OXFORD

*Human Molecular Genetics*, 2018, Vol. 27, No. R2 R89–R98

**doi: 10.1093/hmg/ddy186**
Advance Access Publication Date: 16 May 2018
Invited Review

INVITED REVIEW

# Drug screening for human genetic diseases using iPSC models

Matthew S. Elitt, Lilianne Barbar and Paul J. Tesar*

Department of Genetics and Genome Sciences, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

*To whom correspondence should be addressed. Tel: +1 216 368 6225; Fax: +1 216 368 3432; Email: paul.tesar@case.edu

## Abstract

Induced pluripotent stem cells (iPSCs) enable the generation of previously unattainable, scalable quantities of disease-relevant tissues from patients suffering from essentially any genetic disorder. This cellular material has proven instrumental for drug screening efforts on these disorders, and has facilitated the identification of novel therapeutics for patients. Here we will review the foundational technologies that have enabled iPSCs, the power and limitations of iPSC-based compound screens along with screening guidelines, and recent examples of screening efforts. Additionally we will provide a brief commentary on the future scientific roadmap using pluripotent- and 3D organoid-based, combinatorial approaches.

## Introduction

A limited number of genetic disorders have true disease-modifying therapies. On the surface, this issue is counterintuitive as the mutated gene itself provides a node of focus for therapeutic discovery design (1). Unfortunately the costs and challenges in modeling their pathological processes coupled with their rarity precludes them from significant corporate or public investment. While legislation like the Orphan Disease Act has curbed some of these structural issues (2), and has led to groundbreaking drug approvals for diseases like cystic fibrosis (ivacaftor & lumacaftor/ivacaftor) (3,4), spinal muscular atrophy (nusinersen) (5) and Duchenne muscular dystrophy (eteplirsen) (6), drug development for monogenetic disorders still lags that of more common diseases.

In addition to financial impediments traditional human genetic disease models have notable caveats. Specifically the use of human immortalized cell lines and heterologous expression systems presents concerns for faithful disease modeling due to their non-physiological nature (7). Likewise, animal models can be confounded by substantial inter-species differences (8,9). Furthermore, scalability constraints and animal colony-related costs can further restrict animal-based modeling. Primary, patient-derived disease-affected cell types represent the de facto 'gold standard' disease model but accessing such material is often challenging or prohibited, and these tissues often are poorly expandable.

The development of induced pluripotent stem cell (iPSC) technology has enabled new opportunities to identify disease-modulating therapeutics by providing a nearly unlimited amount of pluripotent material from any patient. This unprecedented technology has enabled the precise study of the individual cellular components of each organ in isolation, mixed-cell settings and 3D organoid environments (organ-like contexts). Additionally its *in vitro* tractability facilitates the comprehensive study of development, disease pathogenesis and the impact of exogenous effectors like drugs and toxins (10,11).

The confluence of disease modeling and ease of drug evaluation, in particular, empowers comparatively rapid drug screening for human genetic diseases. In fact, many of the earliest studies using iPSCs from human patients with genetic disorders employed targeted chemical screening to modulate a specific molecular target in an informed or hypothesis-driven manner. Importantly these reports established the feasibility of therapeutic compound-identification for a diverse set of diseases,

**Received:** April 26, 2018. **Revised:** April 26, 2018. **Accepted:** May 10, 2018

including Alzheimer's disease, familial dysautonomia, Long-QT syndrome, Rett syndrome, schizophrenia, spinal muscular atrophy and Timothy syndrome (12–19). These advances led to the full utilization of iPSC technologies in the first high-throughput, unbiased iPSC-based phenotypic screen using a large chemical library in 2012 (20). Importantly this therapeutic discovery approach with the initiation of the first clinical trial using an iPSC-discovered compound (21).

Below we explore the origins of iPSCs, offer guidelines on how to conduct iPSC-based drug screens, present recent (2016-present) examples of iPSC-based drug screening for human genetic disorders and offer our prospective on the future of these screening technologies using 3D organoid models.

## A Brief History of Pluripotent Technology

### Embryonic stem cells

Modern *in vitro* pluripotent technologies were established through multi-decade path of critical discoveries. This process began with the isolation of the embryonal carcinoma (EC) cells, the undifferentiated, pluripotent cell type of teratocarcinomas. These cells provided the first scalable tool to probe developmental transitions to all three germ layers, in a dish, by providing essentially unlimited pluripotent source material (22–27). Recognition of the similarity between EC cells and the cells of early embryogenesis, including the cells of the inner cell mass (ICM), prompted EC cell transplantation experiments in blastocysts, which demonstrated the ability for EC cells to differentiate and contribute to the tissues of a postnatal mouse (28–30). These results prompted the search to find the genuine, non-malignant pluripotent cell that underlined mammalian development. In 1981, two independent groups successfully isolated pluripotent, embryonic stem (ES) cells from the ICM of mouse blastocysts (31,32).

