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Organ-on-a-chip technology: turning its potential for clinical benefit into reality

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Organs-on-a-chip (OOAC) are research platforms containing cellular models designed to recapitulate relevant biological cues and, in some cases, enable communication between 'on-chip' connected organs. With enhanced physiological relevance, improvements in predictivity of the efficacy and toxicity of test compounds are anticipated. However, there are challenges to demonstrate the 'gain of confidence' of this technology for patient benefit. Translational challenges, the opportunities and deficiencies of the organ models, their intercommunication and the platform technology are all issues to be resolved. Sensitive, real-time detection technologies and data-rich readouts are needed to understand OOAC biology. Thus, the validation of normal and disease biology on chip, and modelling to translate these data to patients, will help position this technology in mainstream drug discovery.

Introduction

A current challenge across the pharmaceutical industry is to lower the rate of attrition in the delivery of new drugs to patients [1]. Despite continued high levels of investment in drug discovery, the rate of new drug approvals remains static, and return on investment to drug companies is estimated to be only ~3% [2]. The inadequacy of current drug-screening models that fail to adequately represent the biology of patients is part of the problem. The limitations of traditional immortalised 2D single cell-based models grown on tissue culture plastic are well understood [3], leading to a switch to 3D co-culture cell model development [4]. Extensive literature also highlights the challenges in animal-based assessments of their efficacy, safety and predictivity to humans [5–8].

OOACs, or microphysiological systems (MPS), are miniaturised physiologically relevant, biological testing systems suitable for drug discovery research [9–11]. OOAC technologies involve a single cell or tissue model or multiple connected models, positioned in a microfluidic flow device (Fig. 1). By linking cell models together, more relevant physiology can be captured. These systems are an alternative to conventional cell culture and some animal models, enabling fluid flow, 3D biology, and communication between cells and organs. The development of OOAC technology has the potential to make *in vitro* drug testing more closely resemble the patient who will be treated. This could improve target identification and validation, measurement of efficacious compound effects, and detection of unexpected toxicological issues, thereby accelerating drug discovery [12,13].

Although this technology has great promise, evidenced by new company formation and financial investment [14], the translation of on-chip models to use in preclinical to clinical drug discovery programs is currently an area both of excitement and uncertainty. Consistent with any new technology, the generation of compelling data to achieve a gain of confidence, alongside the new technology 'hype', is necessary to promote acceptance by regulatory authorities [15].

Here, we highlight opportunities and challenges in the translation of OOAC technology. These include the migration of cell models into devices to recapitulate organotypic (or tissue) function, and the subsequent validation of the system as an effective predictor of desired clinical therapeutic outcomes and off-target effects.

OOAC translational cell models

A key goal for OOAC technology is to present translational models that better represent the patient compared with current standard tissue culture models that might provide an enhanced alternative

