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Cell line panel drug screening in organoids and 3D systems

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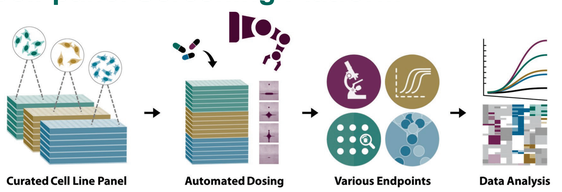
Introduction

To meet the need for cell-panel screening in more complex culture systems, we have investigated 3D cell-based screens to more closely model the complex physiological environment found in tumours. Our existing OncoSignature panel of 300 cell lines was evaluated to produce a 200 strong cell line panel which form 3D spheroids in ultra-low attachment (ULA) plates. Using Horizon's screening 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. Organoids have increased complexity in both structure and cell heterogeneity compared with spheroids, and have historically been challenging to use in high-throughput screening. However, our proof-of-concept study produced robust data that are in agreement with known genotype based compound sensitivities.

Finally, we used our screening platform to evaluate the power of CRISPR-Cas9 approaches in 3D cell-based systems, and are now developing functional genomic screens in organoid models.

Cell-panel Screening Platform



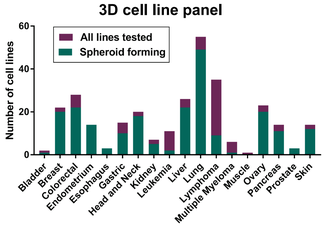
Workflow is managed using LIMS

Development of 3D screen

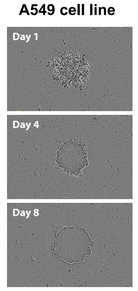
Starting with our 300-strong OncoSignature panel of indication diverse and genetically well-characterised lines, we first evaluated the ability of all cell lines to form spheroids in 384 well ULA plates.

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

Indication distribution of the 3D cell line panel

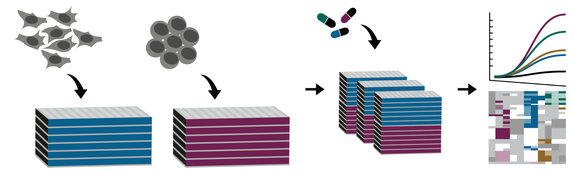


Example imaging: A549 cell line

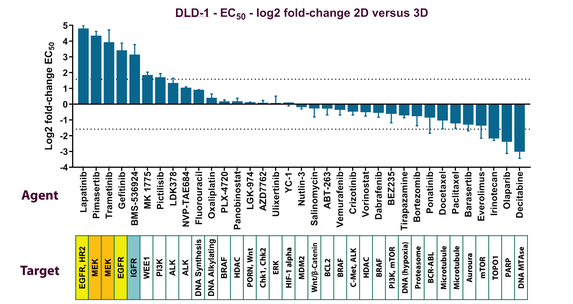


The proportion of lines which formed spheroids were representative of the cancer types that make up the 2D panel of solid tumour derived lines.

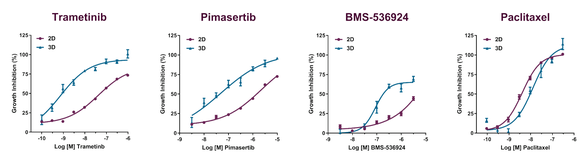
2D versus 3D HTS Compound Screening



To test the ability of our screening platform to differentiate compound activity in 2D versus 3D, growth assays were performed. Example data using the colorectal cancer cell line DLD-1 with CellTiter-Glo2.0™/CellTiter-Glo 3D™ readouts (Promega) are shown.

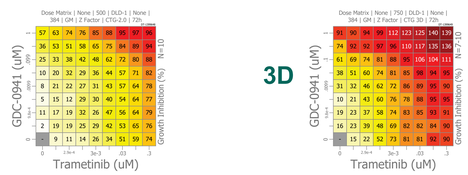


2D versus 3D compound screen in DLD-1 cells. Agents are ranked by magnitude of difference between 2D EC_{50} and 3D EC_{50} values. Dotted lines represent a 3-fold difference in EC_{50} between assay formats.



