A GUIDE TO DRUG DISCOVERY

OPINION

Screening out irrelevant cell-based models of disease

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Abstract | The common and persistent failures to translate promising preclinical drug candidates into clinical success highlight the limited effectiveness of disease models currently used in drug discovery. An apparent reluctance to explore and adopt alternative cell- and tissue-based model systems, coupled with a detachment from clinical practice during assay validation, contributes to ineffective translational research. To help address these issues and stimulate debate, here we propose a set of principles to facilitate the definition and development of disease-relevant assays, and we discuss new opportunities for exploiting the latest advances in cell-based assay technologies in drug discovery, including induced pluripotent stem cells, three-dimensional (3D) co-culture and organ-on-a-chip systems, complemented by advances in single-cell imaging and gene editing technologies. Funding to support precompetitive, multidisciplinary collaborations to develop novel preclinical models and cell-based screening technologies could have a key role in improving their clinical relevance, and ultimately increase clinical success rates.

Although there have been many notable drug development achievements in recent years, several disease areas such as neurodegeneration and aggressive cancers remain largely intractable. For example, substantial investment in the development of novel therapeutics for Alzheimer disease, Parkinson disease and motor neuron diseases has largely failed[1–3]. This failure can be attributed, in part, to our limited understanding of the targets that may prevent or repair neuronal damage, and to a lack of robust disease-relevant preclinical models[1,4].

In the field of cancer, important progress has been made in the discovery of new drugs, including those based on target-directed precision medicine strategies[5]. However, for aggressive cancers, such as glioma, pancreatic, oesophageal and several lung cancers, many promising drug candidates developed from standard cell-line screens and *in vivo* xenograft models did not show clinical efficacy. The poor clinical translation can largely be attributed to the failure of these models to recapitulate key pathophysiological features of the human disease, including complex inter- and intratumour heterogeneity, poor drug penetration through tissue, host-stroma–tumour cell interactions, and the cancer stem cell niche, all of which may have profound effects on the therapeutic response *in vivo*.

In addition, given the periodic emergence of new and old infections[6], and the pressing challenges from antimicrobial resistance and pandemic threats such as Ebola and Zika, modern, disease-relevant, cell-based phenotypic assay methodologies may represent valuable assets for improving our knowledge of the dynamics of host–pathogen interactions in their natural environment[7] and the development of new therapies (BOX 1).

Finally, a substantial proportion of clinical trial failures for novel medicines overall are due to safety issues such as cardiotoxicity and hepatotoxicity[8], and serious toxicity issues are often discovered only after clinical development has been completed[9,10]. Thus, more predictive toxicology models would contribute substantially towards more successful clinical translation and improved patient care.

Despite advances in target- and cell-based screening technologies, the majority of drug discovery projects remain dependent on cell culture systems that were developed several decades ago, incorporating immortalized cell lines; many consider the use of such assay systems to be questionable owing to their poor disease relevance. Although many traditional models provide valuable tools for studying the mechanism of action (MOA) of drugs and have helped identify successful drug candidates in the past, it is our opinion that the widespread use of contemporary cell culture assay systems must be revisited and that current efforts should be directed towards the development of new models, new assay formats and innovative screening technologies that better recapitulate *in vivo* physiology.

In this article, after briefly summarizing the limitations of traditional cell-based models of disease, we discuss how emerging developments in (patient-derived) *ex-vivo* cultures, induced pluripotent stem cell (iPSC) technology, three-dimensional (3D) co-culture and organotypic systems, complemented by advances in single-cell imaging, microfluidics and gene editing technologies, are well positioned to advance preclinical disease modelling and drug screening across challenging disease areas. We outline a set of principles for defining 'disease-relevant assays' (TABLE 1) and highlight methodological and analytical gaps, fundamental challenges and new opportunities to exploit and combine more advanced *in vitro* models with emerging technologies. We conclude by discussing the need to further evolve translational funding schemes and precompetitive research consortia to support the future development of new preclinical models and assay-screening technologies that provide more robust target validation and greater clinical predictivity.

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Box 1 | **Development of improved models of infectious diseases**

Pathogen biology and infectious disease operate on multiple cellular and tissue scales, wherein the biology of the infectious microorganism and its target cell depend on the contextual interplay of pathogen development stages with distinct host tissues, organs and the immune response. One important example of this complexity is the lifecycle of *Plasmodium falciparum*, the parasite that causes malaria in humans. There are several possible strategies to screen for lead compounds using phenotypic approaches, including screening at the human liver-stage, blood cell-stage or the insect-stage of the parasite life cycle. The use of cell-based phenotypic assays is offering promise in the identification of novel therapeutic classes that target multiple stages of parasite development[178].

However, the nature of the targeted cells and the virulence of an infectious agent are intimately linked, and can sometimes only be appropriately recapitulated by very specific host–pathogen combinations. Along these lines, a series of studies to identify new chemical entities that are active against *Leishmania* subspecies, the causal agents of human leishmaniasis, clearly demonstrated that developing an assay targeting the insect promastigote stage of the parasite does not yield viable lead compounds. Instead, a much longer and more sophisticated methodology is required for a primary macrophage assay supporting infection-competent (replicative) parasites, which reduces screening throughput but greatly enhances the quality of results and identification of viable lead compounds[179–181].

A major concern in the field of infectious disease modelling is the use of perpetually cultured laboratory strains of infectious disease organisms despite their poor resemblance to 'real-world' pathogens. Bacterial growth conditions *in vitro* differ fundamentally from the conditions found in natural ecosystems, including infection sites. Indeed, as a result of the high degree of plasticity of their genome, bacterial strains adapt quickly to the optimized laboratory mono-culture conditions and, therefore, rapidly lose key pathophysiological characteristics[182]. In the late nineteenth century, Louis Pasteur had already recognized that laboratory adaptation of bacteria is associated with attenuation of virulence towards the host species, and this idea was exploited by his colleagues Calmette and Guérin, leading to the development of the BCG (bacille Calmette–Guérin) vaccine in 1921 (REF. 183).

It is now well established that bacteria do not exist in isolation but rather live as communities, behaving collectively to adapt to new host environments and modes of growth[184]. Quorum-sensing (QS) — that is, cell-to-cell communication by the production and release of autoinducers in the environment — has a key role in *Pseudomonas aeruginosa* pathogenicity. The large variation of expression of QS genes observed upon culture in different environments emphasizes the importance of clearly identifying and mimicking the actual habitat that bacteria encounter *in vivo* when developing an assay to design new therapies[185]. Along these lines, most bacterial chronic infectious diseases are associated with the formation of polymicrobial biofilms. Biofilms protect the bacteria from the host innate- and adaptive immune response and represent an ideal setting for horizontal gene transfer (HGT), thus creating new virulent strains and resistance by creating a communal distributed 'supra-genome'[186].

A way to develop more physiological infectious disease models is to mimic as closely as possible the *in vivo* ecosystems that the pathogens might encounter in an infected host, to recreate the microenvironments required for the virulence and eventually to identify drugs that might boost the host defence mechanisms. Many pathogens that infect humans have been studied in model animals such as *Drosophila melanogaster*, *Caenorhabditis elegans*, zebrafish or mice; unfortunately, not all of them are easily amenable to higher-throughput drug screening, nor do they properly model the human response to pathogen infection. Nonetheless, in an elegant study, Kim and co-workers describe how host-cell autophagy activated by antibiotics is required for an effective antimycobacterial drug response through conserved mechanisms between fruitflies and mammals[187]. Although small model organisms hold much promise for infectious disease drug discovery, they still have many drawbacks such as their temperature, discrepancies between the anaerobic nature of the human intestine and non-conserved host targets across species. These shortcomings might be overcome using synthetic microtissues such as the human-gut-on-a-chip[188] or small-airway-on-a-chip microfluidic devices[189].

Many pathogens require high biosafety level laboratory confinement (BSL3 and above), which presents several challenges to developing assays under these conditions. The continued development of automated liquid handling and automated microscopy platforms, which enable remote control, including image acquisition and analysis, help support assay development and screening under high-level biosafety restrictions. The lack of an effective cure or preventive measures against emerging antibiotic-resistant bacteria and re-emerging viral pandemics (for example, Ebola, Marburg and Zika) calls for renewed efforts to develop innovative technologies and methodologies for drug screening in the infectious disease area. Comparative phenotypic screening across microbial resistant subspecies combined with relevant host systems may guide drug discovery towards novel therapeutic classes targeting infectious disease resistance mechanisms and new host-oriented therapies[190,191].

Limitations of traditional disease models

Traditional cell culture methods typically rely on cancer cells or immortalized cells grown within artificial environments, on non-physiological substrates such as functionalized plastic and glass. Although these methods have facilitated the discovery of many basic biological processes, they often fail to provide an adequate platform for drug discovery owing to their inadequate representation of key physiological characteristics. These problems can be broadly categorized into the following limitations.

Limitations due to cells. Most cell-based assay screens have traditionally been performed using transformed or immortalized cell lines. These have been cultured for many generations, resulting in a substantial drift in their genetic, epigenetic and physiological characteristics, which means they are not a good model of primary tissue cells[11,12]. The gross genetic and epigenetic abnormalities (characterized by multiple genetic rearrangements and amplified gene copy numbers) associated with long-term culture confound pharmacogenomic and functional genomic studies. Genetic adaptation resulting from long-term *in vitro* cultures also contributes to heterogeneity in cultures of the same cell line between passages, batches and laboratories.

Limitations due to culture conditions. The media most commonly used for cell culture are designed for fast cell growth, incorporating large concentrations of fetal serum and nutrients, which may promote dedifferentiation of primary cell types into more embryonic or fetal-like phenotypes[13]. With the development of primary cell and differentiated stem cell cultures, the use of high glucose and growth factor media has been eschewed for defined culture media to promote cellular identity rather than rapid growth. Cells are often grown in standard incubators under high oxygen partial pressure (approximately 20%), which does not represent the steady-state conditions of human organs and tissues (fluctuating between 1% in the dermis, and 14% in arterial blood)[14–17]. Such conditions poorly recapitulate the distinct microenvironments that define normal and diseased tissue phenotypes. This has a profound impact on cell metabolism, reactive oxygen species (ROS) production, mitochondrial functions and, ultimately, the differentiation and function of cells[18,19]. Additionally,

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conventional tissue culture systems do not readily permit the formation of short-range gradients of nutrients, hormones and oxygen that are often experienced by cells, depending on the distance to the nearest blood vessel. The liver is a well-known example of this, with gradients in the lobules between the central vein and the portal artery leading to zonation[20]. This can be mimicked with the adoption of microfluidic systems that deliver nutrients, dissolve gases and remove waste products[21].

