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Cell Chemical Biology
Review

The Convergence of Stem Cell Technologies and Phenotypic Drug Discovery

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Recent advances in induced pluripotent stem cell technologies and phenotypic screening shape the future of bioactive small-molecule discovery. In this review we analyze the impact of small-molecule phenotypic screens on drug discovery as well as on the investigation of human development and disease biology. We further examine the role of 3D spheroid/organoid structures, microfluidic systems, and miniaturized on-a-chip systems for future discovery strategies. In highlighting representative examples, we analyze how recent achievements can translate into future therapies. Finally, we discuss remaining challenges that need to be overcome for the adaptation of the next generation of screening approaches.

Introduction

Phenotypic screening (PTS) has experienced a renaissance, promising high-throughput access to more complex disease models and unknown disease mechanisms. Combining increased sophistication in high-throughput automation, cell culture, molecular engineering, and hardware and software for data analysis with patient-derived induced pluripotent stem cell (iPSC) strategies has opened up entirely new opportunities for modeling diseases *in vitro*. In addition to addressing conventional therapies focusing on cellular misregulation, PTS can identify new compounds and protocols to drive targeted cell fate switching within tissues. This may allow combating tissue damage including aging and degenerative diseases through induction of local tissue repair via activation/generation of tissue-resident precursor cells, which, in turn, replenish somatic cell populations lost due to injury or disease. However, control of increasingly complex biology requires the codevelopment of a number of upstream and downstream technologies to enable the generation of more homogeneous and closely defined iPSC-derived tissue models in 2D and 3D cultures, to efficiently gather and interpret multidimensional data, and to close the gap between *in vitro* and *in vivo* disease biology.

We highlight examples for the broad utility of PTS approaches in combination with pluripotent stem cell technologies in a variety of established fields including toxicity testing, disease modeling, and conventional 2D high-throughput screening (HTS). We place a significant focus on new and developing technologies that enable modeling and control of tissue and multifactorial disease complexity *in vitro*. Thus, we evaluate the current state of 3D cell culture, organs-on-a-chip, as well as the increasingly complex challenges of data analysis in view of their use in HTS workflows. Further key developments in the future of HTS will hinge on converging the disparate fields of cell culture automation, microfluidics, organic chemistry and library design, reporter systems biology, high-content analysis, data mining, and machine learning. We examine examples of each of these fields in turn as far as they demonstrate relevance to key aspects of future HTS strategies.

Background

Successful drug discovery campaigns often employ one of two general hit identification strategies: target-based screening (TBS) or PTS (Eder et al., 2014; Swinney and Anthony, 2011). TBS identifies small molecules or biologics (large bioactive molecules such as antibodies, nucleic acids, and so forth) that can modulate the biological activity of a known molecular disease target. Target-based approaches first identify molecular targets and then find and optimize drug candidates that specifically engage their targets in cell-based, biophysical, and/or biochemical assays *in vitro*. This is followed by subsequent validation of hit molecules in complementing bioassays as well as by functional profiling *in vivo*. In contrast, PTS measures the functional consequences (i.e., the phenotype) of drug candidates in the context of a disease-relevant cell type or organism, irrespective of the molecular target and mechanism involved. PTS mostly employs cell-based assay formats, which ideally serve as safety and disease models by approximating a particular pathophysiology.

Before 1970, most drugs were discovered by PTS (Pina et al., 2009). However, advances in bioassay technologies, miniaturization, workflow automation, as well as a steep increase in putative drug targets, enabled TBS of large libraries in HTS campaigns (Drews, 1996; Rask-Andersen et al., 2011). This reductionist approach of breaking complex diseases down into molecular drug targets led to a dramatic increase in the number of drug candidates (Imming et al., 2006; Pereira and Williams, 2007). However, TBS fell short of meeting initial expectations for increased numbers of market-approved therapeutics (Harrison, 2016; Overington et al., 2006; Scannell et al., 2012; Swinney and Anthony,

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2011). In addition, with better tools of exploring single targets as the origin of disease, it became apparent that many diseases have multifactorial origins and are thus only inadequately represented in single TBS approaches (Gregori-Puigjane et al., 2012). Further "-omics" analyses and genome-wide association studies (GWASs) revealed that the "one-drug-one-target" reductionist approach of TBS often does not capture the complexity of diseases (Mestres et al., 2009; Sams-Dodd, 2013). By using standardized cell lines, TBS biology often lacks cell-type-specific signaling pathways or posttranslational modifications along with other properties inherent to the diseased cell types. Consequently, drug candidates identified by TBS frequently failed to demonstrate efficacy or safety in clinical trials (Mestres et al., 2009).

These shortcomings of target-based drug discovery have led to a renewed interest in PTS approaches (Eggert, 2013; Haasen et al., 2017; Lee and Berg, 2013; Vincent et al., 2015). Ideally, phenotypic assays measure endogenous, physiological responses of disease-relevant cell types. Therefore, they potentially represent a more appropriate approximation of disease-relevant physiology than isolated biochemical reactions. PTS can also help identify disease mechanisms and novel drug targets that can be later pursued with established, highly evolved TBS workflows (Swinney, 2014). However, a significant limitation of PTS strategies is that the drug target and mechanism of action need to be determined separately in a target deconvolution process. Although knowledge about mechanism of action is not mandatory for drug approval, it is highly desirable for vetting drug candidates for stability, potential adverse effects, and possible delivery options before entry into clinical trials.

Despite considerable progress, PTS and TBS often share one conceptual challenge: the highly streamlined assay biology may fail to recapitulate signaling pathways and physiological traits of disease-relevant cell types *in vivo*, and assay results may not translate well into human clinical outcomes (Horvath et al., 2016). Although PTS is frequently considered superior to TBS in capturing disease biology, the tissue culture conditions for PTS assays differ markedly from the physiological environment of the diseased tissue, where cells interact and communicate with each other and with the extracellular matrix. Standard assay conditions in 2D adherent tissue culture bear little resemblance to physiological conditions but have been necessary to enable automated HTS workflows. Finally, cell lines used in PTS campaigns rarely originate from patients and thus lack the disease-specific genetic background.

Stem Cells Open up New Opportunities for Disease Modeling and Toxicity Screening

PTS using human induced pluripotent stem cell (hiPSC) technologies can address some of these shortcomings. hiPSCs can (1) be differentiated into disease-relevant cell types, (2) form heterogeneous cell cultures that can more closely mimic the cellular diversity of human tissues, (3) be generated from patient-derived tissues that bear the hallmarks of genetic and epigenetic disease states, (4) enable 3D culture to emulate tissue-like environments, and (5) populate microfluidic devices (organ-on-a-chip) (Chen et al., 2018). Advances in iPSC technologies have improved supply of stem cells and have led to the establishment of robust, xeno-free protocols (Chen et al., 2011; Ebert and Svendsen, 2010; Frank et al., 2012; Gunaseeli et al., 2010; Shi et al., 2017) for differentiation of hiPSCs into many human cell types that can serve as disease models and are suitable for drug discovery (Heilker et al., 2014; Takahashi and Yamanaka, 2016). hiPSCs are readily accessible and can be generated easily from many somatic tissues in using commercial solutions. iPSC reprogramming technologies can thus provide cells for HTS campaigns that are otherwise unavailable because primary cells derived from patients are postmitotic and do not proliferate to generate sufficient quantities (i.e., human neurons). In addition, iPSC technologies facilitate genetic modification that may be difficult to achieve in many primary cell types (i.e., hepatocytes). Genome engineering tools such as zinc-finger nucleases, CRISPR-Cas, or transcription activator-like effector nucleases (TALENs) can be used to address genetic variance and generate appropriate isogenic control cell lines for drug discovery in order to conduct HTS campaigns using cell lines with identical genetic background (Chang et al., 2018). This approach will minimize interindividual differences and enable the identification of novel compounds, the discovery of which would have been obscured by genetic variation between nonisogenic cell lines. Furthermore, stem cell databases and repositories, such as HPSCreg.eu, EBiSC, the UK Stem Cell Bank, the International Stem Cell Registry, and Cellular Dynamics International, will offer additional standardization and quality control.

Advances of Stem Cell-Based PTS in 2D Cultures

Building on the strengths and relative maturity of adherent stem cell protocols, several PTS have provided proof-of-concept for iPSC-driven screening approaches (Kondo et al., 2017; Sterneckert et al., 2014) (also summarized in Table 1). At the same time, these approaches highlight the importance of careful hit validation strategies.