Examples of DLD-1 dose response curves for selected agents

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.



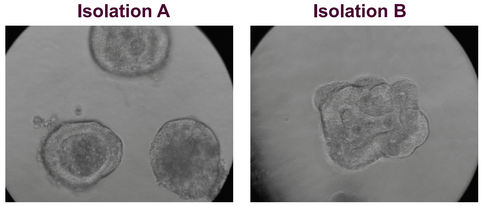
Example of drug combination dosing (Trametinib x Pictilisib) in both 2D and 3D

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 to overcome these challenges.

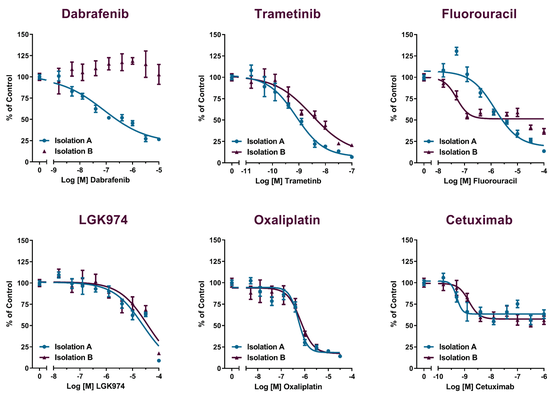
Gene	Isolation A	Isolation B
KRAS	WT	G12D
BRAF	K601E	WT
EGFR	R512K	WT

Selected genetic characteristics of the two organoid lines



Differing morphology of the two colorectal organoid lines

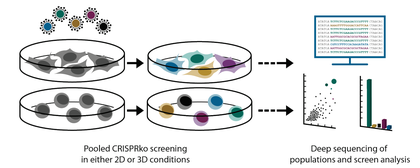
The two organoid lines showed expected morphology and growth in the assay and the morphology reflected that described for colorectal organoids. We found that the BRAF mutant organoids were exquisitely sensitive to the BRAF inhibitor dabrafenib, which is a response that mirrors clinical data.



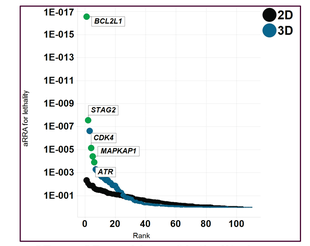
Examples of HTS-format organoid assay dose response curves

CRISPR-Cas9 Pooled Screening in 3D

A pooled CRISPR-Cas9 knockout screen was performed in DLD-1 cells using a tumour suppressor library targeting 110 genes. Cells were grown in standard and low-anchorage conditions.



2D vs. low anchorage CRISPR-Cas9 screen workflow

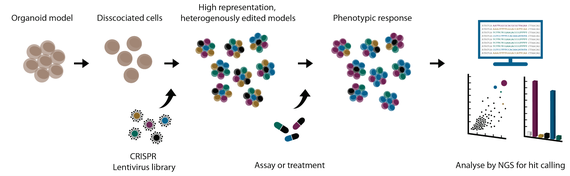


2D vs. 3D ranking of lethality by gene

The screen revealed BCL2L1 as a major sensitiser of DLD-1 cell survival exclusively in 3D conditions.

CRISPR-Cas9 Screening in Organoids

Horizon's screening expertise could also be applied to genetic screening of organoids and we are now developing workflows in this area.



Conclusion

We are developing a 3D cell panel screen using a panel of 200 cell lines suitable for 3D spheroid format drug-profiling.

- 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 (data not shown).
- Our proof-of-concept study using organoids demonstrated recapitulation of clinical observations in this next-generation 3D model.

We have performed a pooled CRISPR-Cas9 screen under 2D/3D conditions and identified BCL2L1 as a sensitiser of cell survival in 3D.

- Utilising our expertise in CRISPR-Cas9 screening, we are developing workflows to facilitate screening in organoid systems.

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