The growth of organoids in cancer drug discovery

Will the automated expansion of organoids help improve the productivity of cancer drug discovery?

Increasingly, complex cell-based assays and tissues are routinely used as part of cancer drug discovery. However, conventional monolayer or suspension cultures can be poorly predictive of the relevant therapeutic effects on a patient’s tumour. As a result, many apparently attractive new drug candidates subsequently fail to meet their primary endpoints in clinical trials. Tumour-derived organoids have the potential to be more predictive earlier in discovery and thus reduce the high rate of compound attrition in downstream development. Tumour-derived organoids have typically been produced in small volumes in specialist research laboratories, using labour-intensive manual processes. Production capacity is constrained and there can be significant batch-to-batch variation. Scalable bioprocessing technologies for the expansion of organoids are now emerging to overcome the bottlenecks of these conventional manual processes. This article will review the challenges and solutions required to expand human cancer organoids to meet the growing demand for the large-scale production of organoids in drug discovery.

According to the World Health Organisation, cancer was the second leading cause of death, accounting for 8.8 million deaths worldwide in 2015, with the number of new cancer cases expected to rise by 70% over the next two decades. Although historic investment in research has resulted in improved cancer survival rates overall, not all forms of the disease have seen such progress and, for some cancers, long-term survival rates have not changed for decades. Consequently, there is an urgent and unmet need for the discovery of novel improved treatments.

More than most other industries, the pharmaceutical sector is highly dependent on the productivity of its research and development (R&D) expenditure to source innovative solutions for their needs, often investing 20% of their revenues back into research each year. Despite these unprecedented levels of investment, current drug pipelines do not always meet the growth expectations of their shareholders for discovering cost-effective new therapies. This increasing pressure on the need to improve industrial productivity is largely due to high levels of pipeline attrition, which is an issue for anticancer drug development with approximately 90% of drugs tested in clinical trials not meeting their primary endpoints. Therefore, the successful products that make it on to the market
are having to compensate for too many failures, which are often weeded out too late in the lengthy development process.

**The opportunity for organoids in cancer drug discovery**

The use of explanted human tumours in drug discovery is not new and, as early as the 1950s, various correlations were claimed between the responses of tumours in tissue culture and their subsequent response to cytotoxic chemotherapy\(^1\). However, such cultures were variable and not consistently available in the quantities required for routine drug discovery applications. So, these explant cultures were typically used later in drug discovery to confirm data that had already been generated to select the most promising compounds for further development. Consequently, such pivotal decisions were often made too late to change the selection of the most promising therapeutic compounds. Immortal cell lines derived from tumours were first developed for scientific research in the 1950s and were used in drug discovery without the variability found with fresh tumour tissue. Although such cell lines often transform over time outside the human body, they represented a significant advance to medical research as, until their introduction, stocks of living cells were limited and took significant effort and time to culture. Since then, numerous other cancer cell lines have been developed but they are typically grown on plastic as flat monolayer cultures. Spheroids can be derived as aggregates from cancer cells or induced pluripotent cells that have been previously grown in monolayers and then assembled into three-dimensional (3D) systems. Spheroids enable cells to communicate with each other as well as their surroundings, providing some similarities to a 3D tumour environment. For example, spheroids can replicate chemical gradients of the various nutrients, oxygen and catabolites found in...
a tumour, leading to the formation of a hypoxic core. However, spheroids do not typically replicate all the different cancer cell sub-populations including, importantly, patient-derived cancer stem cells that are required to drive the development of tumour pathology. Spheroids are, therefore, not entirely faithful representations of the pathology of solid tumours, especially as the heterogeneous and disorganised growth associated with tumours is not easy to recapitulate in vitro.

