ABSTRACT

Novel targeted drugs are already bringing significant benefits to many cancer patients. However, the costs of administering these new treatments often precludes their widespread use in routine clinical practice. The international pharmaceutical industry continues to defend these high prices by emphasising their need to compensate for the high attrition rate of promising new compounds through the drug discovery process and into clinical development. Early decisions on compound selection are often made by using conventional monolayer or suspension cultures of cancer cell lines, which can be poorly predictive of the relevant therapeutic effects subsequently observed on a patient's tumour in the clinic. As a result, many apparently attractive new drugs that elicit positive data early on in cell-based assays or xenograft animal models then fail to deliver meaningful endpoints in clinical trials. Tumour-derived organoid lines grown in vitro from patient biopsies are a novel solution to this problem, as they have the potential to be more predictive earlier in discovery and thus reduce the high rate of compound attrition in downstream development. Bioprocessing technologies for the industrial expansion of organoids are now emerging to overcome this problem. This review will analyse the challenges and solutions required to exploit human cancer organoids to meet the growing demand for their practical application in drug discovery. By reducing the high rate of attrition of compounds in drug discovery, it is expected that the cost of new cancer treatments can be reduced and, therefore, made more widely available across the world. The future economic and medical benefits of this new approach are discussed, along with some consideration of the resultant carbon footprint of cancer drug discovery.
tested in clinical trials are not meeting the primary endpoints that were envisioned in the drug discovery phase. Therefore, the successful products that make it on to the market are having to compensate for too many failures, which are often discarded too late in the lengthy development process. Late-stage failures are particularly costly.

**Application of Organoids in Cancer Drug Discovery**

Although the practical application of explanted human tumours to the testing of chemotherapeutic agents was first instigated during the 1950s, these short-term in vitro cultures were relatively unpredictable to keep alive and were not consistently available for robust and repetitive drug discovery applications [3]. Consequently, these original explant cultures were used later on in the drug discovery process, typically as one-off experiments to confirm data that had already been used to select the most promising compounds for further clinical development. Therefore, these decisions were often made far too late to alter the intelligent selection of the most promising therapeutic compounds for further development and were confirmatory rather than being pivotal in decision making. Immortal cell lines derived from tumours were also first developed for scientific research earlier in the 1950s [4] and were then later adapted for use in drug discovery, without the some of the variability found with the fresh tumour tissue explants described above. Although such cell lines often transform over time outside the human body through the multiple passages required to keep them alive, they did represent a significant advance in medical research. 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 still grown as flat monolayer cultures, which do not always recapitulate the tumour morphology observed in patients.

An organoid is a term typically used to define a simplified version of an organ produced in three dimensions (3D) that grows in vitro to form a realistic micro-anatomy, such as a spherical intestinal-like structure [5]. Organoids are derived from one or a few cells from a tissue biopsy containing adult stem cells, which can self-organise in culture, resulting from their self-renewal and differentiation capacities. Unlike other multicellular models the cells are never grown in 2D but are seeded and maintained in 3D for the entirety of their culture in suitable hydrogels. The technique for reliably growing organoids has rapidly improved and Nature Methods selected organoids as their Method of the Year for 2017 [6]. Recent advances have enabled the long-term growth of organoids to realise their scientific potential as research tools in the general research laboratory [6]. Consequently, organoids are increasingly being used in both basic research and, ever increasingly, in practical drug discovery applications as well [7].