Limitations due to lack of appropriate cell culture substrates and bioengineering tools. The two-dimensional (2D) planar substrates on which cells are typically grown are stiff, demonstrating high (gigapascal) tensile strength and mechanical resistance to deformation, unlike most substrates found in the human body (which are on the millipascal to kilopascal scales), with the exception of bone and cartilage[22]. Hence, the plastic or glass used in cell culture may not accurately represent the normal *in vivo* mechanical environment[23,24]. For example, in the case of liver culture, most differentiation protocols require the use of sandwich cultures, where the cells are grown between layers of either collagen or other extracellular matrix (ECM) proteins. The mechanical properties of these supporting matrices are generally not well characterized, despite the fact that such properties are known to have an impact on cellular function and differentiation in tissues[25,26]. In these types of experiments, where a minimal quantity of deposed hydrogel is used, it is likely that the cells would encounter a stiff environment, which the liver would normally only encounter during fibrosis or cirrhosis. Thus, toxicology assays are typically carried out under pathological rather than healthy liver conditions. Microfluidic devices, which utilize mechanical actuation systems to recapitulate mechanical forces or generate the shear forces that tissues experience in living bodies, are beginning to be used; however, they will require further development and refinement if they are to be used for more general screening applications[27].

A further challenge evident in tissue modelling within current *in vitro* assays is the absence of more physiologically relevant ECM. For example, the popular use of Matrigel and collagen type I as an ECM substrate in hepatocyte cultures does not represent the ECM proteins predominantly found in the liver[28]. Many pathologies are associated with changes in ECM production that have a considerable impact on cell and tissue function. Thus, recapitulating both physiological and pathophysiological ECM composition and structure is an important consideration for *in vitro* cellular models.

Limitations due to lack of appropriate co-culture methods. Cell-culture screening assays traditionally use a single cell type, whereas cells *in vivo* are either in direct contact or communicate over a long range with many different cell types. As most biological processes and pathologies involve the interaction of multiple cell types, these should ideally be incorporated into *in vitro* cellular assays whenever possible. For example, most toxicology assays use only hepatocytes, but although 80% of the liver volume consists of hepatocytes (60% of the cells), other important cell types within the liver include stellate cells, resident macrophages (Kupffer cells), sinusoidal endothelial cells and some non-parenchymal cells. Both stellate cells and Kupffer cells are known to be important for some compound toxicities and should therefore be incorporated into *in vitro* toxicology assays[29,30]. Neurodegeneration, wherein both astrocytes and glial cells are responsible for protecting neurons but are also known to cause neural death, provides a compelling case for the use of mixed cell cultures of distinct cell types[31]. Further development of co-culture methods, which incorporate disease cells with relevant immune subcompartments, is also urgently needed to help better understand and address the role of the host immune system in the pathogenesis and therapeutic outcomes of many diseases[32]. These considerations are of particular importance for pathogen biology and infectious diseases, which operate at multiple cellular and tissue levels (BOX 1).

Tackling the limitations. Efforts to address the limitations of traditional preclinical assays by employing new models face various imposing challenges, including: how to mimic the microenvironment and heterogeneity of normal and disease tissue; how to take into account the environmental and genetic or epigenetic factors governing disease aetiology and therapeutic outcomes; how to understand the effect of drugs upon the whole physiological entity, for example, across multiple cell types and organs of the human body; and how to interpret the role of the host immune system in the pathogenesis of a particular disease. Although it is clear that no single preclinical model or screening assay will faithfully recapitulate the full complexity of human disease, below and in FIG. 1 we outline the latest developments in cell-based models and assay technologies that are beginning to address the limitations of traditional and contemporary *in vitro* assays.

Enhancements in disease modelling

Primary and patient-derived cell models. The adaptation of patient-derived primary cell samples, as well as fresh human tissue samples, for *ex vivo* and *in vitro* translational research applications aims to overcome many of the disadvantages of using transformed cell lines for drug discovery[33]. These samples also offer a more clinically relevant model for testing novel gene and cell-based therapies. However, the lack of culture systems possessing the robustness, scalability and flexibility needed by companies has hampered the adoption of *in vitro* primary cell-based research tools, including patient-derived cell models, at the earliest stages of drug development.

In cancer, highly selective drugs targeted at genetically defined clinical subtypes are needed to support a more patient-centric approach to drug development[34,35]. Potential drugs have been tested *in vitro* and *ex vivo* against well-characterized patient-derived primary cancer subtypes for various cancers, but often without a direct impact on treatment[36–38]. In leukemias, however, where the *ex vivo* material (for example, suspension cells) is more readily available for drug testing than in solid tumours, patient-derived samples have recently been utilized for potential drug repositioning[39,40] and combined with molecular profiling to identify clinically actionable drugs for personalized acute myeloid leukaemia (AML) therapy[40]. Although primary leukaemic cells can be used without further expansion for *ex vivo* drug testing[33,41], the drug responses may vary depending on the cell culture assay conditions. Importantly, several studies highlight the importance of the interaction of leukaemic cells with the bone marrow stromal microenvironment, which can be partly mimicked using co-cultures of leukaemic cells with human bone marrow-derived mesenchymal stem cells[42,43].

The extension of patient-centric primary *ex vivo* drug profiling to higher-throughput applications and primary cells derived from solid tumours or normal tissue presents several challenges[44]. The development of co-culture protocols[45] has enabled a relatively rapid production and scale-up of high amounts of conditionally

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Table 1 | **Defining principles of disease-relevant assays**

No.	Principle	Activity	Justification	Disease-specific considerations
1	Define the translational research objective of the assay	Ensure that assay selection is linked to a definitive clinical question or actual clinical scenario	Accurately inform disease positioning and enhance successful translation of cell-based screening programmes	Generic
2	Adopt the human physiology assay checklist	Acknowledge assay limitations by adopting a human physiology assay checklist (see main text), which would enable unbiased evaluation of which assay conditions accurately recapitulate human tissue physiology and pathophysiology and which do not	Supporting appropriate assay selection and interpretation of assay data to guide subsequent preclinical studies and further assay development to enhance translational success	Generic
3	Retrospectively analyse and measure the predictive value of each assay	Recapitulate poor drug response or known clinical resistance mechanisms through retrospective analysis of approved and failed drugs to determine the positive and negative predictive value (PPV and NPV, respectively) of each assay. Retrospective studies support reverse engineering of preclinical assays to improve PPV and NPV	Validation of assay predictivity to enhance clinical translation	Generic
4	Ensure the tissue context is appropriate	Accurately represent the appropriate tissue context of normal or diseased tissue through the development and detailed characterization of patient-derived, primary human cell and induced pluripotent stem cell (iPSC) differentiation protocols	Generate cell-based screening assays with appropriate tissue context and endogenous pathway network biology across disease areas to support target discovery, target validation and drug mechanism-of-action studies under the most relevant conditions	Generic
5	Ensure the genomic context is appropriate	Ensure the genomic characteristics accurately represent patient populations and disease subtypes	Design bespoke suites of cell-based screens representing disease subtypes or patient populations to inform disease positioning of assay screening hits and new drug targets	• Generic • Cancer subtypes • Hereditary and spontaneous neurological disorders • Personalized cardiovascular disease
6	Capture heterogeneity within cell populations	Define the heterogeneity of cell-based assays, ensuring that a heterogeneous phenotypic response within individual cell subpopulations is measured	Embrace disease heterogeneity by analysing the drug's mechanism of action and target activity at a single-cell level and across subpopulations in complex assay formats to inform future preclinical and clinical development	• Generic • Cancer • Inflammation and immune disorders • Regenerative medicine
7	Use mixed cell culture models and relevant host biology	Use mixed cell culture models that represent the multicellular composition of normal and diseased tissue. Incorporate appropriate host cell biology relevant to the disease	Develop more relevant multicellular screening assays to support novel target discovery and drug combinations exploiting both disease and host cell biology	• Generic • Cancer • Inflammation and immune disorders • Infectious disease • Liver toxicity and disease • Cardiovascular disease
8	Use defined cell growth media	Use defined cell growth media that represent steady-state levels of human tissue nutrients under normal and pathophysiological conditions	Accurately model the environmental conditions of normal and diseased tissue to enhance the translation and prediction of *in vivo* efficacy or toxicity	Generic
9	Ensure substrate tension and mechanical forces are appropriate	Ensure that substrate tensions and mechanical forces are representative of human tissues under normal and pathophysiological conditions	Accurately model the environmental conditions of normal and diseased tissue architecture and mechanotransduction to enhance the translation and prediction of *in vivo* efficacy or toxicity	• Generic • Cardiovascular disease • Musculoskeletal disorders • Fibrosis • Cancer
10	Ensure the atmospheric conditions are appropriate	Ensure that the atmospheric conditions of physiological and pathophysiological gaseous tensions, including oxygen pressure and pH conditions, are tailored to specific tissue types	Accurately model environmental conditions of normal and diseased tissue, including different physiological levels of normoxia and pathophysiological levels of hypoxia within *in vivo* tissues to enhance the translation and prediction of *in vivo* efficacy or toxicity	• Skin diseases • Cardiovascular disease • Lung and respiratory diseases • Liver zonation • Ischaemia • Cancer hypoxia

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Table 1 (cont.) | **Defining principles of disease-relevant assays**

No.	Principle	Activity	Justification	Disease-specific considerations
11	Ensure the extracellular matrix composition is relevant	Extracellular matrix compositions should be relevant and represent the physiological and pathophysiological composition and architecture	Accurately model extracellular environments of normal and diseased tissue to ensure appropriate cell and tissue architecture, cell differentiation and cell function in order to enhance clinical translation of *in vitro* assay screens	Generic
12	Ensure clinically equivalent dosing is used	Mimic drug uptake and retention within normal and diseased tissue by simulating tissue perfusion. Incorporate short-term dosing and drug wash-out protocols that predict *in vivo* pharmacokinetic properties of specific drug candidates or predictions of common drug-like properties	Improved prediction of *in vivo* and clinical activity, supporting more accurate scheduling and dosing of drugs and drug combinations	Generic
13	Simulate systemic multi-tissue effects	Design organ-on-chip or multi-tissue models that mimic *in vivo* compound metabolism, paracrine signalling between tissue and systemic tissue effects, and associated co-morbidities	Enhanced modelling of complex human physiology and whole-organism systems to predict clinical efficacy and toxicity across multiple organs and tissues	• Generic • Metabolic
14	Incorporate appropriately aged cells to model human disease	Utilize primary cells from appropriately aged donors or adapt cell differentiation protocols to generate appropriately aged cell models	Ensure the assay represents the expected age of the patient (for example, embryonic, paediatric, adult and late-onset degenerative disease)	• Generic • Developmental malformations • Neurodegeneration • Cancer
15	Model distinct stages of the disease life cycle	Design models of precursory, early-stage, mid-stage, late-stage and dormant disease	Ensure design of assays that appropriately guide targets, hits and candidate drug therapies for prevention, cure, stabilization and palliative use	• Cancer: premalignant tumours • Arthritis • Neurodegeneration: early-onset and late-stage disease
16	Incorporate appropriate disease-causing 'perturbagens'	Accurately model the environmental and physiological toxins that are responsible for disease initiation and progression. Ensure relevant levels and types of toxin, as well as exposure duration, are incorporated into cell models	Support the development of assays representing disease progression models that accurately reflect disease aetiology to inform clinical positioning and enhance clinical translation	• Drug-induced liver injury • Cancer • Cardiovascular disease • Lung and respiratory disease
17	Define relevant assay end points	Ensure assay end points translate to resolution of disease pathophysiology or guide therapeutic interventions towards desired clinical outcomes	Enhance clinical translation of new targets and candidate drugs derived from preclinical assay systems	Generic

