# SARDRICS

### Simplifying Progress

### Development and Optimization of Matrigel-Based Multi-Spheroid 3D Tumor Assays Using Real-Time Live-Cell Analysis

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#### Summary and Impact

- The tumor-associated extracellular matrix (ECM) micro-environment provides critical biochemical cues as well as an essential structural scaffold for solid tumors to survive and grow.
- Here we describe a robust 3D ECM-based technique for culturing multiple tumor spheroids formed of lung, ovarian or breast cancer cell lines in a 96-well format.
- Incucyte<sup>®</sup> depth of focus (DF) brightfield image acquisition tool enables the ability to monitor and quantify changes in spheroid size and morphology (brightfield) as well as viability (fluorescence) using real-time live-cell analysis.
- The use of Incucyte<sup>®</sup> Cell Health reagents such as Incucyte<sup>®</sup> Annexin V Dye to label apoptotic cells elucidates mechanism of action of compound treatments.
- Furthermore, this approach should facilitate more translational investigation of primary- and patient-derived organoid tumors.

#### Incucyte<sup>®</sup> Live-Cell Imaging and Analysis: Methodology







Incucyte<sup>®</sup> Live-Cell Analysis System

Incucyte<sup>®</sup> Software

Incucyte<sup>®</sup> Reagents and Consumables

#### Novel DF-Brightfield Image Capture



- High-definition (HD) phase and DF-Brightfield (BF) images of multi-spheroids (MS) formed from a range of tumor cell lines (5 days post seeding) on a Matrigel<sup>®</sup> base.
- Incucyte's<sup>®</sup> proprietary image acquisition technique, DF-BF for 3D cultures, generates high contrast, extended depth of focus images.
- 3 days post seeding, A549, SKOV-3 and MCF-7 cells formed round aggregates, while MDA-MB-231 MS exhibited stellate branching distinctive of an invasive morphology.

#### DF-Brightfield Enables Label-Free Quantification



- A549, MCF-7, and MDA-MB-231 cells were seeded (2K cells per well) and allowed to form for 3 days. MS were subsequently treated with
- the cytotoxic agent camptothecin  $(CMP, 1 \mu M)$  or vehicle control (0.1%) DMSO) and images (DF-BF) collected every 6 hr for 7 days.
- Incucyte<sup>®</sup> Spheroid Analysis Software enables kinetic quantification of growing and shrinking MS via size and measurements (Total-BF Area). • A549, MCF-7, and MDA-MB-231 MS
- increased 1.9-, 2.0- and 1.8-fold in size over 3 days respectively.

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

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

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.



#### Quantitative Pharmacology Using Label-Free and Fluorescent Readouts



## Integrated Intensity Camptothecin Cycloheximide Oxaliplatin Controls

Yellow Line = BF Segmentation

#### Cell Number Dependent Multi-Spheroid Size



• Treatment with CMP (1  $\mu$ M) inhibited

growth of all MS.

Time (hr)

Camptothecin (CMP) Cycloheximide (CHX) Oxaliplatin (OXA) 500 500 500 <u></u>400 <u>-</u>400 <u></u>400 .... A 10 300 A 8 300 -- 005 <u>16</u> <u>A</u> Total BF AUC 0-1-001 (AUC 0-1-മറ്റ ਸ਼ੁ <u>ੱ</u> 200 ਹ Size at T = 0 hr - <, 100 ₹ 100-

-9 -8 -7 -6 -5 -4 -9 -8 -7 -6 -5 -4 -10 -9 -8 -7 -6 -5 log [CMP] (M) log [CHX] (M) log [OXA] (M)

Incucyte<sup>®</sup> Nuclight Red MCF-7 MS were allowed to form for 3 days prior to treatment (7 days) with known cytotoxic compounds.

• Time-course plate-views enable rapid visualization of treatment effects on

both MS size (Total BF Area) and viability (FLU Intensity within BF Boundary).



• Concentration response curves represent area under curve (AUC) analysis of the time-course data. • All compounds caused a concentration-dependent inhibition of growth and viability with rank order of potency  $CMP > CHX \ge OXA$ .

#### Label-Free and Fluorescence as a Measure of MS Cytotoxicity



#### FP Expression as an Alternative Measure for Cell Viability



 MCF-7 MS stably expressing nuclear restricted RFP (Incucyte<sup>®</sup> Nuclight Red MCF-7) were treated with CMP (1  $\mu$ M) or vehicle (0.1% DMSO) for 7 days. Incucyte<sup>®</sup> Spheroid Analysis Software reports both MS size (BF Area) and viability (Fluorescence intensity within BF Area) without the need to mask the fluorescent object. Fluorescence (RFP) intensity measurements provide a potential surrogate for MS health.





-9 -8 -7 -6 -5 -8 -7 -6 -5 -4 log [CMP] / M log [CHX] / M

• Incucyte<sup>®</sup> Nuclight Red A549 cells (2K cells per well) were seeded in the presence of Incucyte<sup>®</sup> Annexin V Green Dye (1%) and MS allowed to form for 3 days. • MS were treated with CMP, CHX or vehicle control and images (DF-BF, red and green fluorescence) acquired every 6 hours for 7 days. • Both CMP (cytotoxic) and CHX (cytostatic) caused a concentration-dependent inhibition of MS growth (Total BF time courses). • A loss of RFP signal, lack of growth and a simultaneous increase in Incucyte<sup>®</sup> Annexin V green fluorescence intensity (apoptosis) was observed in CMP treated MS. • Despite CHX inhibiting MS growth, RFP expression remained high, while little or no increase in Incucyte® Annexin V fluorescence was observed, suggesting minimal cell death. These observations are consistent with the cytostatic properties of CHX. • CRCs compare the cytotoxic vs. cytostatic mechanisms of CMP and CHX respectively.