Combining PTS with GWASs can establish links between genetic variants and disease traits across a population. CRISPR-Cas9 gene editing was used to generate human isogenic pancreatic beta-like cells that carry biallelic null mutations in susceptible genes, such as CDKAL1, which were associated with type 2 diabetes mellitus in GWASs (Zeng et al., 2016) (Figure 1A). The study revealed that susceptible genes modulate proper function and survival of pancreatic beta cells. The generated CDKAL1$^{-/-}$ human cell population exhibited insulin secretion impairment *in vitro* and showed defective glucose homeostasis in a mouse model. Moreover, the CDKAL1$^{-/-}$ cells were hypersensitive to glucotoxicity and lipotoxicity. Screening 2,000 US Food and Drug Administration (FDA)-approved drugs by measuring the number of insulin-containing cells using an insulin-specific antibody yielded compound T5224 as a hit. This inhibitor of FOS/JUN activator protein-1 (AP-1) was found to protect cells against induced glucolipotoxicity in a dose-dependent manner and rescue CDKAL1-associated defects both *in vitro* and *in vivo* by inhibiting the FOS/JUN pathway. Oral administration of T5224 or transplantation of CDKAL1$^{-/-}$ cells with RNAi-mediated loss of FOS rescued pancreatic beta cell dysfunction in an *in vivo* mouse model. These findings suggest that FOS/JUN inhibition via pharmacologic or genetic intervention may represent an attractive therapeutic option for patients carrying the CDKAL1$^{-/-}$ risk allele.

Combining a high-content-based screen for the expression of fragile X mental retardation (Fmr1) protein with iPSC-derived human neural precursor cultures, a group at Novartis screened

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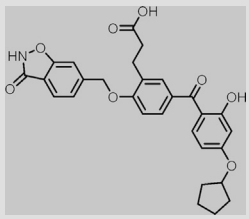
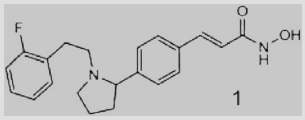
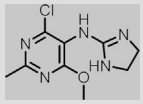
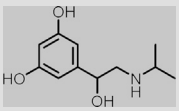
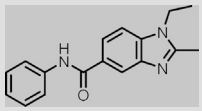
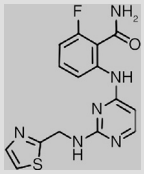
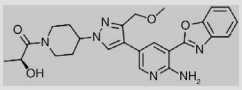
Table 1. Summary of the High-Content Phenotypic Screens Described in the "Advances of Stem Cell-Based PTS in 2D Cultures" Section

Process	Initial Cell Population	Format	Readout	Library (Number of Compounds)	Z′	Hit(s) (Bioactive Concentration) (μM)	Chemical Structure	References
Type 2 diabetes (pancreas)	human pancreatic beta-like cells	384 well	insulin PI	(2,000)	NA	T5224 (16.2)		Zeng et al. (2016)
Fragile X syndrome	human neuronal progenitor cells	384 well	FMRP	(50,000)	0.8/0.4[a]	epigenetic modifiers, e.g., compound 1		Kaufmann et al. (2015)
Tauopathies (brain)	human i^{3}Neurons	384 well	Tau TUJ1 Hoechst	LOPAC (1,280)	0.4	moxonidine (30)		Wang et al. (2017)
						metaproterenol (100)		
Heart failure (heart)	human primary epicardium-derived cells (EPDCs)	384 well	DAPI	internal stem cell set (7,400)	0.4	compound 2 (3)		Paunovic et al. (2017)
Muscle repair	mouse skeletal muscle progenitor stem cells (satellite cells)	384 well	DAPI	Stem Cell Toolbox (800)	NA	GSK2226649A (10)		Billin et al. (2016)
IPF (lung)	human multipotent cells	96 well	proSP-C	NA	NA	2D08 (10)		Fujino et al. (2017)

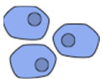
FMRP, fragile X mental retardation protein; IPF, idiopathic pulmonary fibrosis; Ki-67, FHA domain-interacting nucleolar phosphoprotein; LOPAC, Library of Pharmacologically Active Compounds; N/A, not applicable; PI, propidium iodide; proSP-C, prosurfactant protein-C; Tau, microtubule-associated protein tau; TUJ1, neuron-specific class III β-tubulin.
[a]Depending on the type of analysis algorithm.

50,000 small molecules for their ability to epigenetically correct abnormal silencing of the Fmr1 gene (Kaufmann et al., 2015) (Figure 1B). A multiparametric, machine-learning-based image analysis yielded hit rates between 1.1% and 2.8%, depending on the type of analysis algorithm. These efforts highlight the potential of iPSC-derived PTS to identify modulators of epigenetic misregulation.

TALEN-mediated genetic engineering was used to generate hiPSCs that inducibly express Neurogenin 2 to ultimately generate hiPSC-derived postmitotic neurons with high efficiency and homogeneity (Wang et al., 2017) (Figure 1C). These neurons were used to search for small molecules that can reduce the levels of human Tau protein. High-throughput-screening of the Library of Pharmacologically Active Compounds on these i^{3}N neurons identified moxonidine and metaproterenol as hits. Both molecules are adrenergic receptor (AR) agonists, suggesting that AR signaling is associated with Tau homeostasis. This study identified AR activation as a potential therapeutic approach for the treatment of tauopathies by lowering Tau levels.

AstraZeneca performed a phenotypic screen with adult human primary epicardium-derived cells (EPDCs) to identify small molecules that can activate the endogenous regenerative capacity of cardiac progenitor cells and thus restore the heart's limited ability for self-repair upon injury (Paunovic et al., 2017)

A Type 2 diabetes (T2D), Zeng et al., 2016



hESCs

1. CRISPR-Cas9
2. Differentiation

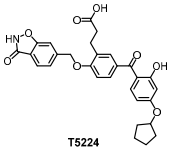
↑ *glucotox*
↑ *lipotox*

CDKAL1$^{-/-}$ pancreatic beta-cells

2000 compounds

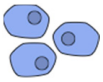
HCS

↓ FOS/JUN



AP-1 inhibitor

B Fragile X syndrome (FXS), Kaufmann et al., 2015



hiPSC

Differentiation

↓ Fmr1

Neuronal progenitors

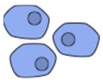
50000 compounds

HCS

↑ *Fmr1*

Epigenetic modifiers

C Tauopathies, Wang et al., 2017



hiPSC

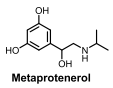
1. TALEN
2. Differentiation

cortical glutamatergic neurons

LOPAC

HCS

↓ *Tau*



AR agonists

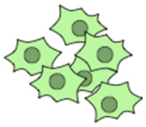
D Post-myocardial infarct, Paunovic et al., 2017

Primary human epicardium-derived cells (EPDCs)

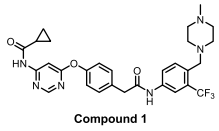
7400 compounds

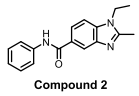
HCS

↑ *Proliferation*



Primary human epicardium-derived cells (EPDCs)





E Muscle atrophy, Billin et al., 2016

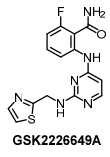
Aged murine satellite cells

800 compounds

HCS

↑ *Proliferation*

Aged murine satellite cells



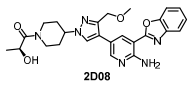
F Idiopathic pulmonary fibrosis (IPF), Fujino et al., 2017

Alveolar epithelial type II cells (AEC2)

Kinase-focused screening

HCS

↑ *ProSP-C*



Axl tyrosine kinase inhibitor

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(Figure 1D). The screening campaign yielded hits belonging mainly to two distinct chemotypes with disparate biological activities. Chemotype 1 activated proliferation in both EPDCs and cardiac fibroblast cells, the latter of which served as a control. On the other hand, a different compound representing chemotype 2 selectively activated proliferation only in EPDCs. These findings emphasize the importance of appropriate triaging of hit molecules obtained by PTS. Evaluation of previously reported biological targets of both compounds did not reproduce this phenotype. However, target deconvolution approaches employing *in vitro* kinome profiling and functional evaluation of additional 147 biological targets revealed a rather promiscuous activity profile for chemotype 1. This result suggested that its mode of action might involve polypharmacology. In contrast, chemotype 2 showed no significant activity on additional targets within this same panel, suggesting that it is less prone to off-target effects.