reprogrammed cells from surgical and accessible biopsy specimens, from both healthy and tumorigenic tissues such as the lung, breast, prostate, pancreas, colon and kidney[44,46]. *In vitro* cell culture conditions modify cells over time, and may even lead to loss of expression markers of the original sample and enrichment of specific cell populations. It is therefore essential to ensure that these cells represent the original tissue and genomic background of the individuals from whom they were derived by extensive genotypic and single-cell phenotypic characterization. Living organoid biobanks for solid tumours can complement cell line- and xenograft-based drug studies by providing an improved model for complex tissue architecture. This was demonstrated in a recent proof-of-concept study, in which the living organoids of 20 patients with colorectal cancer, sharing identical gene expression profiles and genetics with the corresponding original tumours, were screened against 83 compounds; the findings revealed good reproducibility and correlation with individual oncogenic mutations[47].

Overcoming the challenges related to the expansion of primary patient-derived *ex vivo* cultures for higher-throughput screening across distinct patient cohorts will require access to numerous representative patient samples for simultaneous testing, scale-up of limited primary cell material and effective integration of drug sensitivity phenotypic data with the molecular characterization and clinical data associated with each patient sample. Access to high-quality patient-derived primary samples requires close collaboration between researchers, clinics and biobanks to find representative samples combined with relevant clinical data, and to establish standardized sample handling procedures for sensitive live tissues and cells. The Finnish Haematology Registry Biobank (FHRB) provides an exemplar of an operative biobank, functioning as a valuable source of patient material for precision medicine approaches in leukaemia[40,48]. The exploitation of biobanks to support drug testing of *ex vivo* patient cells collected from across both large and smaller patient cohorts can help to prioritize and de-risk drug candidates for larger-scale clinical testing, to support patient-stratified medicine strategies and to systematically identify novel drug-repositioning opportunities[48].

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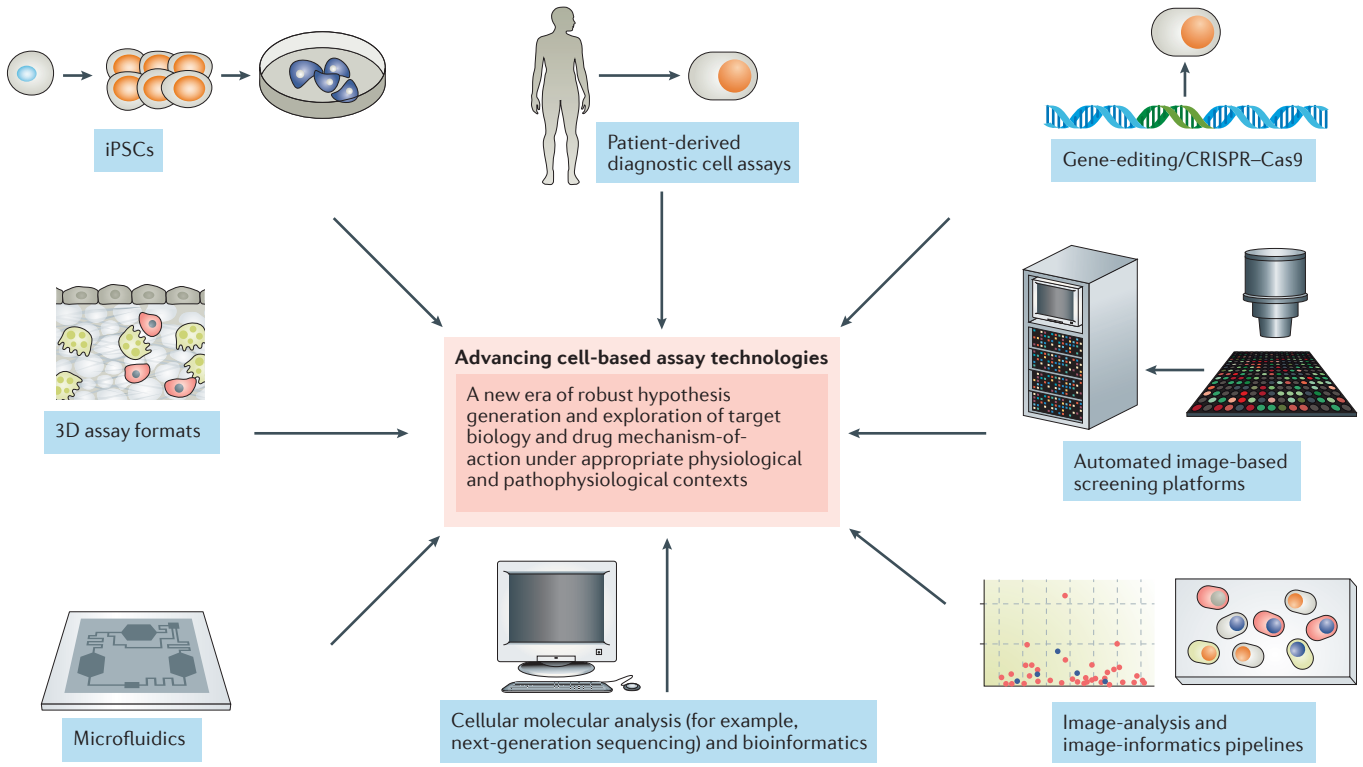

Figure 1 | **Novel assay technologies and their integration.** Advances in patient-derived primary cell models; induced pluripotent stem cell (iPSC) technology; three-dimensional (3D) *ex vivo* and multicellular models, and microfluidic devices; CRISPR–Cas9 gene-editing; automated imaging and image analysis platforms; and molecular cell profiling technologies, including advanced proteomic and genomic methodology (such as next-generation sequencing and bioinformatics) individually and together present new opportunities for incorporating more relevant physiological models into drug discovery.

Further expansion of primary cells for high-throughput screening (HTS) of small-molecule or antibody libraries remains challenging, however, and will require the development of new technology platforms, including miniaturized assay screening formats and defined culture conditions, that provide sufficient sample throughput and stability.

Until these challenges are met, HTS of compound libraries across transformed and immortalized cell line models, when integrated with molecular profiling, may still provide useful opportunities to advance drug MOA studies, target identification and patient stratification hypotheses. A recent study used correlation-based analyses to associate the sensitivity of 481 compounds tested across 860 human cancer cell lines with the basal gene expression profile of each cell line to reveal new target mechanisms for several compounds[49]. Furthermore, the application of multiparametric genetic or image-based phenotypic profiling assays in established cell lines, combined with multivariate statistics and machine learning methods, has been used to pattern-match compound-induced transcriptomic or phenotypic fingerprints with reference data sets to predict the MOA of a compound and postulate new disease indications[50–52]. Thus, although transformed cell line assays may poorly represent disease, integration with in-depth genomic and phenotypic profiling to understand MOAs and elucidate new targets may represent the best use of these well-characterized transformed or immortalized cell line culture resources.

Induced pluripotent stem cell technology. Although primary human and patient-derived *ex vivo* models are considered to be of high value, the availability of the relevant tissue is a limiting factor for modelling many disease phenotypes. The ability to scale up and expand primary cell-derived cultures for HTS applications, while still maintaining the relevant genomic epigenetic and tissue architecture of the original tissue, also remains a challenge. These limitations have hampered drug discovery in several disease areas, most notably in neurodegeneration and psychiatric disorders.

A major breakthrough in the ability to develop tissue-specific cell-based disease models, including patient-derived cell assays at a sufficient scale, has been achieved through the development of iPSC technology[53]. New opportunities presented by iPSC technology in disease modelling and translational research have recently been reviewed in depth[54,55]. We therefore focus our discussion below on advantages and some limitations specifically related to cell-based assay development and screening, and selected exemplar applications for neurodegenerative diseases, cardiotoxicity testing and metabolic diseases.

iPSCs have several advantages as a platform for drug screening. They represent normal primary cells with a mostly stable genotype compared with transformed cell lines, and they possess an intrinsic capacity for self-renewal, facilitating their propagation and expansion for drug screening. iPSCs can also be reprogrammed into many different tissue-specific cell types and they can be derived from any patient in unlimited quantities. Importantly, iPSCs are amenable to detailed genetic

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characterization and to new gene editing technologies, thus presenting an excellent opportunity for directly linking phenotype to genotypes. These properties facilitate pharmacogenomics studies and the development of matched pairs of genetically defined disease phenotypes and isogenic controls for screening.

However, iPSCs also have several limitations. First, the persistence of residual epigenetic memory, from the somatic cells from which the iPSC cells were derived, may adversely influence or confound the phenotypic response to candidate therapies and in drug screening[56,57]. Second, iPSC disease models have tended to focus on rare, monogenic, hereditary forms of disease rather than more common, spontaneous forms that are characterized by complex genetic traits involving multiple unknown genetic and epigenetic factors. New advances in multigene editing and synthetic biology approaches in patient-derived iPSC models may begin to address these challenges[58–60]. Third, the rapid differentiation protocols and embryonic nature of iPSCs and their derivatives may not be optimal for modelling late-onset disorders associated with ageing. Long differentiation protocols have been applied to help develop mature differentiated cell types, and exogenous stressors or ectopic expression of age-related genes have been used to induce ageing-like features of iPSC-derived models of Parkinson disease[61]. However, these approaches only partly address this issue of cellular ageing. Finally, a practical limitation of iPSC models is the long differentiation protocols required. Despite these limitations, iPSC-derived cultures of cardiomyocytes[62–66] and neurons[66–68], as well as intestinal[69] and lung[70] tissues, have been developed as heart, cerebral, intestinal and pulmonary disease models, respectively, and have been used in drug screening[62,63,65–67,71].