The key feature of organoids is that they can self-assemble to re-form with the original architecture and function of the structures from which they were derived, which means they can faithfully model disease pathologies in vitro. For example, there is accumulating evidence that organoids are likely to be better at predicting clinical efficacy than conventional cancer cell lines, since they replicate key aspects of solid tumours: genetic diversity, differentiation, multicellular structure, drug penetration and complex signalling pathway interactions [7]. Organoids derived from metastatic biopsies predict responses to drugs that are subsequently observed in patients from whom the organoids were first derived [8]. In 100% of cases in this study, if a drug did not have a significant effect on a patient’s organoids, then it also did not have an effect in that patient. Furthermore, in nearly 90% of cases, if a drug had a discernible action on the organoids, then it also demonstrated efficacy in the patient as well. Importantly, organoids can potentially be generated for all major classes of solid human cancers, including carcinomas in colorectal, breast, prostate and lung cancer. Organoids are, therefore, a new, disruptive platform technology solution that could transform in vitro pre-clinical drug-screening and lead to improved, specifically targeted cancer treatments. A previous article has reviewed how organoids can now be expanded at scale for the widespread use in drug discovery [7], so this new review will now focus on the practical use of tumour-derived organoids in high-throughput screening applications and the consequent practical and macroeconomic implications of this research.

**The Application of Tumour-derived Organoids in High-throughput Screening**

The simple iterative drug discovery cycle of “design, make and test” drives the optimisation of novel compounds [9] and typically needs around 10,000 new molecules to be synthesised and screened over a 5- to 10-year period to discover a new drug. 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 [9]. A “quick win, fast fail” approach in comparison with the more traditional linear sequence of drug discovery, requires pivotal decision-making to be introduced much earlier in the drug discovery process [10]. Early pivotal decisions are when the long-term development processes that are more closely regulated, less flexible and significantly costlier. As tumour-derived organoids can effectively mimic human cancers in the laboratory, they can be used as a new disruptive 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. Subsequently, precious research resources can then be focused on more promising compounds with a greater probability of success in the clinic.

To initiate the iterative medicinal chemistry optimisation described above, new chemical starting points are required to begin this compound optimisation process, which are typically found by screening targeted compound libraries, such as those for kinase inhibitors [11], which are thought to represent up to 50% of cancer drug targets [12]. Diverse libraries of compounds of well over 100,000 can also be screened, when suitable targeted libraries are unavailable or unsuitable. The screening of such compound libraries also requires larger batches of organoids for screening in high-throughput screening systems, which used to be constructed as large cumbersome systems with bespoke robotics and mostly used for cell-free assays [13]. However, more up-to-date, high-
Consequently, the provision of expanded organoids as effective research tools can now be reliably supplied as frozen organoid lines in cryovials, ready for seeding into various assay formats required for multiple drug discovery applications. For example, the usual requirement is for compound screening being performed in 96- or 384-well conventional screening formats. The thawed organoids can then be re-suspended in a hydrogel in 96-well plates and incubated at 37°C and exposed to a test compound for 2 days or more, before being assessed for cell viability or some other relevant assay endpoint. A recent publication demonstrates the benefits of using patient-derived organoids in such screening systems with novel kinase inhibitors [16]. This study highlighted the limited predictive power of 2D cancer cell lines, which can sometimes fail to fully recapitulate intra-tumour phenotypic heterogeneity. In particular, the relationship between 2D cancer cell biology and cancer stem cell function is poorly understood. By contrast, 3D tumour organoids provided a screening platform in which complex cell-to-cell interactions could be studied. Tumour organoids from colorectal cancer patients were tested to ascertain their responses to known kinase inhibitors. Using compounds with 3 orders of magnitude difference in cellular mechanistic potency together with image-based assays, the study demonstrated that morphometric analyses can capture subtle alterations in organoid responses to inhibitors that are consistent with activity against a cancer stem cell subpopulation. The study also highlighted the value of phenotypic readouts as a quantitative method to assess drug-induced effects in a relevant preclinical model [16].

With the bioprocessing technology required to expand human cancer organoids now becoming reliably available [7], 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 analysis [14]. The next key question is how can these advances produce future benefits for the overall efficiency of the cancer drug discovery process?