An organoid is a simplified tissue model that is cultured in 3D such that it grows in vitro to form a realistic micro-anatomy, such as that seen within a tumour-derived organoid (Figure 1). They are derived from one or a few cells from a tissue, such as adult stem cells, which can self-organise in culture owing to their self-renewal and differentiation capacities. Importantly, and unlike other models, the cells are never grown in 2D but are seeded and maintained in 3D for the entirety of their culture in hydrogels. The technique for reliably growing organoids has rapidly improved since the early 2010s and it was named by The Scientist journal as one of the main scientific advancements of 2013. More recently, the journal Nature Methods hailed them as their Method of the Year for 2017. Recent dramatic advances have enabled the long-term growth of organoids to realise their considerable potential as research tools in the laboratory. Consequently, organoids are increasingly being used in both basic research and drug discovery.

A key feature of organoids is that they can self-assemble to preserve the original architecture and function of the solid tumours from which they can be derived. There is accumulating evidence that organoids are likely to be better at predicting efficacy than conventional cancer cell lines, since they replicate key aspects of solid tumours: genetic diversity, differentiation, multicellularity, drug penetration and complex signalling pathway interactions.

![Figure 2](image-url)

Example images of four different patient-derived colorectal organoid lines. The confocal images were captured using a 20x objective on a CellInsight CX7 High-Content Screening Platform. The organoids are stained for nuclear (blue) and cytoskeletal (red) markers for imaging. The scale bars represent 50µm.
For example, organoids derived from metastatic biopsies predict responses to drugs that are subsequently observed in patients from whom the organoids were derived. In 100% of cases in this study, if a drug did not work on a patient’s organoids, then it also did not work in that patient. Furthermore, in nearly 90% of cases, if a drug did work on the organoids, then it also worked in the patient. Importantly, organoids can potentially be generated for many major classes of solid human cancers, including carcinomas in colorectal, breast, prostate and lung cancer. Organoids are, therefore, a new and potentially disruptive platform technology solution that could transform in vitro pre-clinical drug-screening and lead to improved, specifically targeted cancer treatments.

The simple iterative drug discovery cycle of ‘design, make and test’ drives the optimisation of novel compounds and typically needs at least 10,000 new molecules to be synthesised and screened over a 5- to 10-year period. There is an operational need to reduce the number of cycles required to optimise a development candidate by increasing the predictive power of the biological assays. The alternative ‘quick win, fast fail’ approach, in comparison with the more traditional linear sequence of drug discovery, requires pivotal decision-making to be introduced earlier in the drug discovery process. Early decisions then set in motion the long-term development processes that are more closely regulated, less flexible and significantly costlier. As tumour-derived organoids can mimic human cancers in the laboratory, they can be used as a new technology platform to enable pivotal decisions to be made by identifying the most promising compounds early on in drug discovery, by discarding the less attractive molecules even earlier.

To initiate medicinal chemistry, new chemical starting points are required to begin the process of compound optimisation. These are typically found by screening targeted compound libraries, such as those for kinase inhibitors, which are thought to represent up to 50% of current cancer targets. The screening of such compound libraries also requires larger batches of organoids. Consequently, there is now an opportunity to insert tumour-derived organoids much earlier in the drug discovery process but this would require them to be produced consistently at scale to support both hit finding strategies and long-term medicinal chemistry programmes. Until recently, however, capitalising on this opportunity was constrained by the reliance on the existing manual processes carried out by individual scientists in specialist laboratories.

**Growing organoids at scale for cancer drug discovery**

If grown on an industrial scale, tumour organoids can be used routinely for compound screening but
these advances are derived from the basic underpinning science, which originated three decades ago. Researchers have devised new methods to utilise different types of stem cells to generate organoids resembling a multitude of different organs. In 2009, the laboratory of Hans Clevers at the Hubrecht Institute demonstrated that single LGR5-responsive stem cells can build crypt-villus structures \textit{in vitro}, opening up the field to produce immortal colorectal organoid lines. This emerging science is driving the growing interest in organoid-based drug discovery, especially in colorectal cancer. Some examples of the different morphologies observed in such colorectal cancer organoid lines are shown in Figure 2, which were grown from patient-derived tumours.