For example, the development of automated phenotypic screening assays incorporating differentiated iPSCs that address specific neurodegenerative diseases has recently begun to yield new potential therapeutic targets and lead compounds. Neurons made from iPSCs, derived from a patient with Rett syndrome, exhibiting reduced spine density and smaller cell bodies, were used in a drug screen that identified two molecules, insulin-like growth factor 1 (IGF1) and gentamycin, that were able to rescue synaptic defects[72,73]. In a recent study of a large cohort of healthy controls and patients with amyotrophic lateral sclerosis (ALS), fibroblasts were reprogrammed into pluripotency. The cells were subsequently configured into a high-content chemical screen, resulting in the identification of several US Food and Drug Administration (FDA)-approved small-molecule modulators, demonstrating the feasibility of patient-derived iPSC-based disease modelling for drug repurposing and screening[74]. In another study, α-synuclein-defective cortical neurons were generated using iPSCs from patients at risk of developing Parkinson disease. By identifying pathogenic phenotypic end points suitable for cell-based screening assays, these studies identified new potential therapeutic targets, such as the ubiquitin ligase NEDD (neural precursor cell-expressed, developmentally downregulated), which rescues the α-synuclein toxicity associated with neurons derived from patients with Parkinson disease[75]. Another recent small-molecule chemical screen using human iPSC-derived dopaminergic neurons in a rapid 96-well screening format identified several potential neuroprotective candidates for Parkinson disease[76].

Cardiotoxicity testing has traditionally focused on *in vitro* electrophysiology assays to assess the risk of arrhythmia, yet a major limitation of these assays has been the dependence on the use of cell lines engineered to express single ion channels[77,78]. These reductionist approaches are poor predictors of the risk of arrhythmias as a cardiac action potential *in vivo* involves the cooperation of multiple ion channels. Stem cell technology can be used to provide an unlimited supply of cardiomyocytes that more faithfully reproduce human cardiac electrophysiology and that can be used for *in vitro* HTS approaches, which are beginning to revolutionize the field[79].

However, current HTS approaches are still limited by the maturation state of stem-cell-derived cells, which do not recapitulate completely the contractile function of adult cardiomyocytes[80]. *In vitro* engineered 2D[81] and 3D[82] cardiac tissue models have now been developed as low- or medium-throughput screening platforms using stem-cell-derived cardiomyocytes with an improved maturation status, and these models hold great promise for the future study of cardiotoxicity and myocardial dysfunction. Screening platforms have also been developed from neonatal rat primary cardiac myocytes[83,84]. Although highly efficient, some functional differences to human models exist. Medium- and high-throughput screening technologies for recording cardiomyocyte function currently span from the classic radioligand binding assays[77] to automated patch clamp[85,86] and microelectrode arrays (MEA)[78,83,86]. Optics-based high-throughput assays have been developed for monitoring voltage or ion-sensitive dyes using kinetic plate readers and high-content imaging platforms[78,87–89].

Stem-cell-derived cardiomyocytes, in combination with image-based high-content screening technologies, have proven to be very effective for analysing the structural cardiotoxicity associated with anticancer therapies[90]. Drug-induced heart failure can also arise from impaired cardiac function characterized by changes in cardiomyocyte contractility, which can be studied using real-time cell analysis (RTCA), an impedance-based high-throughput technology[91]. However, cardiomyocyte contractility is rarely monitored in preclinical toxicology studies and remains a particularly challenging task owing to the high speed of cell beating and the complexity of the process, which requires advanced phenotypic approaches. Several new methods have been developed to quantify cardiomyocyte contractility based on digital holographic microscopy (DHM)[92], muscular tissue films (MTFs)[93] or dynamic monolayer force microscopy (DMFM)[94]. With the exception of label-free DHM, which is relatively inexpensive, the complexity and cost of many of the other techniques currently limit their application to HTS.

Cultures of patient-derived fibroblasts have been used extensively to characterize and/or to find new treatments for inherited metabolic diseases, such as genetic enzyme deficiencies and lipid storage diseases (for example, Niemann-Pick disease type C[95]). The advantage of fibroblasts is that they are easy to collect, store and expand for several passages and, in general, they do not require complex culture conditions. Importantly, the fibroblasts can be reprogrammed into iPSCs and further differentiated into the most appropriate cell types relevant to specific metabolic disorders. In diabetes, this approach has been utilized for *in vitro* production of functional stem cell-derived β-cells from fibroblasts of patients with diabetes[96], which can be used to search for new diabetes targets and molecules that promote pancreatic β-cell proliferation and function or suppress β-cell apoptosis[97]. Similar approaches exploiting iPSC technology are being applied to other diseases such as muscular dystrophies[98], cardiovascular disease[99] and aldehyde dehydrogenase 2 deficiency[100]. Importantly, many of these iPSC models can be readily expanded for HTS.

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Overall, it is too early to accurately measure the impact of iPSC technology. However, differentiated iPSC assays, combined with more informative functional screening technologies, provide improved models of normal and diseased tissue compared with traditional assays, leading many investigators to anticipate that these assays will ultimately improve clinical success rates.

Precise genome editing. Advances in genome-editing tools have opened new avenues for developing cheaper, faster and more translatable *in vitro* and *in vivo* models of human diseases. The most prominent is the recent discovery of CRISPR–Cas9 technology, and its exploitation as a gene editing tool in mammalian cell systems[101]. Briefly, the CRISPR–Cas9 gene editing technology consists of two components: a DNA sequence-specific 'guide' RNA (gRNA) and a nonspecific CRISPR-associated endonuclease (Cas9). These components are introduced into model cells and, once expressed in cells, the Cas9 protein and the gRNA form a riboprotein complex, which binds and cleaves the DNA if sufficient homology exists between the gRNA and target sequences. Subsequent insertion of new 'designer' DNA sequences into the targeted site is then possible through endogenous DNA repair mechanisms. More detailed information on the CRISPR–Cas9 technology can be found in recent reviews[102,103].

The great advantage of CRISPR–Cas9 gene editing is the precise nature of the editing; initially, CRISPR was applied to 'knock out' target genes in various cell types and organisms, but modified versions of the CRISPR–Cas9 system have recently been developed to recruit heterologous domains, including transcriptional co-regulators that selectively activate or repress target genes[102]. Additionally, to assist in genome edit detection, purification and visualization, epitope tags and reporter molecules have been incorporated into the genome editing constructs to further expand the utility of this technique to image DNA editions and their protein products in live cells[104]. In contrast to traditional genetic engineering approaches such as site-directed mutagenesis and gene targeting in embryonic stem cells, the CRISPR–Cas9 system is more efficient, faster and cheaper, and has been used to efficiently modify endogenous genes in a variety of cell types and organisms that have traditionally been challenging to genetically manipulate.

The ease of generating gRNAs makes CRISPR one of the most scalable genome editing technologies, and it has been utilized for genome-wide screens to identify new target hypotheses and elucidate the MOA of existing drugs or mechanisms of drug resistance[105–107]. Further development of arrayed libraries will facilitate the application of gene-editing technology to a broader range of cell-based phenotypic assays[108]. Combining technologies — such as CRISPR–Cas9 with iPSC technology and advancing phenotypic assay formats — presents a further opportunity to generate genetically defined cell-based assay models at a sufficient scale, recapitulating precise genetic drivers of disease aetiology. For a more detailed discussion of how CRISPR–Cas9 tools are being combined with iPSC technology to generate new disease models, high-fidelity isogenic pairs for counter-screening and lineage reporters, as well as to progress target identification and validation studies, we refer readers to REFS 109–111. CRISPR–Cas9 has been used *in vivo* to directly mutate tumour suppressor genes and oncogenes in the mouse liver[112], demonstrating the potential to develop new *in vivo* models of disease and custom-designed preclinical drug discovery cascades that bridge the gap between *in vitro* screening assays and *in vivo* proof-of-concept studies.

Three-dimensional cell culture models. Culturing cells in 3D environments can favour the formation of multicellular tissues with the appropriate cell–cell and cell–ECM interactions and architecture that are important drivers of tissue differentiation and function. The use of 3D cellular models for *in vitro* disease modelling and screening is especially useful in instances where aberrant tissue organization is associated with disease pathology and progression: for example, in neurodegenerative disorders, fibrosis, solid cancers and cystopathies.

Many options for 3D *in vitro* and *ex vivo* models are emerging that utilize both natural and synthetic biomaterials, each with advantages and limitations (BOX 2; FIG. 2). New 3D assay formats have been developed specifically for medium- to high-throughput screening, including commercially available options such as microtissue products (InSphero 3D InSight), nanoculture spheroid plates (SCIVAX), micropattern plates (Cytoo), aligned (NanoAligned) or randomly oriented (NanoECM) polymer nanofibre plates and low-density 3D cell suspension media (Happy Cell). These assays provide robust 3D cell culture architecture in 96- and 384-well formats. Examples of advanced multicellular 3D spheroid screening assays, designed to address specific clinical scenarios, include the application of a co-culture model composed of normal human dermal fibroblasts (NHDFs) growing together with red fluorescent protein (RFP)-labelled breast cancer cells for high-throughput phenotypic screening of radiation-resistant tumour cells[113]. This assay was quantified by real-time high-content imaging in a format that is suitable for scale-up to HTS of drug combinations that sensitize cells to radiotherapy, chemotherapy or both[113]. Further high-content image-based 3D spheroid assays have been applied to small-molecule screens investigating compounds that specifically target dormant tumour cells within the inner core of tumour spheroids or compounds that prevent either fibroblast or immune invasion into tumour spheroids[114–116]. Such 3D spheroid assay formats have also been used as tissue surrogates to study immune infiltration into specific tissue types and represent a rapid and cost-effective alternative to animal models for studying host immune responses[114,115].

Although various successful studies have demonstrated the practical implementation of 3D formats to small-, medium- and high-throughput screening assays[113,117], the adoption of 3D tissue culture into routine screening has been sluggish, in part owing to a number of remaining technical issues. First, although animal-derived basement membrane extract (BME) hydrogels often support the growth of difficult-to-culture cells such as primary cells, their physical and chemical properties are fixed, their composition is undefined and there is inevitable batch-to-batch variation associated with these natural products, which is considered a major hindrance to obtaining reproducible results[118]. To promote more cost-effective and reproducible 3D cell culture screening platforms, synthetic biomaterials have been developed (BOX 2; FIG. 2). However, the lack of organic ECM proteins and appropriate extracellular environmental signalling cues mediated by the binding of ECM proteins to cell-surface receptors limits the physiological relevance of synthetic 3D biomaterial substrates. Even peptide-derived gels have yet to recapitulate sufficient functionality for the development of 3D tissues from most cells. Those cells that do grow in inert hydrogels, scaffolds or in hanging drop or low-attachment plates may do so through the secretion of

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endogenous ECM proteins or as a result of oncogenic mutations that confer anchorage independence. The adoption of hybrid matrices combining synthetic and organic biomaterial has recently gained popularity for drug testing in cancer cell models[119], tissue-engineering matrices[120–123] and the development of more complex innovative immunocompetent 3D culture models comprising dendritic cells co-cultured with fibroblasts and keratinocytes[114]. Indeed, the addition of cells that are responsible for producing the ECM *in vivo* (for example, stromal fibroblasts and stellate cells in the case of the liver) into 3D co-culture systems represents an alternative approach for incorporating more physiological ECM constituents into synthetic 3D scaffolds.