Another example describing the challenges of hit validation following a phenotypic screen was reported by GlaxoSmithKline (Billin et al., 2016) (Figure 1E). Enhancing proliferation of skeletal muscle progenitor stem cells (satellite cells), which generate new skeletal muscle upon injury, can be a therapeutic approach to treat acute and/or age-associated muscle loss. This approach used aged murine cells to screen 800 target-annotated compounds and found that GK2226649A, an inhibitor of c-Jun N-terminal kinase 1 (JNK-1), can restore the proliferative capacity of satellite cells. This hit was confirmed *in vivo* using a contraction-induced muscle-damage mouse model. However, structure-activity relationship studies could not establish a functional connection between JNK-1 inhibition (target) and satellite cell proliferation (phenotype). Neither kinome profiling *in vitro* nor chemical proteomics experiments in HeLa and muscle-derived A204 rhabdomyosarcoma cell lysates revealed any other targets. These findings further underscore the importance of appropriate, and, ideally, human cell models for compound screening and validation that can capture key differences between animal and human physiology.

PTS was recently used to identify compounds with activity against idiopathic pulmonary fibrosis, a severe lung disease characterized by progressive lung dysfunction due to alteration of the alveolar epithelial barrier of epithelial cells in the respiratory system (Fujino et al., 2017) (Figure 1F). The increase in levels of prosurfactant protein-C, a marker specific to alveolar epithelial type II cells, was used to monitor the response of mesenchymal lung-like stem cells from adult human patients after addition of inhibitors from a kinase-focused compound library. Target deconvolution using kinase profiling identified a single hit, 2D08, an inhibitor of the receptor tyrosine kinase Axl as a potential target, which was confirmed by small interfering RNA loss-of-function analysis. Further investigations revealed that pharmacological or genetic reduction of Axl kinase activity led to fewer mesenchymal traits in the starting cell population, leading to increased cell adhesion. As Axl activation was observed in lung regions with a compromised epithelial barrier, this study revealed a novel opportunity for improving lung function by Axl inhibition in the treatment of idiopathic pulmonary fibrosis.

Stem Cell-Based Phenotypic Toxicity Screens

Adherent iPSC-based PTS are ideally suited to form the basis for more sensitive and physiologically relevant toxicity screens for human tissues. A major cause for phase I clinical trial failure of drug candidates is unanticipated toxicity, such as cardiotoxicity, hepatotoxicity, and neurotoxicity, with up to one-third of drug failures due to cardiotoxicity alone (Csöbönyeiová et al., 2016). Thus, there is a need for improved, clinically predictive assays. Leveraging iPSC-derived cells to develop cell-based *in vitro* assays for predictive safety assessment offers the potential to deliver higher-throughput, physiologically relevant cell-based assays to interrogate a range of key tissue-specific toxicities earlier and at reduced cost to industry-standard preclinical animal models.

Taking a step toward more predictive cardiotoxicity assays, an HTS protocol assessed the effects of 21 FDA-approved anticancer tyrosine kinase inhibitors (TKIs), known to possess cardiovascular side effects, on viability, contractility, electrophysiology, calcium handling, and signaling of hiPSC-derived cardiomyocytes, endothelial cells, and cardiac fibroblasts, generated from 11 healthy individuals and 2 patients (Sharma et al., 2017). Vascular endothelial growth factor receptor 2/platelet-derived growth factor receptor-inhibiting TKIs caused a compensatory increase in cardioprotective insulin and insulin-like growth factor (IGF) signaling and cardiotoxicity could be diminished via upregulation of cardioprotective signaling with exogenous insulin or IGF1.

More complex toxicity models allow modeling of multiorgan interactions. Since induction of cardiotoxicity or hepatotoxicity often occurs by way of a cascade of mechanisms in different tissues, simultaneous detection of different phenotypes through multimodal combination of high-content screens in different tissues is required to ensure that tissue- and pathway-specific effects of compounds can be captured. Using hiPSC-derived cardiomyocytes and hepatocytes, various high-content and molecular assay combinations provided simultaneous insight into cell viability, mitochondrial integrity, and formation of reactive oxygen species (Grimm et al., 2015). For cardiomyocytes, calcium flux measurements to test effects on beat frequency were combined with competitive ELISA to determine intracellular cyclic AMP (cAMP) levels as indicator of G protein-coupled receptor activation and to assess induction of chronotropic effects. For hepatocytes, high-content imaging was used to assess cytoskeletal integrity and lipid accumulation as an indicator of hepatocellular steatosis. Another study with hiPSC-derived hepatocytes evaluated various assays and phenotypic markers, including cell viability, nuclear shape, average and integrated cell area, mitochondrial membrane potential, phospholipid accumulation, cytoskeleton integrity, and apoptosis, for assessing multiparametric readouts of general

Figure 1. Graphical Representation of the Examples Mentioned in the "Stem Cells Open up New Opportunities for Disease Modeling and Toxicity Screening" Section

Abbreviations: AP-1, FOS/JUN activator protein-1; CDKAL1, CDK5 regulatory subunit associated protein 1 like 1; Fmr1, fragile X mental retardation 1; HCS, high-content screening; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; LOPAC, Library of Pharmacologically Active Compounds; ProSP-C, prosurfactant protein-C; TALEN, transcription activator-like effector nucleases.

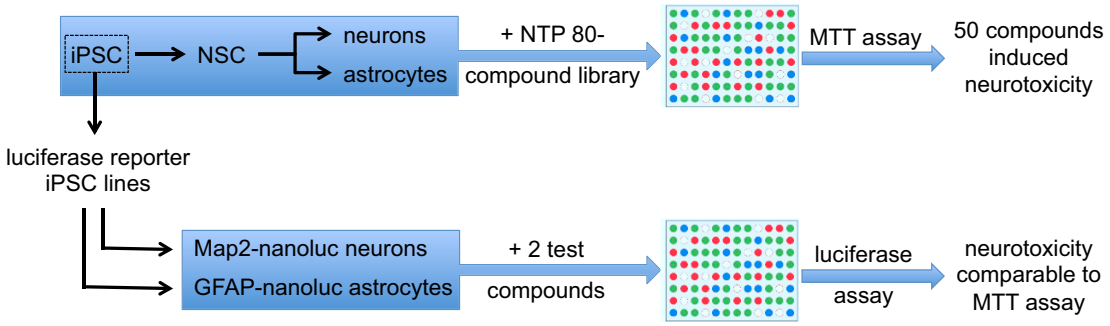

Figure 2. Schematic Outline of Neurotoxicity Study by Pei et al.
The NTP 80-compound library was screened with isogenic cells at four neural differentiation stages (iPSCs, neural stem cells [NSCs], neurons, astrocytes) using the MTT assay. Lineage-specific luciferase reporter iPSC lines were used to simplify the neurotoxicity readout (Pei et al., 2016).

and mechanism-specific hepatotoxicity (Sirenko et al., 2014). An automated high-content screening (HCS) combined general toxicity assessment by the multi-dye assay with a mechanism-specific assay using the mitochondrial JC-10 dye to screen the 240-compound Screen-Well Hepatotoxicity Library. These multiparametric HCS for quantitative *in vitro* profiling of cardiotoxicity and hepatotoxicity provide both descriptive and mechanistic toxicity data in a time- and resource-saving format.

In a comparative cytotoxicity assay, the NTP 80-compound library including drugs, pesticides, and environmental compounds, 37 of which possess reported developmental neurotoxicity and/or neurotoxicity, was tested on isogenic cells at four stages of neural differentiation (hiPSCs, neural stem cells, neurons, and astrocytes) (Figure 2). The screen used the MTT assay (Pei et al., 2016) and identified 50 compounds that significantly induced cell-type-specific neurotoxicity. The neurotoxicity assay could be simplified by measuring luciferase activity in lineage-specific luciferase reporter iPSC lines. To distinguish between chemically induced specific effects on neurite outgrowth and nonspecific cytotoxicity, the same compound library was investigated in a high-throughput, high-content screen with hiPSC-derived neurons via quantification of total neurite outgrowth, branches, and processes as well as cell viability (Ryan et al., 2016). Sixteen out of 38 active compounds showed selective inhibition of neurite outgrowth, and only for 6 of these 16 compounds could the selectivity be reproduced in a repeat experiment with larger sample size. However, neurite outgrowth is only one of multiple mechanisms of developmental neurotoxicity and general neurotoxicity, necessitating other assays to cover diverse toxic effects on the nervous system.