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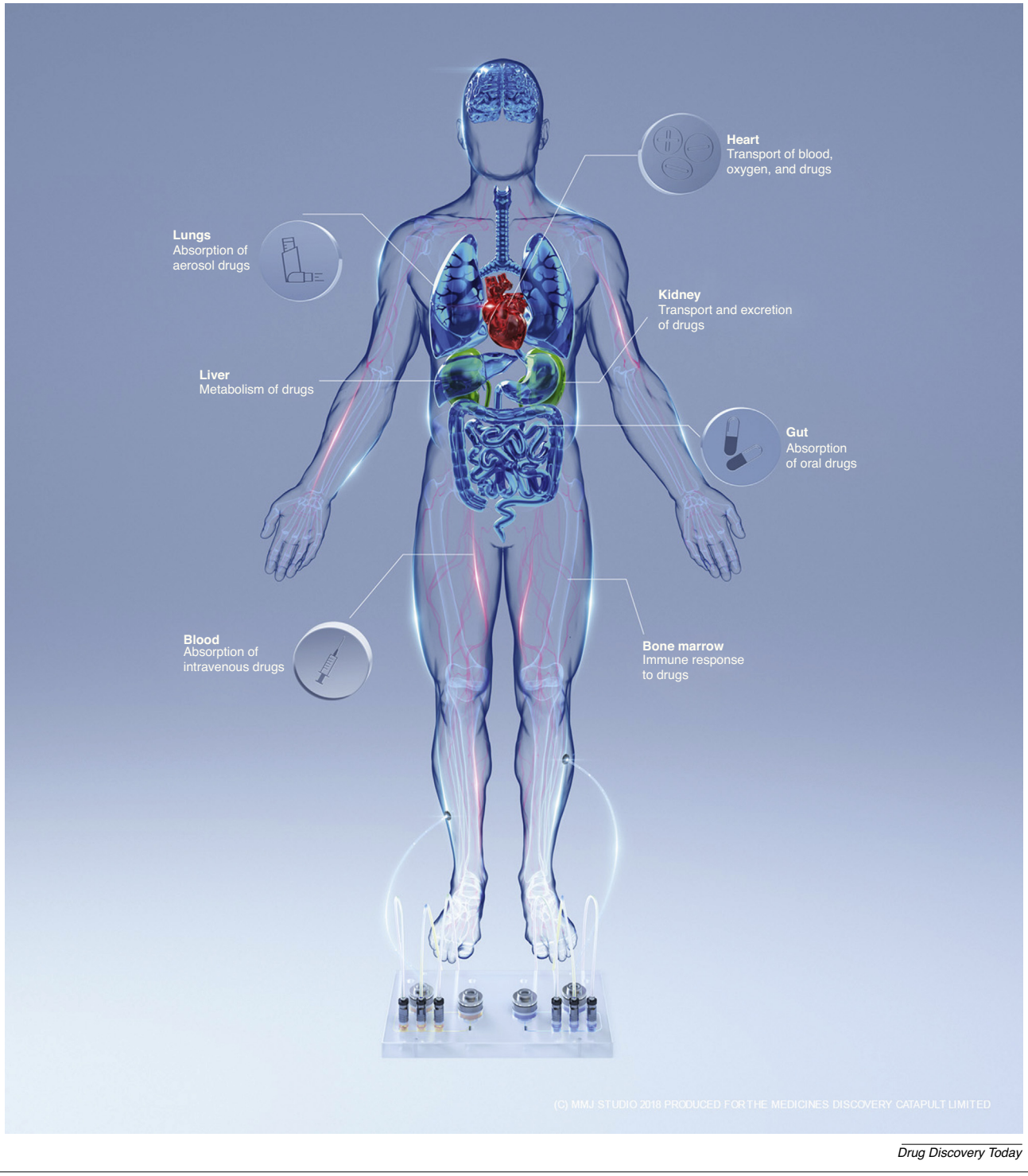

FIGURE 1

On-chip recapitulation of major organ functionality could enable more relevant and predictive drug testing approaches.

(or complement) to animal studies. OOAC technology is not limited to any particular disease area; indeed, it can allow the incorporation of multidisease phenotype models aligned to real-world multipathology and drug-experienced patient populations. The cell models need to reflect either healthy or diseased tissues, ideally representing the diversity of the patient population that is the target for therapy [12]. Prolonged on-chip maintenance of tissue or organ structure can make disease models more amenable to slowly developing or mixed pathologies.

The cell models should include the correct proportion of several cell types and be capable of at least some of the functions of the organ or tissue that they represent. For recapitulation of human biology, the inclusion of primary cells can be desirable, as isolated cells, explant cultures, organoids [16] or potentially recellularised

mini-organs [17]. Induced pluripotent stem cell (iPSC)-derived cell models present an attractive opportunity in terms of larger scale studies and uniformity over time; on-chip iPSC differentiation protocols are currently under investigation [18]. The possibility of genetically matched controls (diseased and wild type) can be important for target evaluation and could help with overcoming potential rejection-like complications in multichip configurations. Even standard immortalised cell lines have been shown to reacquire lost functions in OOAC configurations more reminiscent of *in vivo* [19]. Driven by these advances and new opportunities, an update to the existing Good Cell Culture Practice guidelines is underway reinforcing the importance of quality and rigour across the cell models and OOAC technology [20].

