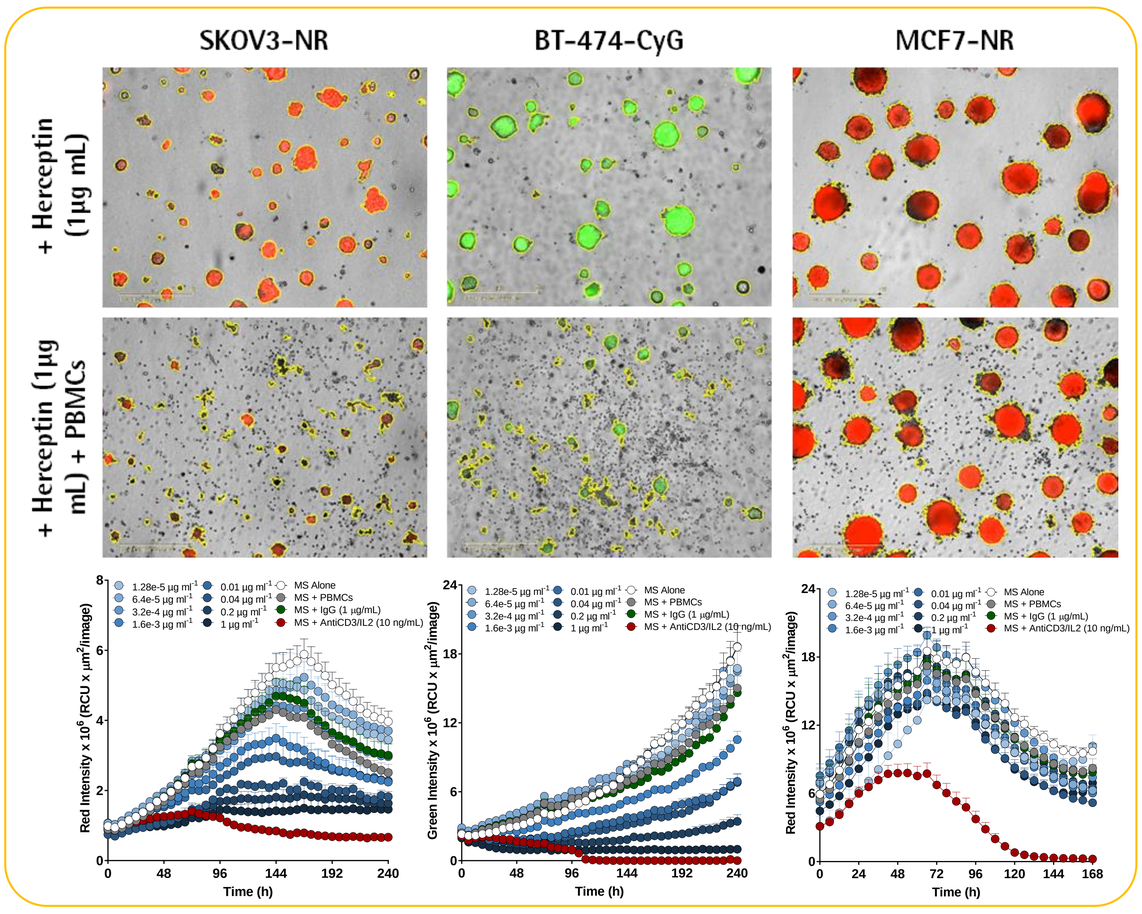
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and data visualization.

IncuCyte S3 Spheroid Software Module

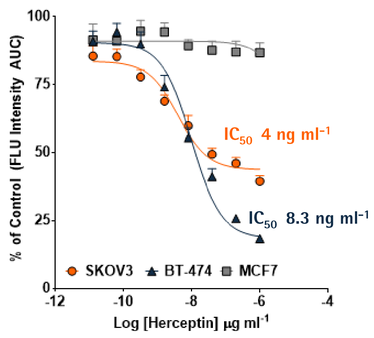
IncuCyte® Reagents and Consumables

A suite of non-perturbing cell labeling and reporter reagents. Includes nuclear-targeted GFP and RFPs for cell counting plus no-wash cell health reagents for apoptosis and cytotoxicity.