Challenges of Stem Cell-Based PTS in 2D Cultures

Despite all enthusiasm, however, challenges remain. Stem cells in culture tend to form heterogeneous populations during differentiation, an outcome that can affect statistical metrics of HTS campaigns (Kaufmann et al., 2015). Therefore, differentiation protocols need further optimization to improve efficiency, reproducibility, and eliminate heterogeneity in terms of cell-type-specific differentiation, biomarker expression, and features that determine HTS compatibility (Calder et al., 2015; Hoing et al., 2012). hiPSCs and derived cell lines exhibit variable properties depending on the cell type of origin (Luu et al., 2018). This variability contributes significantly to the observed lack of reproducibility of results among studies. Furthermore, accuracy and completeness of stem cell differentiation need to be determined and carefully controlled. Typically, phenotypes are defined by biomarker patterns (protein or mRNA), the definition of which can be inconsistent between groups of researchers, leading to accepted patterns changing over time. Rigorous use of iPSC technologies will require tighter and more broadly accepted definitions of iPSC-derived phenotypes used in screening campaigns. Based on these tighter definitions, selection protocols can be refined to select specific cell subpopulations from cell mixtures with varying degrees of maturity or functionally distinct cell-type-specific properties. These measures will help address heterogeneity issues and improve accuracy as well as biological relevance of screening results (Charwat et al., 2015; Steinbeck et al., 2016; Xu et al., 2016). Consequently, high-throughput fluorescence-activated cell sorting and mass spectrometry analysis are increasingly used in HTS workflows for extraction of quantitative information (Ding et al., 2018; Edwards and Sklar, 2015; Zhou et al., 2016).

Stem cell differentiation protocols often require several days to weeks, and even months, for the generation of desired cell types. Long protocol durations drive up cost and complexity of stem cell-based screening campaigns. Although much progress has been made (Heilker et al., 2014; Qi et al., 2017), differentiation protocols need further improvement to reduce the overall duration of differentiation from months to days. One option is predifferentiating stem cells into highly specialized precursor cells, such as neural precursors, to further reduce the time to generate the desired somatic cells as well as the cost (Reinhardt et al., 2013). Another advantage of tissue-restricted multipotent precursor cells is that they can give rise to only a more limited subset of cell types, further reducing potential heterogeneity among differentiated cells.

In summary, iPSC technologies may solve key issues of PTS: they provide patient-derived material, allow generation of scarce or postmitotic cells, facilitate genetic engineering, and hence allow the use of isogenic cell lines for screening. However, they still need optimization to reduce heterogeneity, protocol cost, and duration, and more stringently defined cell-type-specific biomarkers as broadly accepted consensus among researchers in industry and academia. Lastly, iPSCs can rarely be differentiated into cells that resemble an adult phenotype and thus, maturation protocols need to be developed that address this issue. Currently employed HTS models cannot yet

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mimic the complexities of a multiorgan, 3D, interconnected organism, but efforts are starting to approach recreating heterogeneous, multicellular biologies *in vitro*.

Advanced Tissue Culture Methods Allow More Complexity and Greater Control in Phenotypic Disease Models

Advantages and Challenges of Next-Generation Screening Approaches

The ultimate clinical success of PTS hinges on the ability of *in vitro* systems to replicate relevant physiological processes contributing to health and disease *in vivo*. The multifactorial nature of many diseases has so far prevented addressing them in straightforward 2D PTS. Tackling novel diseases may require integrative solutions for modeling cells, tissues, organs, and pathogens *in vitro* that act in concert to bring about disease states. Basic research has been developing and refining techniques such as microfluidics, 3D/organoid cell culture, and organs-on-a-chip, which can mimic ever more complex biological processes *in vitro*. These next-generation screening approaches can combine several organotypic cell types in a more tissue-like configuration to emulate physiologically interdependent cell populations as relevant functional screening units in 3D and over time. Adapting these technologies at their current proof-of-principle state for industrial high-throughput workflows represents a new generation of scalable biological screening approaches for narrowing the gap between *in vitro* and *in vivo* scenarios (Takebe et al., 2017b).

Despite their promise, however, these technologies have so far not found widespread adoption in large pharmacological screening campaigns, and the relevance of 3D models remains yet to be rigorously ascertained. More complex disease models require more sophisticated and multifaceted cell culture and readout systems, each with its own set of issues, compounding difficulties. Thus, the principal challenge lies in the functional convergence of disparate fields in settings that foster the interaction of experts from each discipline, while providing funding to push through initial challenges. Once achieved, this integrative approach may allow the industrialization of complex cell-based models to power the next generation of drug discovery.

3D Tissue Culture and Organoid Technology

Bioactive small molecules are often identified in biological formats that are cultured as monolayers on 2D surfaces. These setups facilitate downstream analysis but show little resemblance to *in vivo* conditions. Over the past few years, easy access to hiPSCs has enabled the assembly of *de novo* prototissues in the form of organoids (Clevers, 2016; Eisenstein, 2018; Pasca, 2018), and protocols have been developed for growing 3D aggregates that bear major hallmarks of many tissues (Huch et al., 2017; Lancaster and Knoblich, 2014; Luo et al., 2016; Qian et al., 2016). As the cellular niche governs cell behavior (Murrow et al., 2017), it is not surprising that many features of developmental biology manifest only in 3D cultures (Centeno et al., 2018; Gibbons, 2017; Lancaster et al., 2013; Passier et al., 2016). Thus, hiPSC-based 3D organoid cultures may provide a more biomimetic 3D tissue-like context for cells in drug discovery campaigns (Bershteyn and Kriegstein, 2013; Mills et al., 2017), reviewed in (Dutta et al., 2017; Fang and Eglen, 2017; Ho et al., 2018).

First reports toward using organoid technologies for the development of HTS approaches in 3D tissue culture are starting to show both the feasibility of this approach as well as highlight the challenges before this technology can mature (Fatehullah et al., 2016). Screening campaigns for cancer therapeutics are already in use for testing the efficacy of various chemotherapeutics against numerous tumor tissues (Drost and Clevers, 2018), as tumor size can be easily measured with current assay readout technologies. In addition, more sophisticated readouts are starting to emerge (Bian et al., 2018; Czerniecki et al., 2018; Vergara et al., 2017; Zhang et al., 2017). Small-scale toxicity studies in 3D promise more physiologically relevant results. For example, a cardiac microphysiological system was developed that allows culture of viable and functional hiPSC-derived cardiomyocytes in an aligned 3D μ-tissue over multiple weeks and offers multiple modes of analysis (e.g., biological, electrophysiological, and physiological) (Mathur et al., 2015). Better consistency of half maximal effective concentration/half maximal inhibitory concentration values of four model drugs with data obtained from tissue-scale compared with cellular-scale studies suggests a significant improvement to predict drug efficacy and toxicity *in vitro*.

However, small-molecule screening with more complex 3D cell assemblies has not yet reached its full potential, as it adds another layer of complexity on top of the challenges of hiPSC-based screening (Arlotta, 2018; Horman et al., 2015; Moffat et al., 2014). Industrial implementation requires extreme reproducibility and efficient, automated workflows to leverage the economy of scale. Formation of organoids relies to a significant extent on spontaneous self-organization of hiPSCs into complex tissues (Di Lullo and Kriegstein, 2017). This process occurs inherently at random and produces an even higher degree of heterogeneity than most advanced protocols in 2D culture. Also, most organoid workflows require discarding a significant portion of the resulting aggregates because they did not spontaneously form structures of interest. Most organoid work to date shows data that have been generated from samples that have undergone a rigorous manual cascade of selections. If organoid approaches are to be adopted in screening workflows, they need to become robust enough to self-organize into well-defined, homogeneous, reproducible 3D tissues, or screening in quantity will not be possible. In addition, many standard HTS readout strategies may not be suitable for use in 3D. For example, it is difficult to obtain optical readouts in large, often opaque cellular aggregates, as conventional microscopy cannot fully penetrate large-scale aggregates. Currently, only easily accessible parameters such as aggregate diameter have been measured in HTS formats, with few exceptions (Czerniecki et al., 2018; Mills et al., 2017; Takebe et al., 2017a; Vergara et al., 2017). Organoids are not yet suitable for the fast data acquisition of high-content live-cell imaging procedures, and may never be. Therefore, readouts either are limited to outer layers of aggregates or require significant additional sample processing. Some of these issues are addressed via tissue-clearing approaches that reduce light absorbance and scattering in tissues, allowing for the acquisition of optical readouts at greater depths (reviewed in Richardson and Lichtman, 2017). Finally, the presence of a large number of

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cells in aggregates and the secretion of extracellular matrix components impair the diffusion of nutrients, oxygen, and potential pharmacological drug candidates to the core of the aggregate. Cells in 3D culture may therefore experience distinct mechanical and physiological conditions depending on their location within a cellular aggregate. Part of this issue may be mitigated by the introduction of blood vessels into various organoid models, the proof-of-principle of which was recently demonstrated (Wimmer et al., 2019).