The importance of environmental cues

A key differentiator for the OOAC approach is the inclusion of physical stimuli, which are usually absent in standard cell culture systems. Although these are present at the higher complexity of *in vivo* models, in these environments they are typically neither readily accessible nor controllable. The ability to establish microcirculatory systems, perfusion, and shear stress on chips enables cells to experience relevant pseudophysiological cues that directly affect their biological function. Fluid flow is essential for nutrient supply, waste removal, diffusion of molecules into cells, and cell polarity. Importantly, it exerts physical stress essential for the correct biological functioning of, for example, endothelial cells [21] by the activation of cell surface molecules and associated signalling cascades. Similarly, the incorporation of flow into an OOAC device influences biological capability even within a single organ. For example, iPSC differentiation into hepatocyte-like functionality [22] is valuable for hepatotoxin identification [23–25]. OOAC systems can recapitulate flow by simple 'rocker' type on-chip fluid movements, or by more complex programmable 'pulsatile' formats arranged in a single loop through to tissue-specific configurations [26].

Precisely controlled microfluidics in more sophisticated OOAC technologies enables cellular nutrient supply, and waste products, to be managed. This is an important consideration, because on-chip cell health is fundamental to model performance and the basal OOAC metabolomic profile [27]. On-chip fluidic circuits permit the interorgan exchange of relevant biological signals, feedback loops for homeostasis, and determination of the effects of drug treatment. Bioengineering expertise has enabled the prolonged maintenance and viability of up to ten organs in a single interconnected chip device [28]. Also, functional coupling (i.e., the physical transfer of media from one component model to another in the correct physiological sequence) has enabled a study to show clinically relevant multiorgan ADME processing [29]. The potential integration of micro-dosing technology [30] in which very small volumes can be added or removed from culture systems, provides an opportunity for drug compound administration to OOAC cell models to mimic *in vivo* drug exposure and pharmacokinetic/pharmacodynamic (PK/PD) profiles. It has been proposed that such microformulator devices could even supply the surrogate function of 'missing' organs in a connected system.

The inclusion of biomimetic substrates and their arrangement can be used to create structural configurations on chip that are representative of those found *in vivo*. These support structures can be fine-tuned for a specific tissue or application, such as cardiovascular disease extracellular matrix remodelling [31], or can be configured as 'seed' points for the on-chip development of a vascular network [32]. Existing 'stand-alone' assay platforms, such as transwells, can be incorporated as part of a connected OOAC system [33].

Routine real-time measurement of temporally dynamic, clinically aligned biomarkers could give OOAC technology a clear line of sight to prediction of clinical responses. To make this a reality, high-sensitivity, low-volume, and high content detection technologies will be required, to ensure that the rich on-chip biology is matched by rich data sets for biomarker identification or target hypothesis testing.

The specific on-chip platform design, and the biology configuration of cell types being cultured, drives the biology of each OOAC platform and, therefore, its translational applicability. Understanding the relative value and impact of each OOAC platform is important: the concept of a 'best' model is unhelpful, because the relative value depends on the context of use.

Translational design challenges

The best chance for increased translational relevance from implementing OOAC is by combining a biologically relevant on-chip environment, recapitulation of functionally appropriate cell models, and the ability to quantitate readouts by sensitive detection technologies.

Key to wider OOAC model acceptance is clinical validation, or at least regulatory qualification. For clinical validation, the reliability and relevance of a model needs to be established for a defined clinical purpose with known predictivity by comparative testing of relevant well-annotated drugs. Regulatory qualification is drug or model approval within a stated context of use (i.e., the results from a specific model can be relied upon as decision-making data, but only within acknowledged boundaries or limitations of the data).