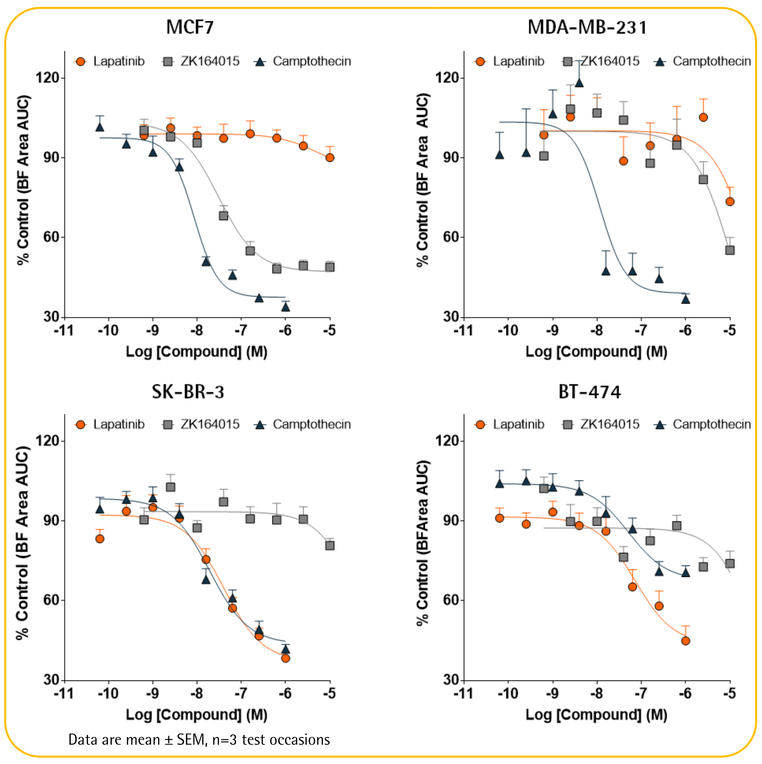
Herceptin-induced ADCC in HER2-positive multi-spheroids



- Tumour multi-spheroids (MS) either stably expressing nuclear restricted RFP (NR) or cytoplasmic GFP (CyG) were co-cultured with PBMCs (E:T, 5:1) & treated with Herceptin (mAb targeting HER2 receptors).
- MS proliferation & immune cell-mediated cytotoxicity was quantified kinetically using the IncuCyte® S3 metrics (fluorescence intensity within BF boundary) which does not require masking of fluorescent tumour MS.
- Herceptin induced a concentration-dependent cytotoxic effect in HER2-positive (SKOV3 & BT-474) but not HER2 negative (MCF7) MS.
- BT-474 MS appear more sensitive to Herceptin cytotoxicity with the highest test concentration of Herceptin (1 µg ml^{-1}) producing ~ 80% inhibition compared with ~ 50% in SKOV3 MS.



