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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 Oncodisc signature panel of 300 cell lines was evaluated to produce a 200-storing 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.

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.

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.

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

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.

To take 3D cell-based screening beyond cell line based spheroids, we are developing a 3D cell panel screen using a panel of 200 cell lines and with considerable batch-to-batch variation, but bioprocessing technologies developed by our partner Cellesce have enabled us to overcome these challenges.

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 sensitizer 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.