Further practical limitations of 3D cell-culture models include: the high cost of biomaterials; higher viscosity and temperature-sensitive gelation hindering automated handling of gels in the liquid state; sample processing (for example, antibody staining and sample washing) for high-content analysis; and the challenge of defining optimal cell ratios, culture conditions and ECM constituents for 3D co-culture models. However, the integration of factorial design strategies and evolutionarily inspired genetic algorithms, together with advances in cell culture automation and phenotypic analysis, are well placed to advance complex assay optimization[124–126]. Perhaps the most challenging aspect of high-content screening of 3D cultures, however, is image capture and analysis, which requires new advanced microscopy and image-informatics solutions. Nevertheless, emerging microfluidic and high-resolution 3D imaging technologies such as light sheet fluorescence microscopy (LSFM) and selective plane illumination microscopy (SPIM) hold great promise for advancing 3D culture-based assays, although they are not yet adapted to a screening setting[127]. Such technologies are discussed in more depth below.

The poor penetration and perfusion of drugs into 3D *in vitro* models can present limitations for drug testing and screening but it also presents new opportunities to mimic fibrotic and poorly vascularized tissues associated with several diseases in which poor drug perfusion contributes to poor clinical efficacy[128,129]. This aspect of pathophysiological drug resistance is not recapitulated in 2D cell culture models and may only be partly addressed in 3D multicellular spheroid models. This more complex aspect of disease pathophysiology can be recapitulated in some *in vivo* models, such as genetically engineered mouse models of pancreatic cancer[128], but *in vivo* models are not practical or cost-effective for screening larger numbers of candidate drugs in a sufficiently rapid manner. However, 3D organotypic *in vitro* co-culture assays (for example, composed of stromal fibroblasts and cancer cells) are faster and recapitulate the fibrosis and poor drug penetration observed in genetically engineered mouse models of pancreatic cancer and in the human disease[130]. Such assays can predict the poor clinical response of solid tumours to small-molecule kinase inhibitors such as dasatinib, and they are suitable for identifying new drugs and drug combination strategies that combat poor tissue perfusion[131,132]. The development of such predictive preclinical assays into higher-throughput and reproducible screening formats is imperative.

Organ-on-a-chip and microfluidic technologies. New approaches that can offer a satisfactory level of biological complexity and clinical relevance while taking into account the issues of throughput, scale and cost are in great demand. One such approach is the organ-on-a-chip technology. These devices are essentially miniaturized microfluidic perfusion systems that permit long-term *in vitro* growth and the propagation of primary stem cells and tissues in a format that is both economically and ethically viable with the potential to scale up for high-throughput discovery campaigns. Although still early in their development, several organ-on-a-chip assay formats have been evaluated; two such examples include a multi-organ chip comprising liver, tumour and bone marrow cell lines[133], and a four-organ-chip system that mimics human liver, skin, intestine and kidney[134]. The advantage of these systems is that they offer a means of modelling the complex tissue microenvironment and the communication between distinct tissues *in vivo*. These systems are reported to produce levels of tissue and organ functionality that are not possible with conventional 2D or 3D

Box 2 | Two-dimensional versus three-dimensional cell biology

Cellular growth in two-dimensional (2D) versus three-dimensional (3D) *in vitro* culture models differs with regard to critical environmental factors. First, the mechanical factors differ; cells grown in 2D cultures are subject to stiffer conditions (that is, less compliant mechanical conditions) than those grown in 3D cultures, which better resemble the mechanical forces exerted on cells *in vivo*. Second, the biochemical environment differs; access to nutrients, oxygen, ions, gradients and drugs is critical within tissues *in vivo* and is clearly distinct between 2D and 3D culture models. Third, the environmental context differs, as physiological cell–cell and cell–extracellular matrix (ECM) interactions are severely compromised in most 2D cultures. These factors can influence intracellular signal transduction pathways, leading to differential gene expression patterns, with important implications for the polarization and differentiation status of cells[192–194]. Accordingly, screening run in parallel in 2D and 3D assays has led to different results[193].

As reviewed in REFS 195–204, there are multiple static and microfluidic systems that facilitate the development of new 3D *in vitro* models of disease (FIG. 2). A range of biomaterial scaffolds for improving the physiological relevance of *in vitro* assays are increasingly being adopted using different natural and synthetic materials in 3D or 2D cell culture models, and for bioprinted organotypic tissue and organs[197,202,205]. Scaffold-based 3D cultures can be generated by seeding cells on an acellular 3D matrix or by dispersing cells in a liquid matrix followed by solidification or polymerization.

The most common scaffolds used fall into two broad categories. The first is biologically derived materials or natural hydrogels that commonly include, but are not limited to, collagen, fibrin, hyaluronic acid, Matrigel and derivatives of natural materials such as chitosan, alginate and silk fibres[206]. The second category is synthetically derived matrices, including polyvinyl alcohol (PVA), polylactide-co-glycolide (PLG), polycaprolactone (PLA) and polyethylene glycol (PEG) hydrogels, which offer more flexibility for tuning chemical composition and mechanical properties as they can be selected or tuned to be hydrolysable or biodegradable[206,207].

To enrich their potential as 'bioactive' materials, those scaffolds are generally supplemented with ECM proteins[122], active peptide sequences[208,209] or nucleic acid aptamers[210]. Magnetic nanoparticles such as magnetite (Fe_3O_4)[211] are used to create magnetic hydrogels, which allow for greater control of the swelling and collapsing properties of the hydrogels using an external magnetic field[212]. These 3D systems better reflect the *in vivo* scenario, allowing, for example, epithelial morphogenetic processes, including the formation of tubules and cysts, and modelling epithelial acini (reminiscent of those found in lung alveolae, mammary and salivary glands, and in pancreatic and kidney cysts), which in many instances have been reported to be functional[27,213,214]. Thus, different 3D assays can be considered to bridge the gap between 2D cultured cells and *in vivo* models.

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Inorganic synthetic matrix cell culture scaffolds (nanofibres, hydrogels and polymers)
- Improved physiological relevance over plastic or glass substrates but limited functionality and relevant ECM–cell communication relative to organic ECM
- Cost-effective and commercial supplies amenable to standard 96- and 384-well formats support high-throughput application and adoption across laboratories

3D microtissues and multicellular spheroid models
- Improved physiological relevance over 2D mono-culture assays
- Many 96- and 384-well assay formats amenable for high-throughput/high-content screening
- Commercial supply of standard plate-based consumables and reagents supports adoption across laboratories

3D organic matrix (for example, Collagen, Matrigel) cell assays
- Improved physiological relevance over standard 2D and synthetic substrates especially if appropriate cocktails and native structure of ECM constituents considered
- Amenable to standard 96- and 384-well assay formats and commercial supply supports adoption across many laboratories. However, high costs and batch-to-batch variability limit high-throughput application

Multicellular organotypic and air–liquid interface assays
- High physiological relevance when using appropriate cell types and organic ECM preparations
- Current formats are not suitable for high-throughput screening
- Established protocols widely published; however, complex assay set-up and lack of commercial supplies limit widespread adoption

***Ex-vivo* tissue culture assays**
- High physiological relevance when using freshly isolated human tissue samples and appropriate assay end points
- Primary-derived tissue formats are not readily amenable to scale up for high-throughput screening
- Limited supplies of fresh human tissue, short lifespan of tissue viability and complex analysis of assay endpoints limit widespread adoption

Organ-on-a-chip and microfluidic assay systems
- High physiological relevance when using appropriate combinations of multiple cell types, tissues, matrix substrates, mechanical stimuli and perfusion or excretion of nutrients
- The most relevant formats are currently restricted to low-throughput applications and not compatible with standard automation and assay screening platforms, limiting adoption across laboratories

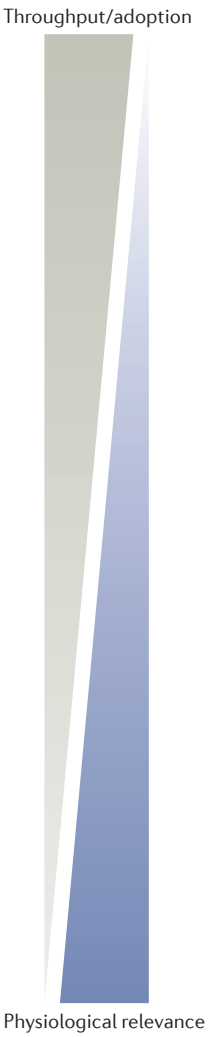

Figure 2 | **Evolution of more physiologically relevant cell-culture assay systems.** In contrast to traditional two-dimensional (2D) cultures of cells as monolayers on flat surfaces, three-dimensional (3D) assays allow cells to grow by forming more complex 3D structures, which better reflect the physiological architecture of tissues and organs *in vivo*. Several new technology developments and culture methods have enabled the design of more consistent and informative 2D and 3D cell culture assays, which can be tailored to address specific biological and clinical questions (BOX 2). Specific developments that are relevant here include: synthetic nanofibre, hydrogel and polymer scaffolds for 3D culture[209,218–221]; spheroid and microtissue assays[222]; 3D organic matrix assays[223]; multicellular organotypic assays[130,172]; *ex vivo* tissue assays[224]; and microfluidic and organ-on-a-chip devices incorporating 3D cell culture substrates, defined mechanical stimuli and controlled perfusion with nutrient media[225–228]. Comparative models of increasing complexity and physiological relevance, although not always suitable for primary screening, may be better positioned further down the cascade as secondary assays or target validation tools to provide increased confidence in the translational potential of novel lead compounds and new target hypotheses. ECM, extracellular matrix.

culture systems, such as kidney tubular epithelial cells and, as previously discussed, hepatocytes[21].

Another advantage of these microfluidic systems is their ability to recapitulate the haemodynamic forces generated by blood flow, which are important in governing the normal homeostatic function of the endothelial cell layer lining the inner lumen of the vascular wall and the sub-endothelial vascular smooth muscle cells. The development of microfluidic perfusion chambers that recapitulate the pulsatile nature of blood flow and regions of high and low shear stress known to regulate endothelial and smooth cell function have helped to more effectively model normal homeostatic vascular function and the pathophysiology associated with cardiovascular disease[135,136]. The latest advances in microfluidic designs, such as the multi-organ tissue flow (MOTiF) chip to enable more precise supply of nutrients and discharge of catabolic metabolites under controlled shear stress, contribute to the increased utility and physiological relevance of microfluidic cardiovascular models[137].

