High-Throughput Cell Panel and Organoid Screening in 3D

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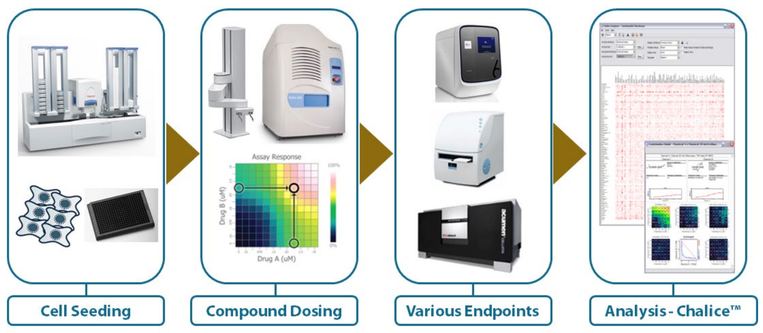
Introduction

To meet the need for cell-based screening in more complex culture systems, we set out to develop 3D cell-based screens to more closely model the complex tumour micro-environment. Our existing OncoSignature panel of 300 cell lines was characterised to produce a 200 strong panel cell lines which form 3D spheroids in ultra-low attachment (ULA) plates. Utilising Horizon's high-throughput platform, we performed compound screens comparing activity in 2D and 3D systems and identified compounds showing differential 2D versus 3D activity.

We also extended our 3D screening into organoid cultures. Organoid cultures have increased complexity in structure and cell heterogeneity compared to spheroids and have historically been challenging to utilise in high-throughput screening, however our proof-of-concept study showed encouragingly robust data which recapitulated recognised genotype based compound sensitivities.

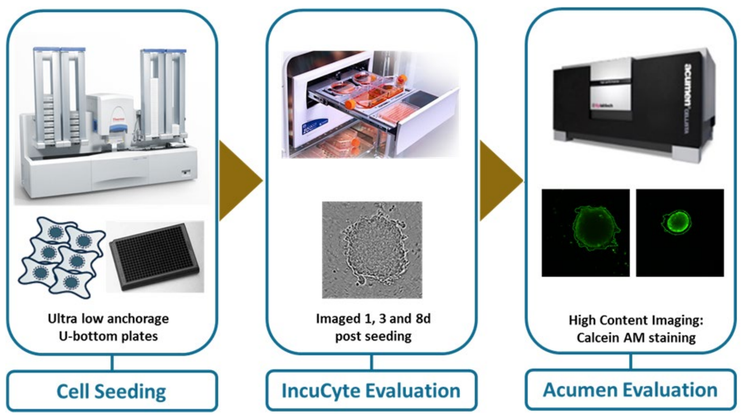
The Horizon Discovery HTS Platform



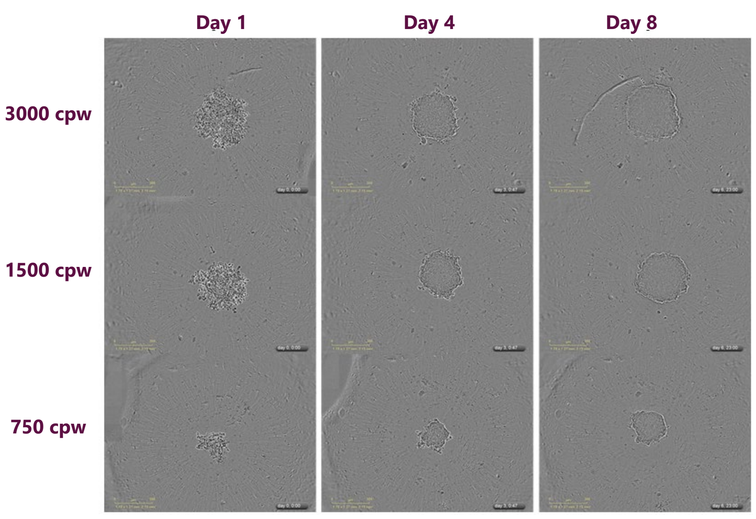
Development of OncoSignature 3D

Starting with our 300-strong OncoSignature panel of indication diverse and genetically well-characterised lines, we first evaluated the ability of all cell lines to form spheroids in 384 well ULA plates.