Translational constraints in OOAC systems derive from the individual cell models, platform limitations, data able to be captured, and the assumptions needed for extrapolation to *in vivo*. Most current data are from simple OOAC models without an holistic integrated response across multiple organ functions. As the number of organs connected increases, technical, biological, and translational complexity escalates. A 'physiome' or 'human-on-a-chip' currently remains a distant reality that, because of cost and technical complexity, might well not be desirable for most studies [34]. Some fundamental physiological responses remain particularly challenging to formulate on chip, such as the immune response, the endocrine system, and gut–microbiome interactions. Each cell model is a compromise containing known or unknown deficiencies; these accumulate with each cellular component of a single organ and multiply as the number of connected organs grows. In addition, the absence of a universal common media able to adequately support all connected organs is also only a partially solved issue and might require a project-specific media formulation [35]. As a result, there is a risk of artefactual and irrelevant nontranslatable data being generated from overly complex OOAC systems. In practice, preclinical studies will often be performed on the least complex model system that can be chosen.

Primary cells or explants could be the preferred tissue of choice; however, access to sufficient human material can be problematic,

and phenotypic drift associated with *ex vivo* culture leads to reproducibility issues. Organoids can address these issues in part, although uniform expansion to scale is not trivial and the recapitulation of organ function is incomplete. For iPSC-derived cell models, their mimetic capacity is restricted by differentiation protocols that do not lead to 'adult-like' organ maturity, complicating interpretation to relevant clinical data. Furthermore, the absence of any agreed definition of the desired functionality that constitutes an organ on a chip is limiting and unhelpful; for example, which of the many functions of the liver are necessary to recapitulate or ignore?

Relative scaling, or allometry, across the biological constituents of an OOAC platform is important and can be based on size, surface area, or functionality following extrapolation from *in vivo*. The choice of multifunctional or allometric scaling parameters has been shown to be important for drug exposure modelling [36]. Organs *in vivo* are not homogenous balls of cells; for example, zonal pressure differences and hypoxic areas are important for *in vivo* functionality and are largely unrepresented in current OOAC design [37].

On-chip technical challenges that make wider adoption of OOAC in drug discovery harder include the materials of manufacture. These include the widespread use of polydimethylsiloxane (PDMS), which has an association with high compound binding for at least some classes of molecule [38]. Further challenges include making chips robust, reproducible, and affordable.

The diversity of current commercial and academic-generated chip formats makes it difficult for system integrators to incorporate chips into automated assay platforms and workflows [39]. OOAC systems currently available commercially are also mostly not easily configurable with standard assay detection platforms. If future OOAC systems can have standardised footprints and input/output ports, similar to the emergence of SBS-standard microtitre plates [40], this is likely to drive integration with automation and sophisticated detection technology. In the short term as throughput is likely to remain modest, optimum placement of the OOAC solution within the drug discovery cascade is needed e.g. where initial risks of the project have been reduced [41].

New on-chip label-free, real-time, biosensors are being developed to report and manage model viability (such as pH, temperature, oxygen level, and nutrient availability), building on experience with bioreactors [42]. In time, self-sustaining OOAC configurations that exploit microformulators will be developed for on-chip viability and homeostasis control. Detection technologies that can sense and quantitate a wider range of on-chip biological responses are also an unmet need. Although live/dead imaging of on-chip biology is established and highly valuable, the incorporation of necessary fluorescent probes might be cytotoxic or limit prolonged observations. Approaches to both detect and stimulate cells are available for niche applications, such as heart on a chip [43], and integration of chips with mass spectrometry might in time provide a powerful opportunity to generate rich data sets. However, even for a simple quantitative measurement, such as impedance via integrated electrodes, interpretation of the corresponding biological outputs can be complicated for a single cell model, let alone multiple combined ones, as has been observed for adipocyte differentiation [44].

Many of the challenges described for OOAC technology have an equivalent in preclinical *in vivo* animal model. An appreciation of the biological limitations of the model system is necessary because of fundamental biological inadequacies and species-specific differences. Although the integrated physiological response of preclinical *in vivo* models satisfies complexity, this further represents the animal rather than human response. Clear gaps in current OOAC capabilities, such as the endocrine or immune system, are a given for *in vivo* models. Similarly, it is hard to imagine long-term repeat dosing or reproductive studies 'on chip' and it is not a given that biomarker responses observed *in vitro* will retain *in vivo* equivalence. Although animal models have been extensively characterised, and some are mandated by regulatory authorities, the equivalent for OOAC needs further development by the engineering of multiple biological, metabolic, and dosing parameters. In this regard, 'animal on a chip' as a crossover activity towards 'human on a chip' might better exploit the decades of available preclinical model data to further the understanding and placement of OOAC technology.