Other examples of recent advances in microfluidic devices for advanced cell culture include the lung-on-a-chip, a microfluidic system that mimics the critical physical and biological features typically found at the alveolar–capillary interface of the human lung. This system can be used to mimic complex pathophysiological responses to stimuli such as those elicited when bacteria and inflammatory cytokines are introduced into the alveolar space[27]. Increasing efforts are being made to produce cardiac tissue on microfluidic devices using cardiomyocyte models as beating heart-on-a-chip platforms that can be used to measure contractility and electrophysiology to test cardiac pharmaceuticals as well as to assess potential cardiotoxic effects during drug discovery[81,82].

The development of microfluidic devices that include temporal and spatial measurements on single cells is further enhanced by methods that manipulate cell movement and collection. Hydrodynamic cell trapping systems have recently overcome the throughput limitations of previous methods for manipulating cells, such as acoustic tweezers or fluorescence-activated cell sorting, by enabling rapid, robust and high-throughput handling of single cells[138]. Microfluidic manipulation of single cells has many applications, including elucidating mechanisms of stem cell renewal and differentiation[139]. Miniaturized methods for manipulating and analysing small populations or single cell phenotypes are complemented by recent advances in ultrasensitive methods for proteomic and genomic analysis[140,141]. As an example, Salehi-Reyhani *et al.*[142] reported the development of a microfluidic antibody capture chip, integrated with total internal reflection fluorescence (TIRF) detection and robust cell lysis to monitor p53 levels within single cells. Progress in whole-genome and whole-transcriptome amplification combined with next-generation sequencing platforms, has facilitated the advancement of single-cell genomics. An integrated microfluidic device, which couples single-cell capture, an enzymatic reaction and quantitative mRNA detection within a single platform, has recently been developed[143]. This platform distinguished stochastic

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variation in gene expression between two distinct cell populations at the single-cell level, which would otherwise be masked when analysed at the population level[143].

Combining microfluidics with image-based or label-free methods for quantifying cell phenotypes at the single-cell level enables miniaturized phenotypic analysis of rare subpopulations and primary cells without the need for bulk expansion *in vitro*. Developments have included the use of optically encoded droplet-microfluidics to enable HTS of compound libraries across single cells[144]. Collaboration between industry and tissue engineering academic groups should encourage further development and adoption of these technologies by a wider community, bringing microfluidic devices, artificial extracellular matrices of tuneable stiffness and mixed cell culture models to a greater number of laboratories.

Advanced microscopy and image analysis tools. One advantage of automated microscopic imaging over other HTS platforms is its provision of information on functional data points together with associated spatial information in *x*, *y* and *z* dimensions. This allows cell-based screening assay formats to progress towards more complex, heterogeneous co-culture and 3D models. Novel *ex vivo* cell models such as those previously described from patients with solid tumours, as well as whole-organism models used in drug testing, are often a source of heterogeneous cell types and present challenges for assay quantification. The heterogeneity that characterizes these models and the original tissues that they represent may drift as a consequence of cell culture conditions, and should be taken into account during assay development and analysis. The widely used whole-well measurements, based on standard luminescence, fluorescence or other similar assays, do not reveal the heterogeneity in response to culture conditions or drug exposure. Such assays also do not recognize or quantify subpopulations of cells carrying specific markers (for example, amplification of a cancer biomarker to inform patient stratification), which can be scored by high-content imaging, and other single-cell technologies.

Microscopy technologies have progressed remarkably over the past few years. Advances in optics, robotics and computational techniques, as well as an expanding repertoire of contrast markers, including functional live-cell reporters, are contributing to the widespread adoption of image-based screening platforms that provide highly dynamic and quantitative fluorescence readouts in cell-based assay systems[145–147]. Non-invasive, label-free imaging techniques have recently emerged, fulfilling the requirements of minimal cell manipulation for cell-based assays in a high-content screening context. Among these label-free techniques, DHM provides quantitative information that is automated for end-point and time-lapse imaging using 96- and 384-well plates[148–150]. Similarly, label-free optical techniques such as phase contrast or differential interference contrast (DIC) can be digitally reconstructed and quantified[151]. Light sheet fluorescence microscopy (LSFM) holds great promise for the analysis of large numbers of samples, in 3D high resolution and with fast recording speed and minimal photo-induced cell damage. LSFM has gained increasing popularity in various research areas, including neuroscience, plant and developmental biology, toxicology and drug discovery, although it is not yet adapted to an automated HTS setting[127,152,153]. Currently, the majority of 3D image analysis software is applied to single images or in a semi-automated low-throughput manner using predominantly customized solutions because no community-wide accepted tools exist[154,155]. Image-based multiparametric phenotypic profiling, including morphology, topology and texture parameters such as wavelet and image moments, have begun to address the challenges of automated segmentation and mathematical descriptor extraction for 3D cell profiling[155,156].

Optimizing bioinformatics solutions and phenotypic analysis towards systems pharmacology. Developments in microscopic imaging provide a strong example of the impact of new technologies on functional biology and cellular pharmacology studies. Rapid and transformative advances in other areas of technology, including proteomic, lipidomic, transcriptomic, epigenetic and mass spectrometry imaging technologies, all support a move away from reductionist approaches in drug discovery to a more holistic 'systems pharmacology' approach. Systems pharmacology describes a broader view of drug activity whereby targets are considered part of integrated biological networks and wherein phenotypic response is linked to genotype and epigenetic considerations, supporting a more in-depth understanding of a drug's MOA and potential personalized health-care strategies[157]. The CANScript technology was recently developed to combine *ex vivo* phenotypic responses of heterogeneous patient-derived tumour tissues with next-generation proteomics and genomic data in order to predict clinical outcomes[158]. Following integration of experimental *ex vivo* data with genomic, proteomic and clinical data, machine learning was utilized to predict the clinical outcomes of chemotherapy in patients with head and neck squamous cell carcinoma and colorectal cancer[158]. Further development of such 'integrative' bioinformatics tools, combining clinical expression or the mutation status of specific targets with cellular networks, chemical tools and preclinical activity, is exemplified by the CanSAR knowledge base, which enables evaluation of the target biology, drug MOA and patient stratification hypothesis within the context of pathway networks and integrated biological systems[159,160].

Although such bespoke bioinformatics solutions demonstrate promise, a major challenge to exploiting the latest functional genomics, proteomics and phenomics technologies is how to integrate large orthogonal datasets in a robust manner to accurately inform the drug discovery process and predict viable clinical development strategies[161]. New advances in network biology and graph theory offer approaches for such integration but require further development and validation[162]. A common weakness of current bioinformatics databases incorporating functional preclinical data is the lack of any standardization of phenotypic assay operation, data reporting and evaluation of the relevance of the phenotypic assay. Indeed, two recently reported large-scale pharmacogenomic studies in cancer cell lines have resulted in follow-up discussion and debate of the inconsistency between study results[163–165]. This could be due to the lack of standardization in experimental assay design, execution and data analysis. However, high correlation for both drug testing and the genomic data could be achieved if common standards for experimental assays and bioinformatics methods are used[166]. These examples further highlight the need for new, better-defined and more robust screening assays through the establishment of strict standard operating procedures and bioinformatics data analytics procedures, allowing statistical comparisons and validations across laboratories[167]. Further

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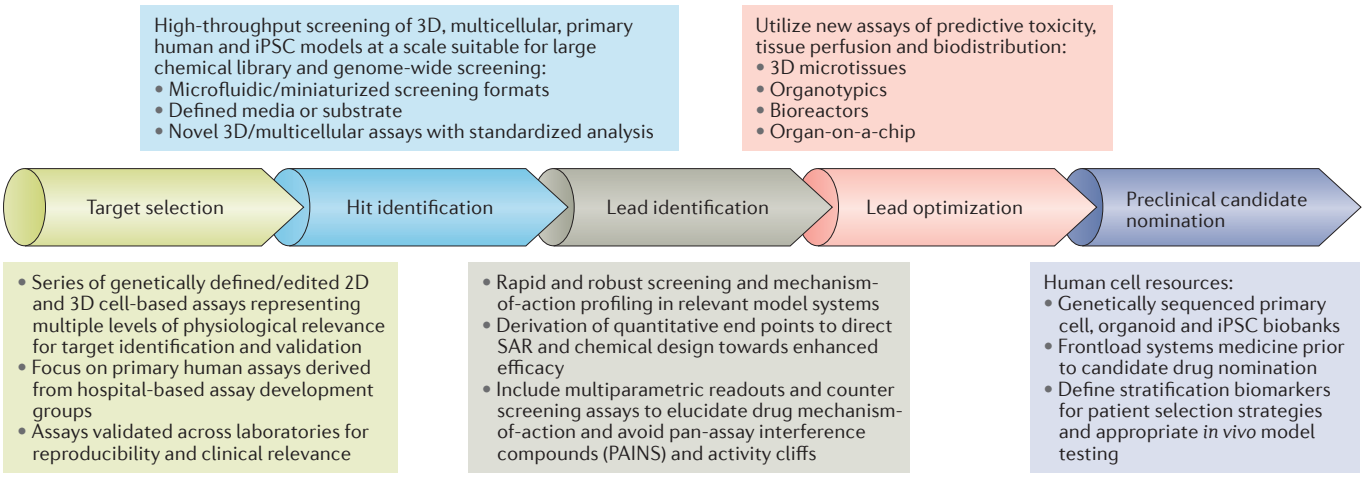

Figure 3 | **Contributions of new cell-based assay technologies to the early-stage drug discovery pipeline.** The figure outlines areas where the application of emerging cell-based assay technologies can enhance the quality and clinical relevance of data derived from cell-based models. Adoption of more robust, informative and relevant assays early within the drug discovery process will support more informed decision-making on which opportunities to progress into preclinical development. Frontloading attrition earlier in the drug discovery process will reduce the substantial costs associated with late-stage attrition due to poor efficacy and toxicity, and contribute to the development of improved treatments for the most challenging diseases. iPSC, induced pluripotent stem cell; SAR, structure–activity relationship.

investment in preclinical assay development, standardization of compound profiling, screening formats and quality control over phenotypic analysis will be required to fully exploit the potential of new integrative drug discovery bioinformatics tools and the systems pharmacology approach.

Principles of disease-relevant assays

Although important progress has been made in developing more physiologically relevant and predictive cell-based assays, as described above, many key aspects of these technologies must be improved to promote adoption, with the ultimate aim of reducing clinical attrition rates. Key considerations are described below.

