Human Cancer Models Initiative goals

- Generate 1,000 new cancer models
- Utilize novel culture techniques that better mimic in vivo physiology
- Models derived from underrepresented tissues and genetic backgrounds
- Supported by patient clinical and genomic data

Pilot phase goals

- Generate 100 new cancer models
- Transfer to ATCC for expansion and distribution

Feasibility study goals

- Replicate and optimize protocols
- Assess potential for scale-up

Results

Table 1. Feasibility study model information. Of the 10 target 10X models for the feasibility study, 14 were received in time for inclusion in the pilot phase feasibility study, 14 were cultured, and 12 completed all testing. Models provided were intended to be a representative selection of the 100 models to be produced during the pilot phase of the HCM Initiative.

<table>
<thead>
<tr>
<th>Total number of models</th>
<th>Number of tissues</th>
<th>Disease states</th>
<th>Model providers</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 received</td>
<td>4 (colon, esophagus, pancreas, mammary)</td>
<td>3 (tumor, normal, other)</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. Scale-up plans for organoid culture. CMOD protocols specified to seeding densities and suggested split ratios were 1:4 or less, if stated at all. We routinely seeded organoids at 5x10^3 - 10^4 cells/mL, which was typically equivalent to split ratios of 1/8 or greater if it varied by model. We utilized a “multi-dome” approach where organoids were seeded in multiple small domes of ~10 μL within each vessel to maximize gas and nutrient diffusion.

Expansion Potential and yield

Type 2 models were evaluated for expansion potential, cryopreservation, and recovery viability.

Table 3. Scale-up plans for organoid culture. CMOD protocols specified to seeding densities and suggested split ratios were 1:4 or less, if stated at all. We routinely seeded organoids at 5x10^3 - 10^4 cells/mL, which was typically equivalent to split ratios of 1/8 or greater if it varied by model. We utilized a “multi-dome” approach where organoids were seeded in multiple small domes of ~10 μL within each vessel to maximize gas and nutrient diffusion.

Figure 2. Estimated model counts per project phase

Figure 3. Standard single dome three dimensional organoid culture. Culture methods were broadly similar between protocols, utilizing a typical “embedded” three dimensional culture system overlaid with medium, though specific dish preparations, media formulations, and ECM varied.

Figure 4. Model growth rates as measured by proliferative cell level (PDL). A subset of models (right) were maintained in culture for ~40 days (equivalent to ~5-10 passages) to assess expansion potential. Average PDL ranged from 1.2–2.4 depending on the model. Models were considered “fast growers” if the average PDL per passage was greater than 2, and “slow growers” if less than 2. No models showed indications of senescence.

Figure 5. Dibridged photographs displaying cultured organoid morphology seven to ten days post-passage. Models exhibited heterogeneous morphology between tissues, donuts, and across the course of culture (not shown). Models could be broadly described as “soft” (larger size and lacking an obvious lumen), “solid” (small and lacking an obvious lumen), or “mixed” (exhibiting a combination of solid and liquid morphologies). Images captured with 10X objective. Scale bars are 400 μM.

Figure 6. Immunostaining of colon models reveals tissue and organoid relevant protein expression. Organoids were fixed and paraffin embedded, and sectioned. Organoids showed expression of CK20 (cytokeratin 20), cytokeratin 18, vimentin (ventilin), epithelial (Ecad), vimentin (ventilin), and CK20 (cytokeratin 20) as colon markers.

Figure 7. Short tandem repeat (STR) analysis. STR profiling was performed after bank passages and compared with source or early passage material, assessing alleles at 10 loci. One of 10 models tested exhibited drift, a gain and loss of separate alleles. Overall this suggests that organized models are largely stable over time in culture.

Figure 8. Viability pre- and post-thaw (left) and viability during culture (right). All models tested were successfully cryopreserved and recovered. No differences were seen between thaw and source viability data (not shown). During routine culture viability was largely stable, ranging from 71% to 94% depending on the model. Across all models the average viability was 83%. Viability was assessed by trypan blue exclusion after enzymatic digestion using an automated cell counting instrument.

Summary

- All models tested in this study were amenable to moderate scale up (generation of at least 1000 cells—in 2 months), and our banks of cryopreserved organoids now totals approximately 1×10^9 cells.
- We developed and demonstrated the feasibility of utilizing a “unified” organoid culture procedure to recreate multiple disparate laboratory/tissue specific protocols. This standardization facilitated larger-scale bioproduction activities and will be published to support the research community in their use of organoid models.
- Remaining challenges for human cancer organoid culture include the need to prepare complex, model-specific culture media, and the requirement for multiple types of undefined media (conditioned media and ECM).

Acknowledgements

Organoids models were provided by Fyza Stefan and the Tavassoli Laboratory from Cold Spring Harbor Laboratory. Robert R. Vines and Sofia Pelletier from the Foundation Institute for Organoid Technology, Mathew J. Guerry and Hosney A. Farouk from the Wellcome Sanger Institute, the Broad Institute, and Vincenzo Corbo and Francesca Lupo from the Center for Applied Research in Cancer at University of Verona.

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Feasibility of manufacturing-scale bioproduction of novel next-generation 3D organoid cancer models in support of the Human Cancer Models Initiative

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ABSTRACT

To meet the need for improved approaches to study cancer in vitro, there has been a surge in the development of novel research models utilizing advanced culture techniques. These methods permit in vitro growth of cancer types previously not possible, and/or models with enhanced in vitro relevance compared to traditional continuous cell lines. However, availability of these early-stage research models is currently limited and there is a lack of data on the ability to scale up production of these models to support the needs of the global cancer community. We sought to investigate the protocols, expansion capacity, cryopreservation ability, genetic stability, and feasibility of large-scale bioproduction of the models generated by the Human Cancer Models Initiative (HCMI), an international collaborative effort between Cancer Research UK, the foundation Hubrecht Organoid Technology, the National Cancer Institute, and the Wellcome Sanger Institute. The HCMI initial goal is the development of 1,000 novel human cancer models, paired with biomathematics and patient clinical data, particularly from rare or underrepresented cancer types. One advanced culture method being utilized, three-dimensional organoid culture, poses challenges for traditional large-scale bioproduction processes. It requires growth embedded within an undefined extracellular matrix and complex media formulations containing multiple small molecules and recombinant proteins with unknown stability and shelf-life. Additionally, organoid growth media typically includes multiple sources of undefined conditioned media containing critical growth factors. We cultured organoid models derived from human colon, pancreas, esophagus, and mammary tissues developed by laboratories contributing to the HCMI. Multiple unique donors were available for all tissues and both cancer and non-cancer models were available for two tissue issues. Most models were maintained in culture continuously for at least 60 days (3–27 population doubling, > 10 passages). Tissue and donor variability was evident in model characteristics, including morphology (assessed by microscopy and immunohistochemistry), genetic stability (measured by short tandem repeat analysis), and genetic characteristics (characterized by sequencing). All models were amenable to scale up beyond multwell plates, and all models could recover from cryopreservation. While organoids represent a significant divergence from typical two-dimensional monolayer culture of continuous cell lines, our results show that these in vitro models are suitable for large-scale bioproduction. This is vital to ensure the widespread availability of these models within the research community to facilitate applications like pre-clinical drug discovery and basic cancer research.