Although 3D cultures conceptually promise to be more physiologically relevant than 2D cultures, solid experimental proof is scarce, with one study showing a negligible difference between hepatic 2D and 3D cultures (Sirenko et al., 2016). Reasons for a lack of data on the relevance of 3D studies may include the comparatively low number of 3D studies with quantitative/screening results. Before becoming more established, 3D culture needs to solve its inherent tendency toward highly heterogeneous samples, to offer novel HTS-compatible fast readout technologies, and careful comparison of relevant clinical biomarkers when compared with existing 2D approaches. Ultimately, the relevance of 3D screening technologies can only be verified once 3D-generated results can be vetted in the clinic. However, the potential is enormous. Building organ-like human tissues for HTS campaigns could help bridge the gap between human *in vitro* and *in vivo* physiology and help cut escalating costs of current drug development.

Microfluidic Systems

Although organoids add tissue-level complexity over the traditional 2D monocultures used in screening, microfluidics offer superior environmental control. Many parameters, such as fluid flow rates, local mechanical and electrical signals, oxygen and nutrient levels, and the pattern of enclosed cells can be controlled accurately and varied independently. The possibility to add (pulsatile) flow can enhance the differentiation, long-term survival, and maturity of many cell types (Huh et al., 2010). Microfluidic experiments are often conducted in small, transparent architectures, which help reduce reagent volumes to the microliter- or picoliter range per reaction, while being able to be read out in real time (Esch et al., 2015; Regnault et al., 2018). This permits the analysis of disease states that cannot be addressed in conventional screening setups, for example the dynamic variation of oxygen tension to invoke vaso-occlusion in sickle-cell disease (Wood et al., 2012).

Many drug metabolism phenomena only emerge after drugs interact with several tissues. Organs-on-a-chip can combine different cell types and configurations under continuous-flow conditions, cultured in reproducible biological architectures in micrometer-sized chambers with a defined size and shape (Bhatia and Ingber, 2014; Picollet-D'hahan et al., 2017; Rothbauer et al., 2018; Skardal et al., 2017; Takebe et al., 2017b) (see Figures 3 and 4). Recent proof-of-principle studies even combine several organs-on-a-chip to form a "body-on-a-chip," including a pumpless 14-chamber multiorgan system representing 13 tissues/organs (Miller and Shuler, 2016). Although these technologies need to mature further to quantitatively emulate complex body chemistry interactions, these approaches represent first and important steps that can provide new approaches for studying the molecular mechanisms of action and the toxicity of drugs in a physiologically relevant yet standardized context (Bhatia and Ingber, 2014; Ronaldson-Bouchard and Vunjak-Novakovic, 2018).

While cumbersome to design, implement, and troubleshoot, microfluidics are in principle compatible with HTS workflows, and may be an option to realize complex biological systems in screens by driving miniaturization, parallelization of reactions, and rapid analysis (Jackson and Lu, 2016). The need and ability to screen more complex biological systems at industry scale is just emerging, and industry-grade small-molecule screens with organs-on-a-chip have not yet been reported, in large part due to several significant challenges that still need to be overcome (for review, see Starokozhko and Groothuis, 2017). However, just as microfluidics have started to revolutionize standard lab techniques such as RT-PCR and the -omics techniques, the ability to mass-produce microfluidic devices combined with the economy of scale will make microfluidic devices more attractive as larger-scale screening systems (Mullard, 2018). Currently, the simplicity and optimization of large-scale screening workflows are custom-tailored to 384- and 1,536-well designs, which are more suited to highly optimized 2D TBS and PTS workflows. Further development of microfluidic devices offers a path forward for assay designs that accommodate more complex biological setups and can help combine emerging techniques such as 3D culture, 3D bioprinting (Yi et al., 2017), or even device-less microfluidic designs (Walsh et al., 2017) to create and analyze spatially defined yet heterogeneous tissues with the rigor and control required for large-scale screening setup.

Phenotypic Screens in Advanced Culture Systems

Despite the challenges that remain, several studies have already outlined the potential of PTS in modeling complex phenotypes that cannot be adequately demonstrated in other TBS-based or 2D culture models, but can be addressed by combining 3D culture, microfluidics, and can even model interactions of tissues with pathogens.

HCS by immunostaining of Zika virus (ZIKV)-infected hiPSC-derived neural progenitor cells during early human neural tissue development (Zhou et al., 2017) identified that the FDA-approved small-molecule drugs, hippeastrine hydrobromide and amodiaquine dihydrochloride dehydrate, reduced ZIKV E protein levels. Further investigations showed that hippeastrine hydrobromide rescued several defects associated with ZIKV infection in human fetal-like forebrain organoids and in the adult mouse brain.

A new combinatorial screening approach using iPSC-derived cardiomyocytes cultured in high-density microbioreactor arrays discovered small-molecule modulators of cardiomyocyte proliferation (Titmarsh et al., 2016). Using 8,100 individual chambers, the study investigated four well-established small-molecule modulators of Wnt, Hedgehog, IGF, and fibroblast growth factor signaling pathways in a combinatorial fashion. It revealed that CHIR99021 (a GSK3β inhibitor) is the most potent inducer of human cardiomyocyte proliferation. These results demonstrate the power of automation, miniaturization, and parallelization technologies for future hiPSC-based PTS approaches.

First proof-of-concept studies combining microfluidics and human neural progenitor-based 3D cultures promise a rapid

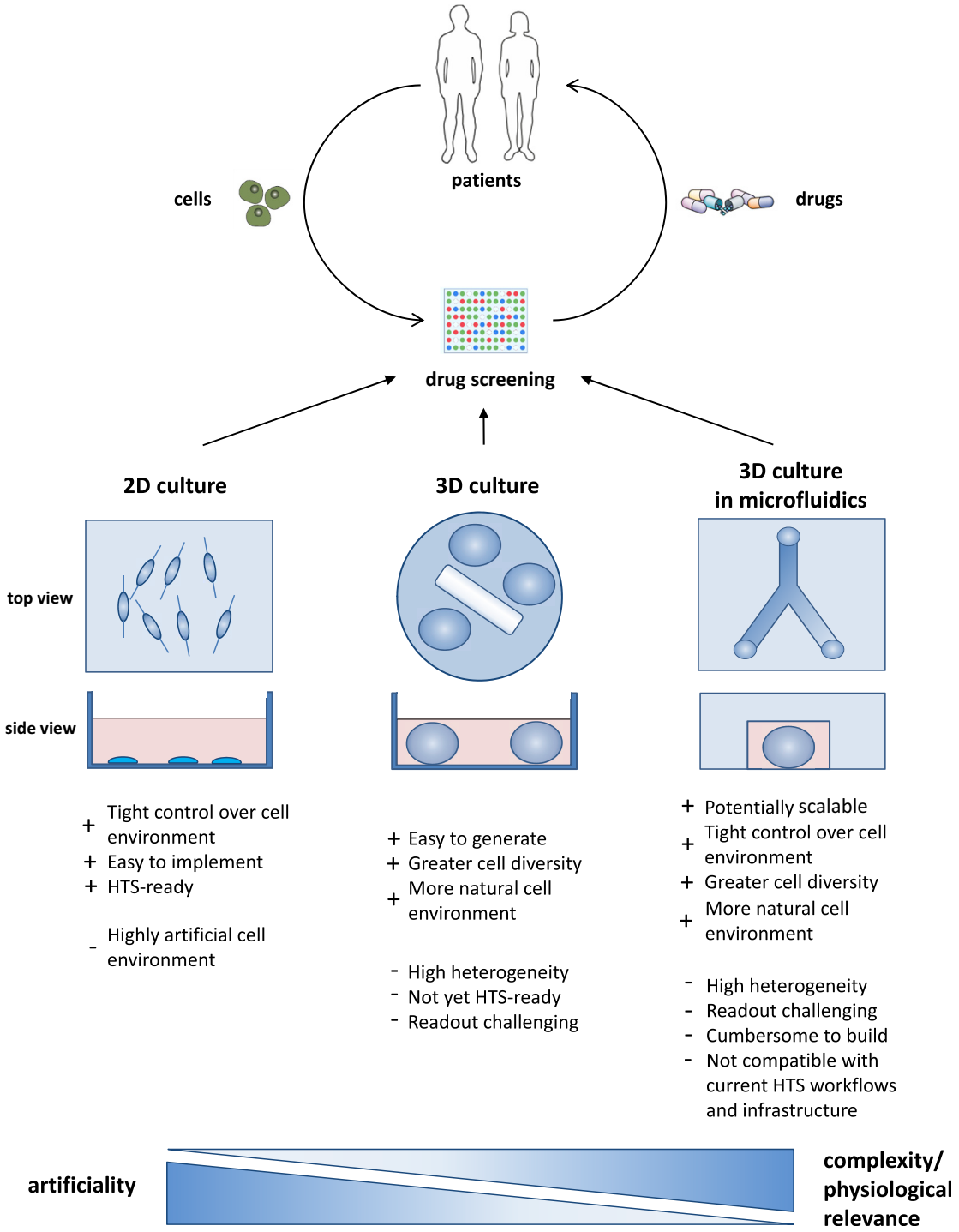

Figure 3. Schematic Overview of Current and Emerging Phenotypic Screening Strategies and their Main Advantages and Challenges

assessment of neuronal cytotoxicity of potential drugs at early stages of drug discovery (Nierode et al., 2016). A chip-based microarray allowed for the combinatorial optimization of culture conditions in 3D at the nanoliter scale by monitoring cell-specific marker expression using immunofluorescence. Upon screening of 24 compounds for cytotoxicity and proliferative effects in undifferentiated versus differentiating progenitors five compounds with distinct activities depending on the differentiation status were identified. Such platforms could be scaled up and used for larger-scale neurotoxicity screening.