**Benefits of Tumour-derived Organoids in High-throughput Screens**

For 2 decades or more, there have been numerous attempts to use strategic outsourcing and technology collaborations to accelerate drug discovery research in the pharmaceutical industry [17]. In summary, there were two parallel outsourcing approaches being adopted around the millennium: firstly, to reduce the cost of labour by moving outsourced research and development to locations where the full time equivalent (FTE) rate of a research scientist was significantly lower; secondly, to invest in new technologies to improve the success rates in drug discovery significantly. Overall, neither of these strategies has yet been successfully proven to have delivered more cost-effective drugs. While FTE rates have undoubtedly been reduced on a global basis, paying scientists less has not produced significant productivity gains that can be translated into reduced costs of new cancer drugs [1]. While the past 70 years have seen huge advances in many of the scientific and technological factors that should, at least in theory, tend to raise the efficiency of commercial drug research and development, this had not been the case in reality. The number of new drugs approved per billion US$ spent on research has halved roughly every 9 years from 1950 to 2012, falling around 80-fold in inflation-adjusted terms [18]. The 2012 review analysed the problem and introduced the concept of “Eroom’s Law”, which is “Moore’s Law” spelt backwards [18]. This new “Law” refers to processes that are getting steadily slower and more difficult to execute over time, the opposite of Moore’s Law, which was originally the observation that the number of transistors in a dense integrated circuit doubled every two years. Admittedly, this may well be an overly pessimistic analysis but there is certainly significant room for improvement within the current “state of the art” in the cancer drug discovery process.

In cancer research, as with all other drug discovery applications, the introduction of high-throughput screening, combinatorial chemistry and biotechnology solutions has not produced the significant productivity gains that were originally envisaged. However, patient-derived organoids grown from individual patients’ tumours now have the potential to be integrated into existing screening technologies to improve the probability of selecting compounds that are more likely to succeed in clinical trials. Even if organoids can enable only modest improvements in success rates in cancer drug discovery, then this could be transformational for the productivity of the pharmaceutical industry.

**Potential Benefits of Organoids in Reducing Carbon Emissions**

Although an understanding of conventional healthcare economics is critical, so is an understanding of the new bioeconomics of the carbon cycle. Despite the heightened urgency of curbing carbon emissions around the world, the healthcare sector in general and the pharmaceutical sector, in particular, have received very little attention from a sustainability perspective in terms of their contribution to the global carbon footprint [19]. A recent analysis revealed that the pharmaceutical industry is significantly more emission-intensive than the automotive industry [19]. Although most of the emissions probably relate to manufacturing of the therapeutic products themselves, drug discovery also significantly contributes to these emissions. For example, millions of animals are used in research and toxicity testing globally, including in drug discovery and development. Although the environmental consequences are yet to be adequately addressed, current evidence suggests that research animal use and disposal contributes significantly to environmental pollution [20]. The quantity of energy consumed by research animal facilities is up to 10-fold more than offices on a square metre basis [20]. Animal research facilities require total...
Conclusion

The costs of administering new targeted cancer treatments often precludes their widespread use in routine clinical practice. The high prices of these new patented medicines are typically justified by the need to compensate for the averaged 90% attrition rate of promising new compounds throughout the drug discovery process and into clinical development. Early decisions on compound selection made by using conventional 2D cultures of cancer cell lines can be poorly predictive of the relevant therapeutic effects subsequently observed on a patient’s tumour in the clinic. As a result, many apparently attractive new drugs that elicit positive data early on in cell-based assays or even in animal xenograft models then fail to deliver meaningful endpoints in clinical trials. Tumour-derived organoid lines grown from patient biopsies are a solution to this chronic problem, as they have the potential to be more predictive earlier in discovery and thus reduce the high rate of compound attrition in downstream development. This article has reviewed the challenges and novel solutions required to allow the widespread application of human cancer organoids into drug discovery. If these challenges can continue to be overcome, it is expected that the cost of new cancer treatments can be reduced and, therefore, made more widely available across the World. The future economic and medical benefits of this new approach are clear, along with an additional opportunity of reducing the resultant carbon footprint of cancer drug discovery.

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