Differential pharmacology exemplified



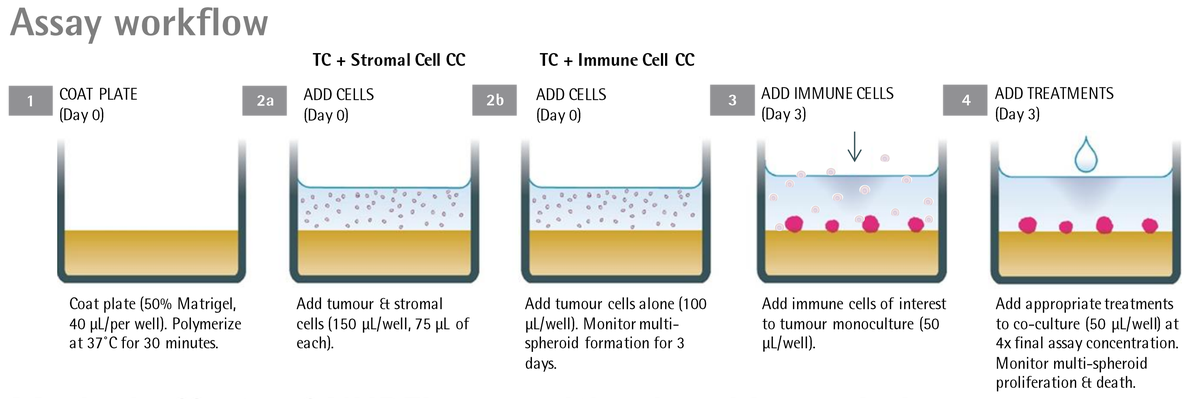
Data are mean ± SEM, n=3 test occasions

- Four tumour breast cell lines were co-cultured with normal human dermal fibroblast (NHDF's) at a 1:1 ratio (1K cells per well of each).
- MS were allowed to form for 3 days prior to treatment (7 – 10 days) with standard of care & cytotoxic agents.
- Concentration response curves represent area under curve (AUC) analysis of time-course data.
- Inhibition of MS growth caused by Lapatinib & ZK164015 aligned with the known expression profile of receptors targeted by these agents.
- The dual EGRF and HER2 tyrosine kinase inhibitor Lapatinib caused a concentration dependent inhibition of SK-BR-3 & BT-474 MS growth, while the estrogen receptor (ER) antagonist ZK164015 was a potent inhibitor of MCF7 MS growth with little or no effect on MS devoid of ER expression.
- The DNA topoisomerase inhibitor camptothecin (CMP) caused comparable inhibition of growth across all MS.

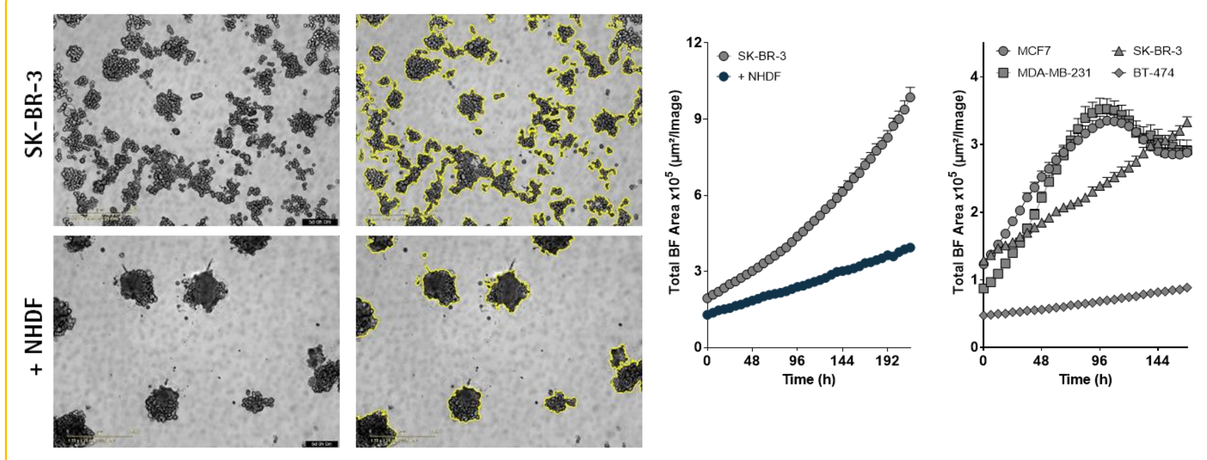
	Pharmacology (pIC_{50})			Relative Expression [1]		
	CMP	Lapatinib	ZK164015	EGRF	HER2	ER
MCF7	8.1 ± 0.04	< 5.0	7.5 ± 0.09	+ (1)	-	+ (6)
MDA-MB-231	8.0 ± 0.04	< 5.0	< 5.0	+ (1)	-	-
SK-BR-3	7.8 ± 0.15	7.5 ± 0.04	< 5.0	+ (2)	+ (3)	-
BT-474	7.6 ± 0.24	7.2 ± 0.17	< 5.0	+ (1)	+ (3)	-

[1] Subick et al., Molecular characterization of breast cancer cell lines. Basic and Clinical Research 2010:4 35 – 41.