Next-Generation Phenotypic Analyses

With recent progress and a wider application of phenotypic approaches, phenotypic data are acquired at a rate higher than can

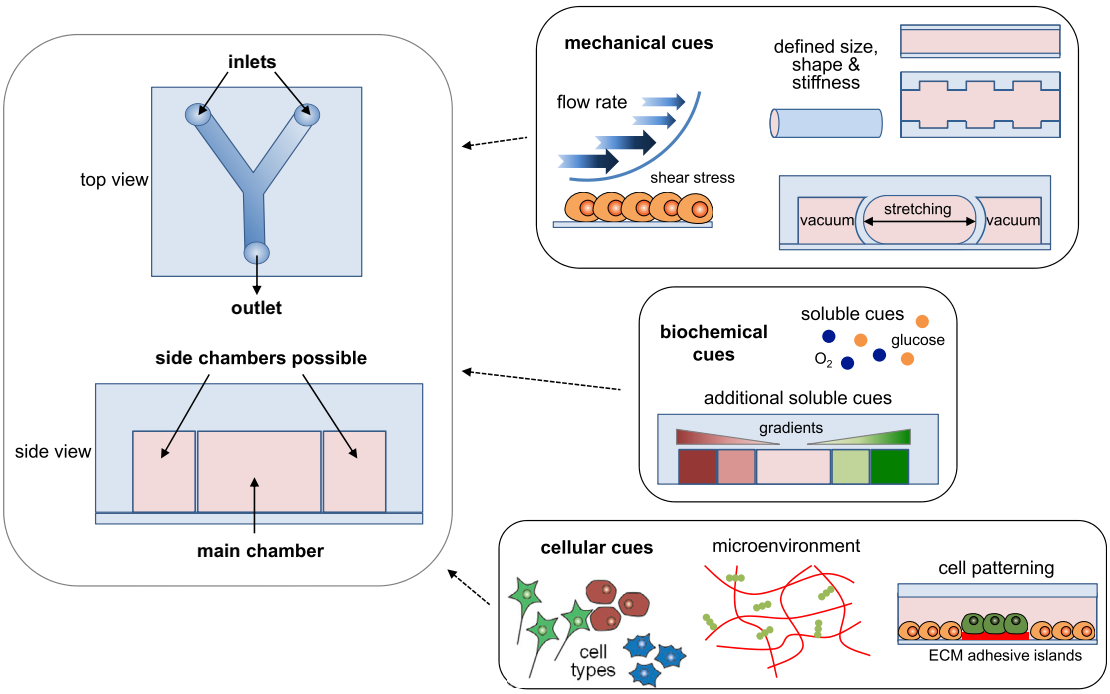

Figure 4. Schematic Overview of Parameters that Can Be Addressed in Microfluidic Setups

be processed (Cech and Steitz, 2014; Gustafsdottir et al., 2013; Pegoraro and Misteli, 2017; Reisen et al., 2015). With automation, genomic, and imaging capabilities soaring, data storage, annotation, classification, and analysis form new bottlenecks in the generation of biological insight. Handling of massive amounts of multidimensional data to unravel complex dependencies creates a new frontier in screening. Machine learning algorithms are amenable to integrating complex interrelated data and extracting relevant parameters from datasets that are too large to analyze by other means, or in cases where relevant parameters are yet unknown. Rapid advances in machine learning algorithms and, in particular, deep learning are expected to advance phenotypic approaches that deconvolve complex systems after recording large swaths of raw data without previous knowledge of a target (reviewed in Smith et al., 2018). The resulting datasets can form the starting point for systems biology strategies that tackle an increasing array of parameters and detect interdependencies (Lee and Berg, 2013; Schirle and Jenkins, 2016). The latest-generation screening approaches allow not only target-agnostic but also phenotype-agnostic strategies. After collecting a large amount of in-depth data either by -omics/-sequencing-based techniques, or by high-content imaging, cellular responses may be clustered into phenotypes after the experiment, and only assembled by machine learning approaches into distinct phenotypes during the analysis stage. This strategy, coupled with spiking compound libraries with substances of known and specific activity, allows for clustering of cellular behaviors into distinct groups, even without knowing the molecular pathways involved (cell painting) (Bray et al., 2016). With a sufficient number of curated datasets, it will become feasible to predict protein/RNA/compound-based phenotypes (*in silico* modeling) (Digles et al., 2016). Particularly the possibility to acquire dynamic/longitudinal datasets in microfluidic formats as part of HTS will require novel data handling and analysis architectures.

Biologists, statisticians, and software experts need to join ranks to tackle this new challenge. Tracking datasets for compound libraries across research institutions and correlating phenotypes acquired by different individuals under different conditions could help turning the vision of *in silico* modeling into a reality by distilling core multidimensional phenotypic changes out of a heterogeneous biological sample space. While we are not aware of such advances in the stem cell field, innovative approaches including patient-specific, high-content immunofluorescence-driven approaches in complex co-culture formats with a cross-patient longitudinal integration of datasets exists in clinical cancer research, in a technique called pharmacoscopy (Snijder et al., 2017). With the growing availability of stem cell banks coupled with in-depth patient records, applying such comprehensive approaches applied to stem cell screens and compound libraries will drive additional insight into health, developmental, and disease aspects across cell lines and patients, thus further broadening the insight and predictability of phenotypic changes in stem cell-based screens.

Novel Approaches to Tissue Maintenance and Repair: Phenotypic Screens to Direct Cell Fate and Generate Tissue-Resident Stem Cells

Frequently PTS approaches aim at restoring cell function or survival of terminally differentiated cells that are *already present* in their tissues and do not function properly because of age, injury, or disease. The co-emergence of the ability to direct cell fate changes with the ability to create and screen tissues in HTS contexts opens up an entirely new purpose for PTS: identification of

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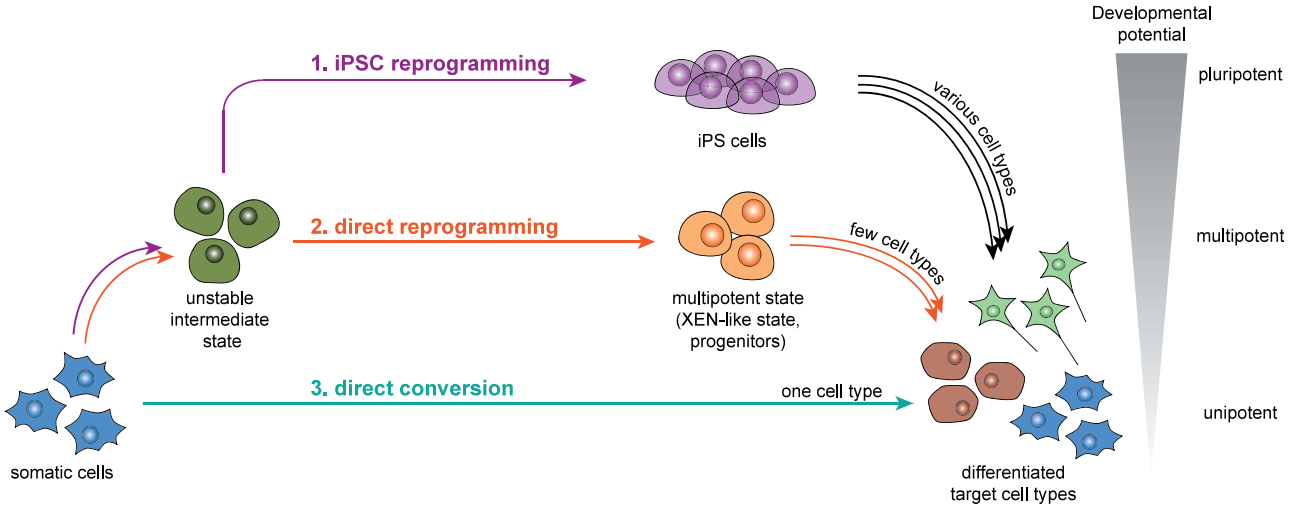