Requirements for *in vitro* to *in vivo* translation

Results from OOAC devices need to be readily quantifiable and relatable to *in vivo* data by translational modelling. OOAC should not be expected to act as an exact mimic or directly predictive of the entirety of *in vivo* effects. A more achievable goal is being more predictive and biologically relevant compared with the assays that it replaces in the drug discovery cascade. New mathematical models are required to compensate for system-encoded effects to enable better extrapolation from OOAC to *in vivo* (animals and patients). In areas such as ADME, these extrapolation relationships are easier to establish based on extensive existing modelling knowledge [45]. For example, accumulation of drug in tissues on a chip can be measured (endpoint determinations, or potentially in real time through microsampling), and then extrapolated to *in vivo* organ accumulation. Greater *in* vitro–*in vivo* translation (IVIVT) challenges arise with less well-understood disease biology and increased on-chip biological complexity. It might also be necessary to apply a mathematical surrogate for a missing organ or crucial functional response in the incomplete system. The additional interpretative steps for IVIVT require quantitative systems pharmacology (QSP) [46] assessment for the integrated and iterative combination of on-chip and off-chip data from diverse sources [34,47]. The requirements here are access to extensive data sources and the know-how to derive and test QSP models, such as modelling of lidocaine metabolism from a liver OOAC and extrapolation to PK variability from patient data [48].

Where well-validated and clinically relevant biomarkers are known, their on-chip detection in real time would enable meaningful transitions away from this baseline to be interpretable for hypothesis testing, within the use case limitations of the chip.

Translational equivalence of OOAC models might be easier to accomplish for efficacy projects with a defined molecular target, because the necessary components of the therapeutic model can be configured in the OOAC device. Passive communication between organs might be enough for project progression needs, allowing the biological effects of a compound to be determined in an environment unable to be established *in vitro* otherwise. Of course, the availability of human data is limited for molecular

characterisation at novel targets and a complete understanding of drug toxicity in humans cannot be achieved preclinically. In toxicological studies, there might be a desire for representation of all possible 'off targets', which would require a human-on-a-chip, with extensive measured outputs to identify *the* toxicity. Identification of an on-chip therapeutic index would help with cross-species prediction or extrapolation of effects to patients.

Working models

There are many hurdles to be overcome to deliver the truly widespread adoption of complex OOAC platforms in preclinical drug discovery pathways. Single-component OOAC models have been reported that mimic major organ functions for heart, lung, liver, kidney, gut, brain–blood brain barrier, skin, vasculature, and extending to key disease areas, such as cancer. Furthermore, connected organ approaches exploiting multiple perfusion, single-pass, or recirculating approaches enable some recapitulation of interorgan-dependent functionality. This enables integrative on-chip testing with encouraging potential utility for drug discovery [26]. With time, the appropriate positioning of these models into drug-hunting cascades will prove their value, or otherwise, as effective predictors of *in vivo* clinical effects.

For single- or two-component tissue or organ devices, there are encouraging examples of good translation to man. For Barth syndrome, a mitochondrial DNA mutation results in cardiomyopathy and reduced contractile performance [49]. Patient-derived (and subsequently engineered) cardiomyocytes were placed onto a PDMS membrane from which sarcomere measurements of contractile function were made. In this 'heart-on-a-chip' model, cells showed a reduced contractile effect consistent with previous *in vivo* measurements. In a lung-on-a-chip membrane barrier model [50], endothelial and epithelial cell layers represent an alveolar capillary interface on which mechanical stimulation imitates breathing. Introduction of bacteria onto the epithelial space was found to lead to activation and recruitment of neutrophils, whereas exogenously added IL-2 led to oedema because of fluid rearrangement. In this example, organ-level translational events were established in a set up that is sufficiently representative to drive relevant cellular activity with valid clinical translation. Also, in a stem cell-derived linked microchannel model of the neuromuscular junction, bungarotoxin, botulinum toxin, and curare compound dose responses were determined at multiple stimulation frequencies. These closely matched human *in vivo* responses in an innovative on-chip configuration, which is now available for routine screening [51].