Currently, specialist academic research laboratories use organoids that are grown manually for basic research, but production is both costly and labour-intensive. However, organoids are not typically produced in the quantities required for the widespread use in drug discovery and lack the uniformity of size and metabolic standardisation required to generate the high-quality data required to drive effective decision making in the drug discovery process. There is a need to grow cancer organoids on an industrial scale for incorporation into both medium- and high-throughput screening protocols and the key features and benefits of this approach are illustrated in Figure 3. To solve the problem created by this bottleneck, organoid expansion processes that can grow organoids in the medium- to high-volume range required for the pharmacological profiling of hundreds to thousands of compounds have been developed. Incorporating these new technologies into a larger-scale bioprocess has developed a capability to considerably increase the productivity of individual scientists working manually. Therefore, with the increased availability of bioprocess-expanded organoids being provided at scale in cancer drug discovery. These organoids can then be used in assay formats compatible with high-throughput screening.

Consequently, with bioprocessing technology required to expand human cancer organoids now available, patient-derived organoids can be used to meet the emerging demand for the large-scale production of organoids in cancer drug discovery. These organoids can then be used in assay formats compatible with high-throughput screening.

Dr Mark Treherne has been actively involved in the biopharmaceutical industry for more than 25 years and previously led the neurodegeneration research group at Pfizer’s research facility in Sandwich, including using stem-cell derived lines for screening compounds. In 1997, he co-founded Cambridge Drug Discovery as Chief Executive, leading the company’s subsequent acquisition by BioFocus plc.

Organoids have been treated with a range of concentrations of trametinib as described in the main text and then fixed in situ and the Promega CellTiter-Glo® 3D Cell Viability Assay was used to determine cell viability in this colorectal cancer organoid cell line. The assay measured ATP as an indicator of viability and generated a luminescent readout.
where he became Commercial Director and drove significant growth of the profitable services business. Dr Treherne joined Cellesce as Chief Executive in 2018 to help drive the commercialisation of organoids for drug discovery. Dr Treherne has a PhD in Pharmacology from the University of Cambridge.

Dr Marianne Ellis co-founded Cellesce in 2013 based on her extensive knowledge of, and expertise in, tissue engineering bioprocessing for cell therapies and in vitro models, particularly scalable bioreactors for cell expansion. She is currently a Senior Lecturer in the Department of Chemical Engineering at the University of Bath and is a chartered chemical engineer. Dr Ellis has held a Royal Academy of Engineering/Leverhulme Trust Senior Research Fellowship and was recognised as a leader of the future with an EPSRC ‘Rising Star’ award in 2014.

Professor Trevor Dale is a pioneering research scientist interested in how cells talk to each other, how this goes wrong in cancer – and how protein machines control it all. The main focus of Professor Dale’s research group is on the Wnt signalling pathway, and his team are growing normal and tumour organoids from a number of human tissue types. Professor Dale studied Biochemistry at Imperial College before completing a PhD on interferon signal transduction at the Imperial Cancer Research Fund (now Cancer Research UK). Following a postdoctoral fellowship at Baylor College of Medicine in Houston, he established a research group at the Institute of Cancer Research in London in 1991. Trevor is a professor at Cardiff University.

Dr Luned Badder is a postdoctoral researcher at the Breast Cancer Now unit at King’s College London and the Institute of Cancer Research. Dr Badder obtained her BSc degree in Biomedical Science at Cardiff University, prior to completing a PhD, to develop patient-derived 3D organoids for colorectal cancer therapeutics (European Cancer Stem Cell Research Institute and School of Biosciences, Cardiff University). Dr Badder is an NC3Rs-funded postdoctoral researcher currently focused on deriving 3D organoid models of Triple Negative Breast cancers.

Dr Andrew Hollins is a postdoctoral researcher at Cardiff University with Professor Dale’s research team. Dr Hollins obtained his BSc degree in Biomedical Science at Cardiff University, prior to completing a PhD in Pharmaceutical Cell Biology based upon a patient-derived model of lung cell differentiation (Welsh School of Pharmacy, Cardiff University; 2001). Dr Hollins has since further broadened his experience with primary cell culture protocol development across a number of human tissues (including colorectal, lung, liver, mammary, prostate and retinal) and is currently an Innovate UK-funded postdoctoral researcher focused on the development of a scalable breast tumour 3D organoid platform.

References