Figure 5. Illustration of the Three Reprogramming Concepts: iPSC Reprogramming, Direct Reprogramming, and Direct Conversion
During iPSC reprogramming (1), cells are reset completely back to the pluripotent state via an unstable intermediate state, and then differentiated into various cell types. During direct reprogramming (2), cells follow the route of iPSC reprogramming up to the unstable intermediate state, but subsequently pass onto a multipotent state rather than heading all the way back to the pluripotent state. Therefore, direct reprogramming gives rise to only a few specific cell types. During direct conversion (3), cells undergo transdifferentiation and do not pass through a pluripotent or multipotent state, but stay unipotent, and hence give rise to only one specific cell type.

compound cocktails to drive cell fate conversion in tissues. New therapeutic strategies could replace damaged or lost cells by converting tissue-resident somatic cells into their respective tissue-resident stem cells, which, in turn, regenerate missing somatic cells locally. This autotherapy approach (Davies et al., 2015; reviewed in Lumelsky et al., 2018) minimizes potential complications from allogeneic immune-incompatibility as well as local tissue damage from cell delivery.

Unfortunately, traditional transcription factor-based lineage conversion requires genetic manipulation of host tissues (Gascon et al., 2017; Graf and Enver, 2009; Tsunemoto et al., 2018), which is technically and ethically challenging. PTS is a powerful tool to study and evolve cell fate conversion approaches focusing on small molecules. Insights gained can help ameliorate duration and efficiency of cell fate conversions to improve yield and quality of desired cell types, e.g., for use in disease modeling or autologous cell therapy, as well as to discover novel direct cell-type conversion protocols for regenerative medicine. Current phenotypic screens have great potential for identifying small molecules that can replace transcription factors and may be more suitable as therapeutic agents (also summarized in Table S1).

Before clinical translation of cell conversion approaches can become clinical reality, several technical and regulatory advances need to be achieved. Technical limitations include low conversion efficiency, heterogeneity of resulting cell populations, and only partial functionality of converted cells. Safety considerations are paramount with reprogramming techniques with clinical uses as goal, as for example in tissue-based regenerative strategies. The death of a patient in a gene therapy clinical trial and therapy-induced cancers (Wilson, 2009) revealed an insufficient understanding of clinical safety and suitability of a radically new therapy approach with unfortunate outcome that hobbled further progress for the following decade. It is vital that stem cell-based therapeutic approaches do not suffer the same fate. Therefore, rigorous safety testing and long-term studies in animal models are indispensable. All concerns about regulatory requirements need to be addressed on the way toward safe application of cell fate conversion approaches in the clinic (Kumar et al., 2017). Even with new tools at hand which direct conversion of somatic cells, these considerations will likely impose significant hurdles to be overcome.

Here, we address PTS approaches to identify small molecules that are relevant for two types of cell fate conversion: (1) direct reprogramming (somatic to multipotent stem or precursor cells) and (2) direct conversion (somatic to somatic cells) (see Figure 5). In direct reprogramming, cells do not retrace the path to pluripotency, but only to a multipotent, intermediate state, giving rise to a more restricted number of lineages; and, in direct conversion, cells do not pass through a pluripotent or multipotent state but stay unipotent and go through a transdifferentiation process to produce only one desired cell type (Graf and Enver, 2009). We do not discuss PTS to direct cell fate toward pluripotency, because pluripotent cells bear a high risk of tumorigenicity, and are thus less suitable for therapeutic approaches.

PTS for Direct Reprogramming Factors—Somatic to Multipotent Precursors

Direct reprogramming bypasses a potentially tumorigenic pluripotent state and leads directly to a multipotent stem cell state. This reduces time and cost spent on reprogramming and differentiation and lowers the risk of tumor formation by residual pluripotent cells in clinical applications. These advantages will be beneficial to improve cell supply for disease modeling as well as regenerative medicine approaches.

Combinatorial PTS has identified a set of nine target-annotated small molecules that were capable of efficiently converting mouse fibroblasts into tripotent neural stem-like cells (Zhang et al., 2016a). In another study, human fibroblasts were converted into cardiomyocyte-like cells by the addition

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of a nine-compound cocktail (Cao et al., 2016). Notably, when transplanted into infarcted mouse hearts, these cells partially remuscularized the infarcted area. Four small molecules were shown to be sufficient for reprogramming mouse fibroblasts directly into highly expandable cardiovascular progenitor cells that robustly differentiated into cardiovascular cells *in vitro* as well as *in vivo* in the diseased heart, improving cardiac function for up to 12 weeks after transplantation (Zhang et al., 2016b).

An emerging concept that bypasses pluripotency altogether is the generation of an extraembryonic endoderm XEN-like state. Cells in the XEN-like state are highly expandable and shown to be present during the chemical reprogramming of mouse somatic cells into pluripotent stem cells (Zhao et al., 2015). Instead of inducing a pluripotent state (and then generating a somatic state after cellular differentiation), a method was developed for directly obtaining functional mouse neurons and hepatocytes via this chemically induced XEN-like state (Li et al., 2017).

PTS for Direct Conversion Factors—Somatic to Somatic

Direct conversion represents a special subcategory of cell fate switching. Directly converted cells retain epigenetic information and aging signatures of the donor cell type, whereas iPSC-derived cell types exhibit features of rejuvenation, as they are epigenetically reset during iPSC reprogramming (Mertens et al., 2015). Thus, direct conversion technologies open up the investigation of age-related disorders to the realm of HTS. In addition, opposed to direct reprogramming, direct conversion is faster, has improved efficiency, avoids pluripotency-associated risks of tumorigenesis, and promises potential use in autologous cell therapies and autotherapy. Further research and safety testing are needed to investigate which approaches will be most promising for clinical applications.

Transcription factor-mediated transdifferentiation of mouse cardiac fibroblasts into cardiomyocyte-like cells can be greatly enhanced by the addition of SB431542 (transforming growth factor β [TGF-β] inhibitor) and XAV939 (Wnt inhibitor). The addition increases conversion efficiency by 8-fold and shortens the conversion process from 6 to 8 weeks to only 1 week (Mohamed et al., 2017). Notably, this combinatorial treatment significantly improves transdifferentiation *in vivo* and results in enhanced cardiac function after myocardial infarction. Also, the efficiency and speed of human cardiac reprogramming was enhanced in the presence of TGF-β and Wnt inhibitors, which reduced the number of required transcription factors from seven to four (Gata4, Mef2c, Tbx5, and myocardin)—compared with three (Gata4, Mef2c, and Tbx5) in mice. In addition, efficiency of direct neural conversion of human fibroblasts was reportedly increased by more than 6-fold by addition of a six-compound mixture comprising kenpaullone (Gsk3β inhibitor), prostaglandin E2 (cAMP/PKA modulator), forskolin (adenylyl cyclase activator), BML210 (HDAC inhibitor), aminoresveratolsulfat (SIRT1 activator), and PP2 (Src kinase inhibitor) (Pfisterer et al., 2016).

Furthermore, a small-molecule approach led to the efficient conversion of human fibroblasts into functional Schwann cells under chemically defined conditions (Thoma et al., 2014). PTS for molecules that drive *in vivo* conversion of mouse myoblasts into brown-like adipocytes yielded the retinoid X receptor (RXR) agonist bexarotene, which enhanced mass as well as function of brown adipose tissue in mice, further establishing selective RXR activation as a potential therapeutic approach for the manipulation of brown/beige fat tissue metabolism *in vivo* (Nie et al., 2017).

Transdifferentiation also plays a critical role in cardiac fibrosis, a process whereby large amounts of connective tissue accumulate upon physical damage and/or inflammatory insult leading to organ malfunction and death. A key aspect of fibrosis is the accumulation of extracellular matrix-secreting activated myofibroblasts. Search for ways to inhibit this process led to the development of a live-cell imaging assay for HTS with primary rat hepatic stellate cells and to the subsequent discovery of antifibrotic agent CBR-096-4, which exhibited excellent activity in rodent and human models of lung, liver, as well as skin fibrosis (Bollong et al., 2017).