With increasing complexity of the connected organs comes both an opportunity to assess translational relevance in a more holistic setting and the challenge of ensuring compatibility of

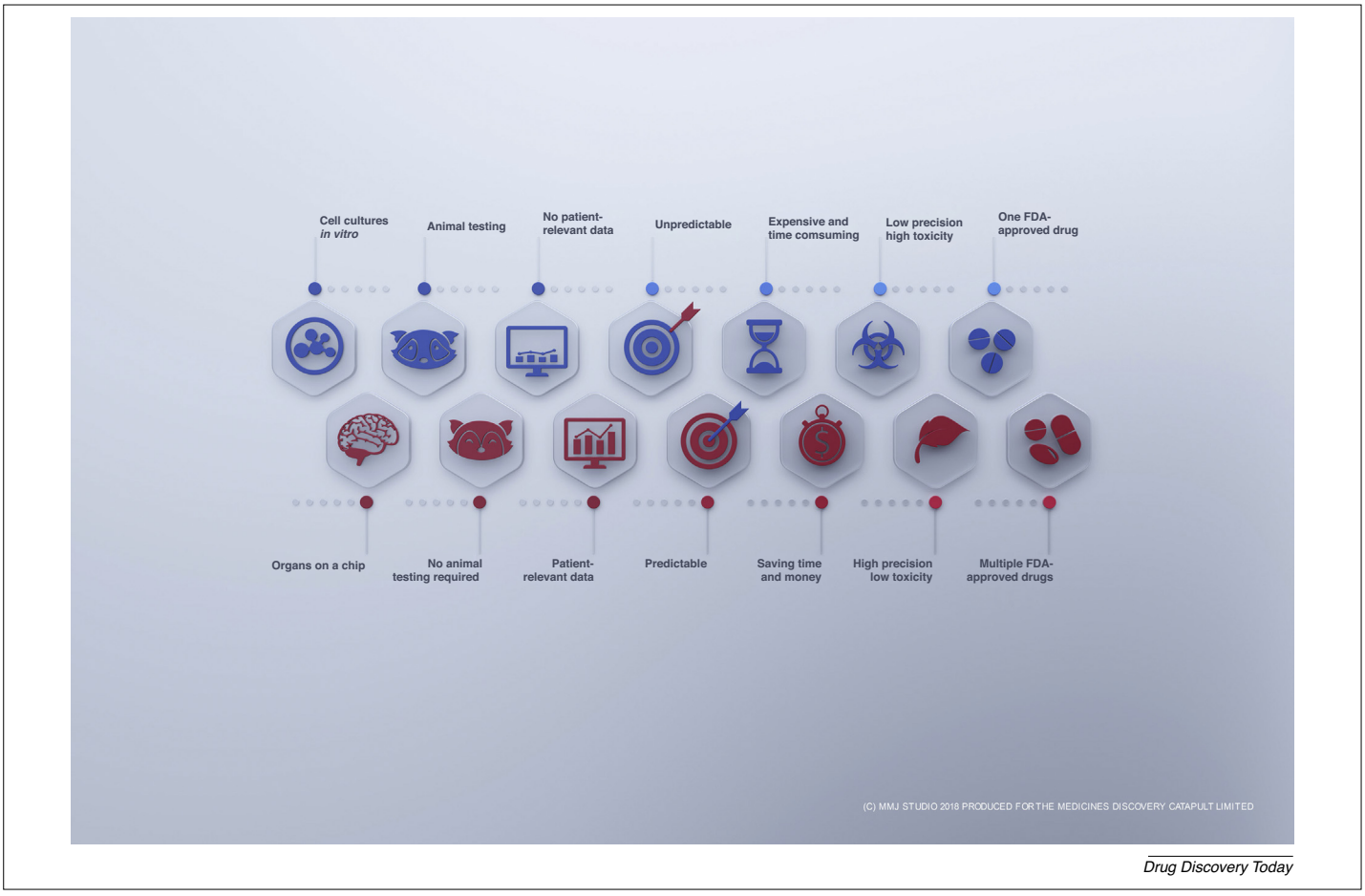

FIGURE 2

Traditional versus potential future organ-on-on-a-chip (OOAC) drug discovery. A revised drug discovery cascade is envisaged because of the uptake of OOAC systems, which are more patient centred, with increased preclinical predictivity of efficacy and safety.