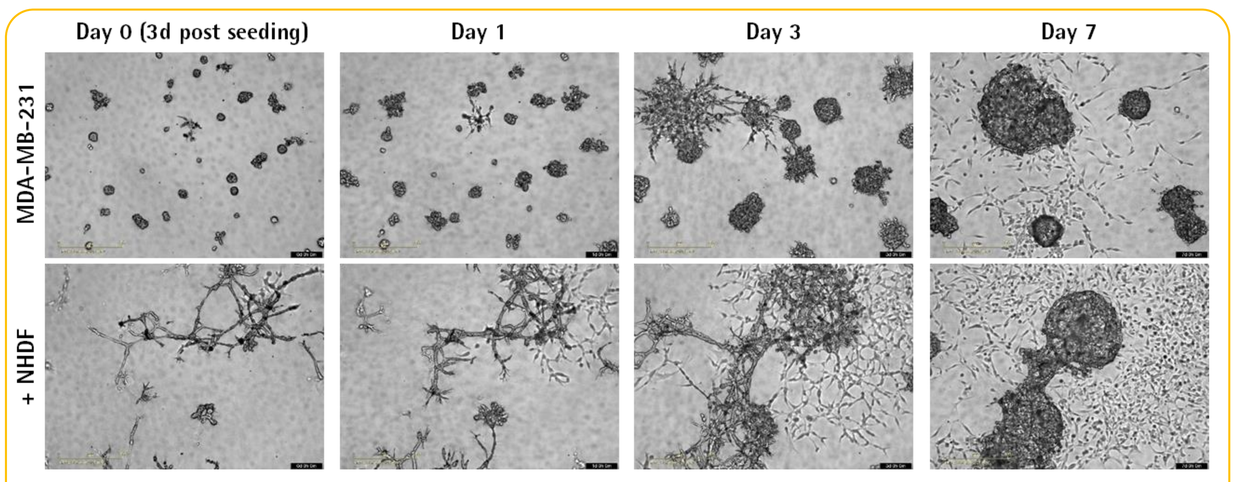
Assay workflow



Marked effects of NHDF's on multi-spheroid morphology

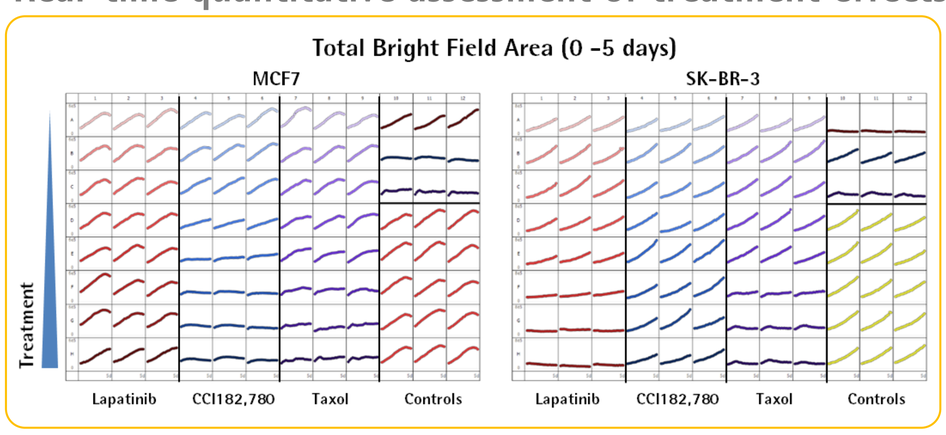


- SK-BR-3 cells were seeded alone or with NHDF's (1:1 ratio, 1K cells per well for each) & images (DF-BF) acquired every 6h. Images shown are captured 8 days post cell seeding.
- In mono-culture, SK-BR-3 cells fail to form compact multi-spheroids. Co-cultured with NHDF's results in the formation of more compact aggregates.
- IncuCyte S3 multi-spheroid software enables kinetic quantification of both morphologies, tracking MS growth via size measurements (Total BF Area).
- Note, the different growth profiles of the four tumour breast cell types in co-culture with NHDF's.



- NHDF's co-cultured with MDA-MB-231 cells (1:1, 1K per well for each) reveal a time-dependent effect on MDA-MB-231 spheroid morphology.
- MDA-MB-231 multi-spheroids transition from a stellate, branched appearance evident at early time-points, before reverting to a more clustered round appearance beyond 6 days.

Real-time quantitative assessment of treatment effects



- Multi-spheroids were allowed to form for 3 days prior to treatment (5 days) with known standard of care and cytotoxic agents.
- Real-time, automated time-course plate-views enable rapid visualisation of treatment effects on MS size (Total BF Area).
- Note, the difference between cell types to treatments (time & sensitivity).