Challenges to Overcome for Adaptation of Next-Generation Screening Approaches

With the current state of screening able to coopt and integrate a number of novel technologies to allow modeling more complex diseases, many challenges remain that we summarize here:

- **Biology**
  - To foster biological reproducibility among batches and cell lines and compatibility of results across different laboratories, the outcomes of cell conversion protocols (differentiation and dedifferentiation) need to be better defined across different laboratories and fields. For example, one needs to have a global, marker-independent metric such as an RNA sequencing-based score that determines how much a given cell resembles a hepatocyte. Each community could define several key phenotypes that would help group and compare results.
  - Firm global cell-type definitions will also help to unify and to understand the degree of cellular maturation of iPSC-derived cell types across different laboratories. In turn, this could help recognize and develop novel ways to advance aging in screening environments, or, in the case of direct conversion, to score individual age characteristics of cells slated for screening. Without a common metric, findings of age-related phenotypes remain largely incomparable between studies.

- **Analysis**
  - Increasing opportunities in data mining require streamlined software and hardware solutions that can handle large-scale multifactorial datasets during acquisition and analysis. Connections between large and diverse types of data can only be found if they are accessible to a single analysis platform. New endeavors need to plan for hardware and software that can store and serve disparate types of data all relating to individual samples and conditions, which can then be analyzed by a unified neuronal network or other central platform with a holistic data analysis capability.
  - Machine learning and artificial intelligence approaches need to be integrated into existing analysis pipelines. Multifactorial datasets increasingly exceed the capacity

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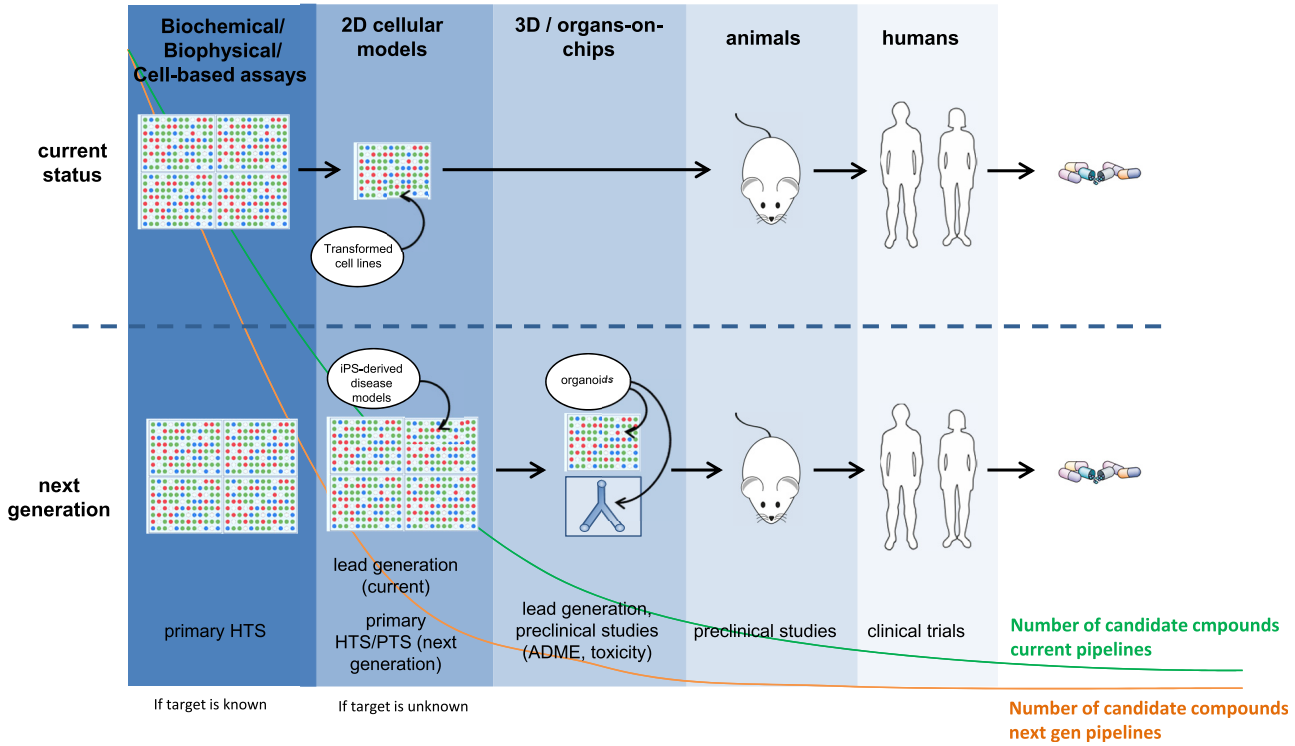

Figure 6. Comparison of Conventional and Next-Generation Drug Discovery and Development Pipelines
As next-generation strategies mature, preclinical animal experiments can be phased out.

of human-based approaches to extract relevant connections. Unsupervised methods could form a first frontier of data sorting, predigesting complex datasets to be then screened by humans or human-supervised algorithms.

- Machine learning, especially deep neural networks, need to be developed to deal with the increased demands of image/data segmentation originating in next-generation 3D multitissue screens. These nonlinear methods could allow access to morphologically parse image sets that so far are not digestible by conventional image analysis methods.

- Automation/Engineering
  - Highly manual approaches such as organoid protocols need to be standardized, automated, and rendered robust enough to function in high-throughput settings. So far, protocols relying on self-organizing tissues do not meet the rigorous standards of reproducibility required for large-scale screening. Outcomes need to be measured on a standardized global metric, see argument above.
  - Microfluidics need to become robust enough to be parallelized in massive screening workflows. Issues such as expensive low-volume production, cumbersome assembly, and lack of standardization, need to be addressed in order to generate cost-effective microfluidic solutions based on the economy of scale.
  - Ultimately, engineering and automation must drop the costs for complex cellular models while minimizing any manual intervention. Cell seeding, maintenance, analysis, and disposal all need to be able to be handled in a system that can process thousands of samples in parallel in a standardized format. Microfluidics holds the promise to provide these characteristics but have not yet passed the proof-of-principle stage in academic environments.

- Overall
  - Next-generation screening technologies including 3D culture and microfluidic approaches will need to be validated in comparison with existing 2D approaches in the clinic to gain widespread acceptance.

Outlook

Over the past decade, hiPSC-based PTS technologies have matured and proven to integrate well with the HTS drug discovery workflows. More complex biological systems require the convergence of more sophisticated cell culture techniques (3D and microfluidics), automation, and analysis methods. For a number of proof-of-principle studies, this has already been achieved. However, transfer to industry scale systems still requires further development to achieve efficient scalability for testing large compound libraries in next-generation phenotypic formats in a robust and cost-efficient manner.

Given their complexity, we expect that next-generation screening approaches will complement rather than replace conventional TBS approaches for primary screens. But we envision that 3D phenotypic assay technologies, including organoids and organs-on-a-chip, or even bodies-on-a-chip (see Figure 6), will

play a prominent role in hit validation and profiling. More integrated and sophisticated models combining organoids, sensors, and microfluidics are already taking shape (Zhang et al., 2017).

PTS is on the rise again as a technology for identification of bioactive compounds. Different fields are coalescing, bringing together iPSC technology, organoids, microfluidics, and advanced analysis pipelines for big data projects. Collaborative efforts are therefore indispensable for achieving interdisciplinary solutions involving experts in chemical biology and medicinal chemistry (Kapoor et al., 2016; Plowright et al., 2017) for exploiting the full potential of small-molecule iPSC-based PTS especially in the pharmaceutical industry (see Figure 1; for examples, see Plowright et al., 2017; Haasen et al., 2017; Ursu et al., 2017). For each individual indication, the stem cell community needs to improve stem cell tissue culture to advance and expand the disease model portfolio and organoid technologies in close collaboration with clinicians and experts in human disease physiology to better approximate the *in vitro* with the *in vivo* models. This outcome cannot be achieved without engineers providing scalable, tunable, and cheaper automated workflow solutions and microfluidic devices that are suitable for large-scale screening campaigns. The chemical biology community needs to develop strategies for target deconvolution of large datasets from complex biological samples involving advanced systems biology strategies as well as machine learning algorithms. In addition, these groups need to interact with experts in disease biology for individual indications. These challenges will be efficiently solved in interdisciplinary academic-corporate partnerships and translational research centers in an open innovation fashion. The synergies created will facilitate the development of more specific and efficient methods for assessing the effects of diseases and drugs *in vitro* and open up new avenues for treating many afflictions that are beyond our reach today.

SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

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