organs and measurement approaches with some line of sight to clinical findings. In an integrated gut and liver model [52], baseline interactions were measured and maintained between the connected organs for more than 2 weeks. Sensitive detection technologies were used to detect 'normal' gut and liver crosstalk measuring bile metabolism, and CYP7A1 expression and inflammatory specific effects because of circulating CXCL9, 10, and 11 chemokines. The translational relevance of connected OOAC systems can also be assessed using well-characterised compounds with understood clinical PK or toxicity profiles. In either a functional coupling approach [29] or continuous flow system [53], compound exposure revealed organ-specific processing and toxicological features of five different drugs, which was consistent with clinical data. Using the wealth of in-patient drug understanding to back translate the predictability and limitations of the OOAC system might assist repurposing of existing drugs to recapitulate clinical observations.

Next steps

Replacing 2D cell lines in which a protein is overexpressed but other parts of the crucial signalling cascade are absent, with a patient-derived, 3D culture system on a biologically relevant substrate, incorporating fluid flow and communication from another organ that would be found *in vivo*, is a major step forward that OOAC can already deliver. Although inherently they are imperfect models for comparison with humans, it is the expanse and availability of decades of animal-testing data that could present the shortest route to establishing gain of confidence for further OOAC translation. Replacing some poorly predictive or intrusive animal tests with an OOAC equivalent has potential and is encouraged from a 3Rs perspective. The extensive data package required to ensure that regulatory authorities are confident about adopting new models, and to drive consistency in OOAC design for such a purpose, requires substantial funding and cross-company working. The potential benefit of such progress is easy to envisage, and precompetitive work is already beginning to address some of the challenges outlined in this review to ultimately realise the promise of patient-relevant OOACs (Fig. 2).

Given the promise of the predictive ability of this technology, and the associated substantial technical challenges, the OOAC community is galvanising to address common issues and promote collaboration across traditional boundaries. Recent developments include the establishment of an Organ on chip in Development (ORCHID) group alongside the pan-European OOAC network containing representatives from 11 countries. At the national level, in the USA, three tissue chip-testing centres have been established by the National Centre for Advancing Translational Sciences (NCATS) to act as independent assessors of OOAC technology. The UK OOAC network and the Human Organ and Disease Model Technologies Network (hDMT) in The Netherlands are both bringing together public and private expertise to facilitate model and technology development, skills exchange between laboratories, communication with regulators, and enhancing public engagement. Furthermore, efforts to democratise the expensive and resource-consuming OOAC technologies are underway via industrial-focussed research companies, such as the Medicines Discovery Catapult (Innovate UK), which aims to facilitate availability, collaboration, penetration, and impact of these approaches to the UK drug discovery community.

Concluding remarks

OOAC technology holds great potential in drug discovery to derive more representative models that better recapitulate patient biology beyond the limitations of traditional cell-based assays. Technological innovation has enabled microenvironments that can sustain multiple different connected cell types in microfluidic devices and mimic aspects of *in vivo* biology previously challenging to establish *in vitro*. These cues, such as flow, enable chip-bound biological models to respond in a way more akin to *in vivo*, although these improvements need not necessarily be dramatic to deliver increased predictivity. It appears likely that the application of connected biological systems to study drug efficacy is easier than capturing more systemic aspects needed for drug delivery or toxicology studies. In common with other new technologies, working collaboratively and sharing successes and failures across the drug discovery community will more rapidly deliver new therapies demanded by an increasing patient population.

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