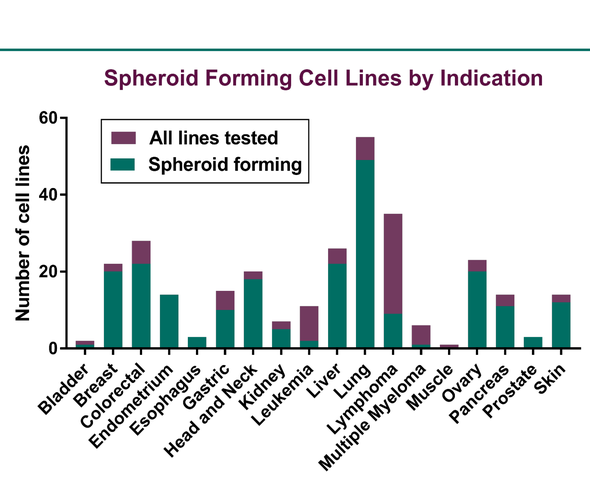
Based on imaging, over 200 lines were identified as being spheroid-forming.



Overview of the cell line characterisation workflow for assessing spheroid growth



Example of spheroid characterisation imaging (A549 cells)

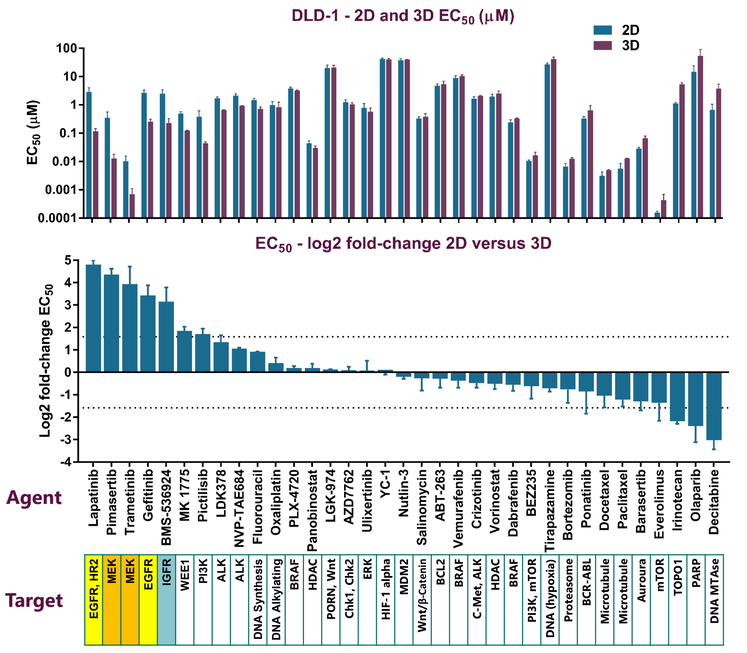


Indication distribution of the OncoSignature 3D cell line panel

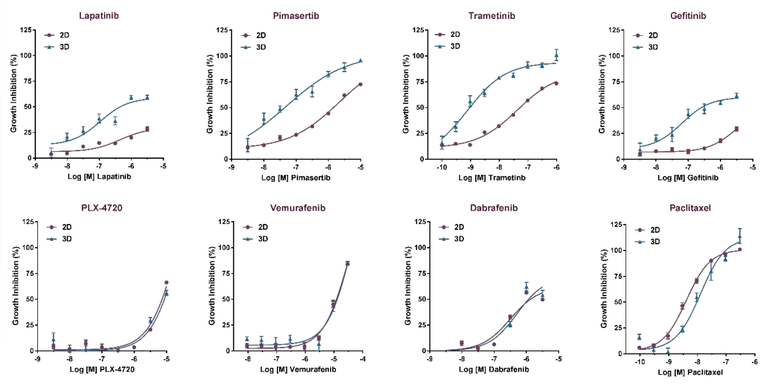
The proportion of lines which formed spheroids maintained the indication representation of the 2D panel for solid tumour derived lines.

2D vs. 3D HTS Compound Screening

To test the ability of our screening platform to differentiate compound activity in 2D versus 3D, growth assays were performed using the colorectal line DLD-1 both in standard 2D culture and in the 3D spheroid assay (CellTiter-Glo2.0™/CellTitre-Glo 3D™ readout, Promega)

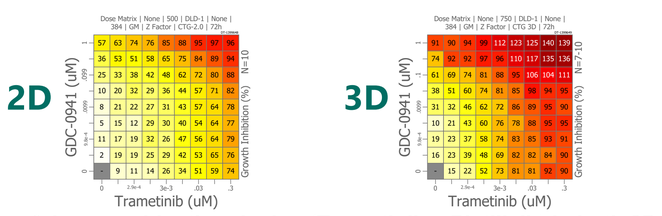


2D versus 3D compound screen in DLD-1 cells. Agents are ranked by magnitude of difference between 2D EC_{50} and 3D EC_{50} values. Dotted lines represent a 3-fold difference in EC_{50} between assay formats.