First, DNA fingerprinting and karyotyping of cell cultures to confirm their origin and genomic integrity could facilitate accurate disease modelling and robust pharmacogenomic analysis and biomarker discovery. Additionally, deep genotyping and phenotyping of patients and healthy volunteers from whom primary cell and iPSC models are derived will help us to understand phenomena such as cell heterogeneity and drug resistance and how *in vitro* culture conditions influence the representation of disease. These efforts could be supported, for example, by the generation or utilization of biobanks comprising well-annotated patient-derived cells, ensuring improved characterization of the genomic attributes of these disease models. Such approaches should also help us to understand the contribution of clonal variation within models by monitoring, quantifying and compartmentalizing cell heterogeneity, leading to a better understanding of the underlying causes of attrition resulting from patient heterogeneity and variation in efficacy and toxicity across diverse human populations. In-depth molecular characterization of patient samples and patient-derived cell models at genetic and post-translational pathway levels would support reverse engineering of assays that recapitulate clinical drug resistance mechanisms. Such detailed characterization of cell models may also partly address the lack of reproducibility across laboratories, which may be due to genomic variation.

Identifying reproducible protocols for the generation and differentiation of consistent somatic cell phenotypes could facilitate the expansion of primary cell and iPSC models for candidate drug profiling and HTS. Such developments will enable evaluation of new target biology and drug MOA across multiple diseases, patient cohorts and healthy donor samples. They will also help identify issues arising from poor experimental reproducibility and therefore facilitate a better understanding of pharmacogenomic effects that may be inherent in these systems. Additionally, it should be ensured that the cell models used in such studies represent the appropriate maturity (for example, embryonic or adult characteristics) for the expected age of the patient, therefore providing a more accurate disease model.

The environmental (macro and micro) conditions of *in vitro* models should be appropriately tailored to represent tissue types, disease pathophysiology and disease aetiology. This will involve accurate modelling of the nutrient and metabolite concentrations, pH and dissolved gases. Special attention should also be focused on the mechanical conditions in which cells and tissues are propagated. These include ECM constituents, specifically those that contribute to the stiffness of growth substrates, the interaction of cellular adhesion molecules, mechanical deformation and shear forces. Also, many culture systems will require the controlled introduction of defined and relevant disease-causing environmental and physiological 'perturbagens' to develop more accurate models of disease progression.

In diseases where the underlying causes are multifactorial, in many cases it will be challenging to isolate the relevant end point (or end points) from *in vitro* cell and tissue-based assay systems. For example, in neurodegenerative diseases that are associated with plaque formation, it is currently a matter of debate as to whether the current readouts (the inclusions) represent a mechanism of protection or whether they are a pathological end point leading to irreversible damage. Similarly, in type 1 diabetes, it is unclear whether assays measuring the production or release or the degradation of granules should be utilized to guide new therapies focused towards stimulating the production and

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release of insulin, or whether the degradation of old insulin-secreting granules should be prioritized to allow newly synthesized granules to fuse with the plasma membrane. Thus, as previously discussed by others[168], the selection of relevant end points, which translate into clinical phenotypes or biomarkers of disease outcome, may be most beneficial.

Additional considerations include:

- development of multiscale tissue assays to mimic organism-level physiology to accurately model drug metabolism, co-morbidities and systemic paracrine effects;
- incorporation of clinically equivalent dosing strategies that mimic known or predict expected *in vivo* pharmacokinetic properties of various therapeutic modalities;
- derivation of iPSCs from multiple patients and tissue types, containing multiple diverse genomic alterations, in order to reflect broader patient populations and more common disorders;
- comparison of the drug–response data between patients and patient-derived cell models to evaluate the predictive value of the models;
- integration of molecular genetic, epigenetic and proteomic data sets with robust phenotypic measurements in both clinical and preclinical settings with computational bioinformatics, supporting multiparametric validation of drug targets and assay relevance through a less-reductionist 'systems pharmacology' approach; and
- adoption of a human physiology assay checklist, based on unbiased evaluation of which assay conditions accurately recapitulate the human tissue physiology or pathophysiology under study, and the limitations that each assay will inevitably have with regard to recapitulating the full complexity of human disease.

With the above considerations in mind, in TABLE 1 we provide our proposal for a set of defining principles of disease-relevant assays and justify their potential impact on drug discovery. Although many principles are generic and can be applied across disease models, certain principles have disease-specific considerations, which require more in-depth investigation and modelling, as indicated in TABLE 1. We anticipate that no single model or assay will be perfect and many of them will fail to recapitulate the full complexity of the human disease conditions that they intend to represent. However, we believe that careful consideration of the limitations of each assay and the defining principles outlined in TABLE 1 will lead to more critical evaluation of the predictive value of every cell-based assay, and also lead to more cautious and appropriate interpretations of the results derived from these assays.

These defining principles are also intended to encourage further discussion and debate on preclinical assay screening standards and their clinical relevance in order to help drive forward the field of preclinical assay development towards improved clinical success rates. We anticipate that broad communication of such principles across diverse scientific disciplines will both stimulate and guide further investment in innovative solutions and the development of new technologies that address these challenges. In FIG. 3, we highlight how integration of the technologies discussed above could contribute to early-stage drug discovery.

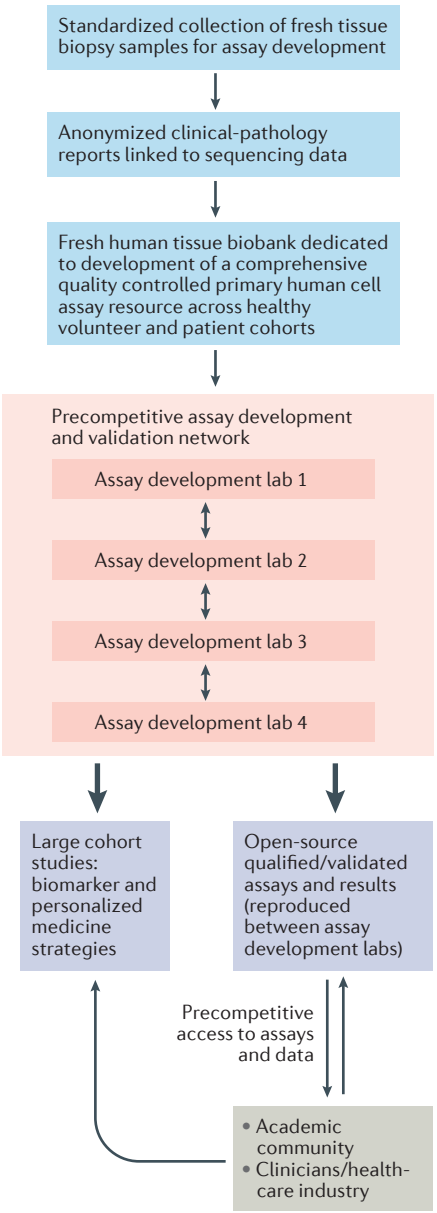

Figure 4 | **Precompetitive consortia facilitating predictive assay development, experimental and personalized medicine strategies.** The development of screening assays and preclinical models that better predict clinical outcomes is a major challenge, and has not received the necessary investment from translational funding bodies and industry. Here, we present a model for the precompetitive development of more predictive and reproducible cell-based assays though academic and industrial consortia. Facilitated by the access to human cell and tissue biobanks, we propose a network of core cell-based screening laboratories that are proficient in assay quality control and automated cell-based screening technology, to develop assays to common standards. New assays will be qualified for disease relevance in partnership with disease area experts and clinicians performing retrospective studies with approved drugs and failed clinical candidates, where possible, to determine the positive and negative predictive value of each assay. Assay protocols and assay results relating to the identification of new targets, lead compounds and repositioning opportunities will be validated for reproducibility by benchmarking assay performance and confirming results between distinct core laboratories within assay development networks. We believe that academic, industrial and government funding of such precompetitive activity will provide open-source access to high-quality assays and results to be exploited by the academic and health-care communities and the biopharmaceutical industry. These activities will drive substantial improvements in the quality of the academic literature, publishing new target hypotheses and new drug combination and repositioning opportunities. Such precompetitive activity will also improve the quality of assays used by academic and industrial groups for screening internal chemical and biological libraries. Follow-up studies on promising leads or drug candidates could be performed on larger cohorts of patient-derived cell models through the consortia and/or access to the academic network of core laboratories.

Assay validation and translation

Validating new preclinical models for physiological and disease relevance is not trivial, and arguably the best validation is evidence of prediction of clinical efficacy or toxicity. The majority of new cellular assay technologies described in this article have not yet reached a level of maturity that would permit any clear conclusions

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Box 3 | **Phenotypic drug discovery versus target-directed drug discovery**

Target-directed drug discovery (TDD) has been the predominant strategy of the pharmaceutical and biotechnology industry for the past 25 years. It is characterized by the identification and optimization of compounds that modulate a pre-nominated target that is implicated in disease progression, often using high-throughput screening to identify initial hits. Phenotypic drug discovery (PDD) has been defined as the generation of hit or lead molecules without any prior knowledge of the target. Such compounds are typically identified and developed through empirical testing in physiological *in vivo* models or from cell-based phenotypic screening assays.

An initial retrospective analysis of all drugs approved by the US Food and Drug Administration (FDA) between 1999 and 2008 indicated that of the first-in-class small-molecule medicines approved, 37% (28 drugs) were initially identified by a PDD approach, whereas 23% (17 drugs) were identified by TDD[215]. Follow-up retrospective studies in cancer[216] and across disease areas[217] produced different results, in part owing to differences in the definition of phenotypic screening and in part owing to differences in the time period studied, with TDD showing substantial success in cancer in more recent years. In cancer, 31 novel small-molecule drugs approved by the FDA between 1999 and 2013 were discovered by TDD (including 21 kinase inhibitors), whereas 17 novel small-molecule drugs approved during the same period were discovered by PDD (including a single kinase inhibitor, trametinib, targeting MAPK/ERK kinase (MEK))[216]. A retrospective analysis by Eder and colleagues[217] across all disease areas, using a refined selection criteria, identified 45 novel small molecules approved during 1999 and 2013 that were discovered through TDD. At the same time, 33 approved small molecules were discovered by non-target-directed methods, including 8 through unbiased phenotypic screening of large chemical libraries and 25 through chemocentric approaches, representing a combination of rational drug design and phenotypic screening/validation. These studies indicate that PDD and TDD can be considered complementary approaches. Further investment in more predictive cell-based assay systems could advance both PDD and TDD strategies, ultimately resulting in improved clinical success rates.

regarding their predictive value. Currently, the adoption of new technologies often depends on an organic rise in utilization and acceptance. However, rather than simply waiting for a sufficiently large body of evidence to be generated by pioneering drug discovery efforts, a concerted effort is needed to compare the predictive power of new cell-based models for outcomes in patients. Such efforts would ideally involve testing collections of known molecules that have demonstrable clinical efficacies and toxicities to support backward translation to determine the predictive value of *in vitro* models. Backward translation studies may also encompass reverse engineering of *in vitro* model systems to predict past clinical trial failures by recapitulating known mechanisms of drug resistance or relapse observed in the clinic.

Recent examples of such work have focused on evolving hepatotoxicity assays utilizing primary hepatocytes, stem-cell-derived hepatocytes and hepatocyte cell line cultures grown on a range of defined organic or synthetic matrix substrates. These systems were then tested against a panel of drugs to predict agents that result in drug-induced liver injury in humans[169–171]. Similar approaches using stem-cell-derived cardiomyocytes to predict cardiotoxicity are also under development, as previously discussed.

Examples of backward translation studies to predict the clinical efficacy of therapeutic agents are rare, which is probably a reflection of the lack of research funding available to support backward translation studies and assay model development. However, to demonstrate the predictive and translational potential of new preclinical models described in this article, we highlight two case studies: the Hedgehog signalling inhibitor vismodegib and an αvβ6 blocking antibody (264RAD). Both of these examples exploit the unique aspects of the 3D organotypic pancreatic cancer model previously described, which is composed of a co-culture of established pancreatic cancer cells cultured on dense collagen matrix contracted by fibroblasts[130,172]. This model recapitulates *in vivo* pharmacology issues relating to poor drug penetration and inadequate clinical efficacy[131,132].

In the first example, studies that used both the *in vitro* organotypic cell culture model and comparative *in vivo* transgenic models showed that cyclopamine, which targets Hedgehog signalling in the stromal cell compartment, leads to increased penetration and hence activity of the anticancer drugs dasatinib and gemcitabine in pancreatic cancer[128,132]. Clinical trials of the approved Hedgehog signalling inhibitor vismodegib have been instigated in pancreatic cancer, and the recently published preliminary results indicate an improved efficacy response[173]. In the second example, pioneering studies demonstrating that targeting αvβ6 suppressed cancer cell invasion in 3D organotypic assay formats[174,175] have contributed to the progression of the novel αvβ6-blocking antibody therapy (264RAD) into a phase I trial in pancreatic cancer; see the Barts Cancer Institute website for further information.

Finally, a recent article published by Scannell and Bosley[176] provides further justification for increased investment in backward translation studies. This work, which describes a quantitative decision-theoretic model of declining R&D productivity, illustrates how small improvements in the positive predictive value (PPV) of preclinical assays can outperform the documented advances in assay automation and throughput with regard to improving R&D productivity as defined by the successful translation of drug discovery to positive clinical outcomes[176]. Thus, a clearly defined pathway to assay validation and a subsequent development of a repository of assays with known positive and negative predictive values (PPVs and NPVs) would be of considerable value. As far as we are aware, there is no unifying systematic approach to measuring and recording the clinical impact of established assays or new assay technologies and approaches. We suggest that a routine evaluation of the PPV or NPV of all assays used in drug discovery would provide a valuable resource to the translational research community.

The need for funding and consortia

Academic–industrial partnerships have existed in some form since the birth of the pharmaceutical industry over 100 years ago, but have been increasingly appreciated and pursued more recently in a wide range of contexts, from identifying and validating novel therapeutic targets through to addressing drug development challenges that are too broad for any one organization to tackle effectively alone, which are often addressed through consortia. There remains, however, a gap in the development of such consortia in the area of preclinical model development, and here we put forward three proposals to address this.

First, we propose that direct funding for the development of preclinical models and assay-screening technologies with improved clinical relevance is an aspect of the drug development process that can be most effectively addressed by

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academic–industrial partnerships. Although target-based drug discovery is directly supported through academic–industrial partnerships such as Bayer's Grants4Targets, GlaxoSmithKline discovery partnerships with academia (DPAc scheme) and the IMI European Lead Factory consortium, the general strategy of drug discovery groups within both industry and academia, at present, is to 'tap into' academic groups that have developed more relevant assays for their own research and apply them to existing drug discovery programmes. Such academic assay systems and models have typically not been developed for the purpose of screening or guiding clinical decision-making, and thus remain to be validated for robustness, reproducibility and clinical predictivity[177]. We therefore feel that translational funding schemes and precompetitive research consortia must be substantially expanded and rebalanced from hypothesis-driven, target-directed research to support the development of new preclinical models and assay-screening technologies that can provide improved prediction of clinical outcomes.

Second, we propose that biobanking should be encouraged and developed. The initiating step in moving towards more clinically relevant primary human assays to support experimental medicine and personalized treatment paradigms is a standardized flow of high-quality samples from the clinic to the research bench. Such samples require relevant and anonymized clinical information, complete pathology reports and timelines from surgery for optimal integration with molecular profile data. Currently, the sample flow is often initiated through disease-specific research projects where ethics boards provide permission for sampling of patients. However, in the long term, biobanking operations with a single consenting procedure provide a much more viable solution. The development of drug-testing pipelines for human cell-based or *ex vivo* samples must at least aim to achieve diagnostic-grade assays with quality standards in place, to enable relative ease of routine procedures and to be scalable in terms of maximizing both sample amounts and screening capacity. Such translational efforts are often nation-centric with the limitations of national laws, which unfortunately confound larger cohort studies and contribute to duplication of efforts with limited standardization.

Third, we propose that international collaboration and consortia to derive primary human-cell biobanks with relevant annotation and standard operating procedures will promote a step-change in the derivation and use of primary human-cell assays to support drug discovery, experimental medicine, drug repositioning and personalized health-care studies. A recent consortia composed of academic and industrial partners led by the Structural Genomics Consortium, entitled ULTRA-DD (Unrestricted Leveraging of Targets for Research Advancement and Drug Discovery), is beginning to address this issue by providing open-source access to high-quality chemical probes and resources for exploring target biology in patient-derived models[177]. However, further investment in the development and validation of the most disease-relevant assay model systems is required to support and expand these activities across drug discovery programmes. Quality control, standardized procedures, standardized data analysis and validation of assay results across reference laboratories will be crucial to the efficient development and acceptance of such assays, by driving improved data reproducibility and greater general confidence in promising early translational research results. Collaboration between dedicated assay development and assay screening groups, together with clinicians, biostatisticians, computer scientists and IT experts adequately supported from industrial, academic, government and charitable funding sources, is necessary to address these challenges (FIG. 4).

Conclusion

The emergence of new, cell-based assay technologies will be important in enhancing conventional target-directed drug discovery (TDD) by supporting more robust target identification and validation, as well as secondary screening assay cascades. Such advances are also well placed to support a new era of phenotypic drug discovery (PDD) (BOX 3). However, in order for these technologies to reach their full potential, there must be an acknowledgement of the value of functional biology and physiological-based assay systems in drug discovery and the need to further develop more predictive *in vitro* models and new assay screening approaches towards greater clinical relevance. We propose that the starting point of any cell-based assay development should involve a consideration of its physiological relevance and the defining principles of disease-relevant assays proposed in this article (TABLE 1). Adhering to these principles may lead to increased research costs, but in our view the benefits justify the increased investment and effort. As a translational research community, we should drive the field towards greater disease relevance in drug development and call for funding bodies to support more advanced cell-based assay development and robust hypothesis-generating translational research as standalone grants. The current focus on reductionist, hypothesis-driven research combined with the lack of robust disease-relevant preclinical models to evaluate targets and novel drug candidates and to predict clinical outcomes is clearly hindering advancement. Our hope is that the principles laid out in this article should function as a signpost for greater investment in the development and uptake of advanced preclinical disease modelling technologies and infrastructures. It is our view that such investment will complement the existing investments in academic and industrial drug discovery and substantially increase the likelihood of success for drug discovery and experimental medicine projects.

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doi:10.1038/nrd.2016.175
Published online 12 Sep 2016

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Acknowledgements

The authors thank H. Ebner for assistance in the writing of this manuscript. E.D.N is supported by the program Paris Alliance of Cancer Research Institutes (PACRI), Investissements d'Avenir, launched by the French government with the reference ANR-11-PHUC-002. N.A. and S.L.S. are grateful for support from the 7th Framework Programme of the European Commission (LEISHDRUG project, 223414) and the French Government (L'Agence nationale de la recherche (ANR)) programmes: Investissements d'Avenir programme ('Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases'; grant ANR-10-LABX-62-IBEID); France BioImaging (FBI; grant ANR-10-INSB-04-01) and the Fondation Française pour la Recherche Médicale (FRM; Grands Équipements Program). N.O.C. acknowledges a fellowship award from Research Councils UK (RCUK). P.H. acknowledges support from the Hungarian National Brain Research Program (grant MTA-SE-NAP B-BIOMAG). V.P. and P.H. acknowledge support from the TEKES Finland Distinguished Professor Programme (FiDiPro) Fellow Grant (40294/13). M.C.M is supported by the Spanish Ministry of Economy and Competitiveness (MINECO) and the The Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC) foundation, and received funding from the Severo Ochoa Center of Excellence (MINECO award SEV-2015-0505), MINECO (grant BIO2014-62200-EXP) and the Innovative Training Networks (ITN) EU Horizon 2020 (EU-H2020) programme (grant 641639 BIOPOL). V.P. and P.Ö. received funding from the European Union's 7th Framework Programme (FP7/2007–2013; grant 258068); EU-FP7 Systems Microscopy Network of Excellence (NoE) project, the Sigrid Juselius Foundation, the Cancer Society of Finland, the Academy of Finland (Centre of Excellence in Translational Cancer Biology), the Magnus Ehrnrooth foundation and the TEKES FiDiPro Fellow Grant (40294/13), and TEKES New Generation Biobanking Grant (40294/11). Research in the Kallioniemi group at the Science for Life Laboratory received funding from K. Wallenberg and A. Wallenberg (grant 2015.0291), and the Karolinska Institutet. G.T. is supported by École Polytechnique Fédérale de Lausanne (EPFL) and the Swiss National Science Foundation/ National Centres of Competence in Research (SNF/NCCR) in Chemical Biology. D.E. acknowledges research support from Cancer Research UK (CRUK) and the Higher Education Funding Council for England (HEFCE).

Competing interests statement

The authors declare competing interests: see Web version for details.

FURTHER INFORMATION

Bart's Cancer Institute ("Promising antibody against cancer cells characterised at BCI to be developed for clinical trials"): http://bci.qmul.ac.uk/news/general-news/item/264rad-antibody-bci-medimmune-cruk-trials
Bayer's Grants4Targets: https://grants4targets.bayer.com/
CanSAR knowledge base: https://cansar.icr.ac.uk/
European Cell-Based Assays Interest Group: https://www.eucai.org
GlaxoSmithKline discovery partnerships with academia (DPAc scheme): http://www.dpac.gsk.com/
IMI European Lead Factory consortium: https://www.europeanleadfactory.eu/
ULTRA-DD (Unrestricted Leveraging of Targets for Research Advancement and Drug Discovery): http://www.ultra-dd.org/

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