Examples of dose response curves for agents showing higher activity in 3D assays (upper panel) or limited or no differential (lower panel)

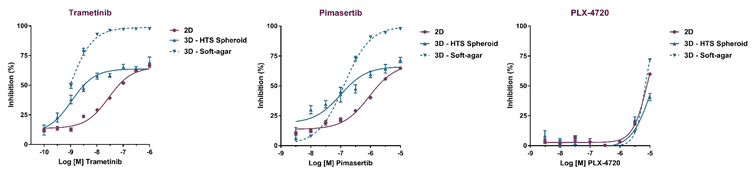
In the 2D versus 3D screen, MEK and EGFR inhibitors clearly showed higher activity in 3D than 2D, with the EC_{50} being >10-fold more potent in 3D.



Example of drug combination dosing (Trametinib x Pictilisib) in both 2D and 3D assay formats

Validation by 3D Soft-Agar Assay

Extremely similar potencies were observed for compounds in the HTS spheroid assay compared to low-throughput soft-agar assays.



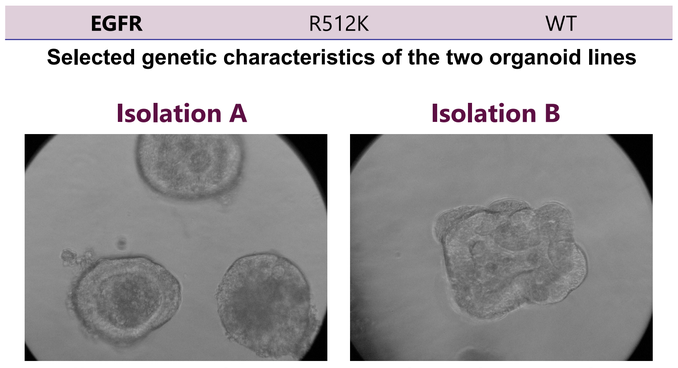
Comparison of dose response curves for selected compounds in HTS spheroid assay versus 96 well-plate based soft-agar assay

High-throughput Format Screening in Colorectal Organoids

To take 3D cell-based screening beyond cell line based spheroids, we performed a proof-of-concept study using colorectal organoids in a high-throughput format. Historically, organoids have been available in limited quantities and with considerable batch-to-batch variation, but bioprocessing technologies developed by our partner Cellesce have enabled us overcome these obstacles.

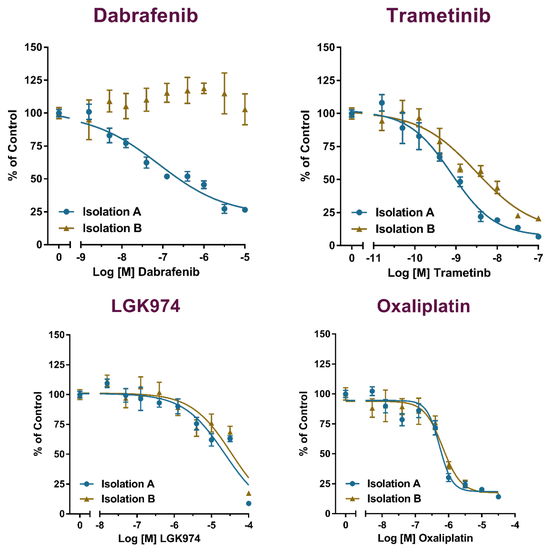
Gene	Isolation A	Isolation B
KRAS	WT	G12D
BRAF	K601E	WT
EGFR	R512K	WT

Selected genetic characteristics of the two organoid lines



Differing morphology of the two colorectal organoid lines

The two organoid lines showed expected morphology and growth in the assay and the morphology reflected that described for colorectal organoids. We found that the BRAF mutant organoids were exquisitely more sensitive to the BRAF inhibitor dabrafenib which is a response that mirrors clinical data.



Examples of HTS-format organoid assay dose response curves

Conclusion

- **We have developed OncoSignature 3D, a panel of 200 cell lines suitable for 3D spheroid format drug-profiling using CellTiter-Glo 3D.**
- Agents known to show greater activity in 3D than 2D, such as MEK inhibitors, were identified in drug panel screens and differential 3D/2D activity for these agents were validated in soft-agar assays.
- Our proof-of-concept study using organoids demonstrated recapitulation of clinical observations in this next-generation 3D model.

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