Similar isolations of human ES cells from the ICM of human blastocysts proved to be challenging. While ethical, regulatory and funding hurdles certainly contributed to the delay (33), technological challenges arising from fundamental differences between mouse and human ES cells were the principle impediment. As it was later discovered human ES cells are distinct from mouse ES cells, and instead mimic mouse epiblast stem cells—a more primed pluripotent state—which require distinct culture conditions (34–37). This latter distinction prolonged the successful isolation of human ES cell lines until 1998 (38), more than 15 years after the first description of mouse ES cells. While human ES cells revolutionized the study of human biology, generating cell lines harboring heritable diseases was restricted to donated embryos discarded after screening a small number of mutations as part of pre-implantation genetic diagnosis (39–43). A pressing need existed to develop pluripotent lines from any patient harboring any genetic disease.

### Induced pluripotency of somatic cells

To address these access issues groups sought ways to reprogram somatic cells to earlier developmental states. Application of Sir John Gurdon's 1958 Nobel Prize-awarded somatic cell nuclear transfer (SCNT)-based reprogramming technique (44–46) was a logical choice. This method facilitated reprogramming of fully differentiated, somatic cells simply by introducing their nuclei to an enucleated oocyte. Strikingly trans-activating factors present in the oocyte could reverse developmental commitment and generate bona fide clones. This phenomenon translated across species and was most famously applied to create the first cloned mammal, Dolly the sheep (47). While these headline-grabbing findings were truly remarkable, the prospect of leveraging SCNT to provide an ES cell source for any human adult or child was arguably more exciting. Although derivation of pluripotent stem cells from human SCNT embryos was eventually demonstrated in 2013 (48), the ethical issues with cloning and destruction of presumptive embryos, as well as the challenges in obtaining donor human oocytes and performing the technically-intensive SCNT procedure presented significant impediments to widespread use of this technology. Fortunately, a groundbreaking alternative was poised to provide a widely accessible alternative to SCNT.

The idea that trans-activating factors could be used to reprogram a somatic cell presented an intriguing possibility: the identification and application of these factors could be used to induce pluripotency in somatic cells without the need for donor oocytes. While the oocyte factors themselves could be characterized and tested for this purpose, work from the early 2000s suggested that pluripotent cell-derived factors could provide an alternative reprogramming approach (49,50). Leveraging these findings, Shinya Yamanaka and colleagues ectopically expressed four transcription factors (Oct3/4, Sox2, Klf4 and c-Myc) in mouse fibroblasts, reprogramming these cells to mouse iPSCs (51). This Nobel Prize winning work was then applied to generate human iPSCs from fibroblasts a year later (52,53), providing scalable access to essentially any diseased tissue from a human patient. Despite some notable transcriptional, epigenetic and potency differences when compared to ES cells (54–64), iPSCs are competent at recapitulating known pathological hallmarks and uncovering new understandings for a wide range of diseases (12–19,65–73), cementing their scientific importance.

## iPSC as a Drug Discovery Tool for Genetic Disease

Derivation of patient-derived iPSCs can be obtained using commercially-available reagents or third-party fee-for-service operations. These methods have greatly increased researcher access to these powerful personalized medicine platforms, enabling previously unattainable *in vitro* disease modeling and subsequent drug screening studies. However, these latter studies can be challenging and their success relies on appreciation of the overall framework and appropriate choices at critical decision nodes (Fig. 1). We also recommend Derek Lowe's Science Translational Medicine blog 'In the pipeline' for further clarity on drug screening-related considerations.

### Patient iPSCs and control lines

Patient somatic cells have typically consisted of fibroblasts obtained by punch biopsy but can also be sourced less invasively using peripheral blood mononuclear cells (74), dental pulp from deciduous teeth (75) and renal epithelial cells from urine (76). These somatic tissues can be reprogramed with integrating viruses but non-integrating episomal-based (77) and Sendai virus (78) now offer the ability to perform footprint-free reprogramming and are now the preferred route.

In the past parallel wild-type control experiments were performed using non-isogenic, well-characterized human ES lines (79) or iPSCs however these comparisons can be challenging to interpret due to differences in genetic background. However with the advent of the highly modular type II CRISPR systems

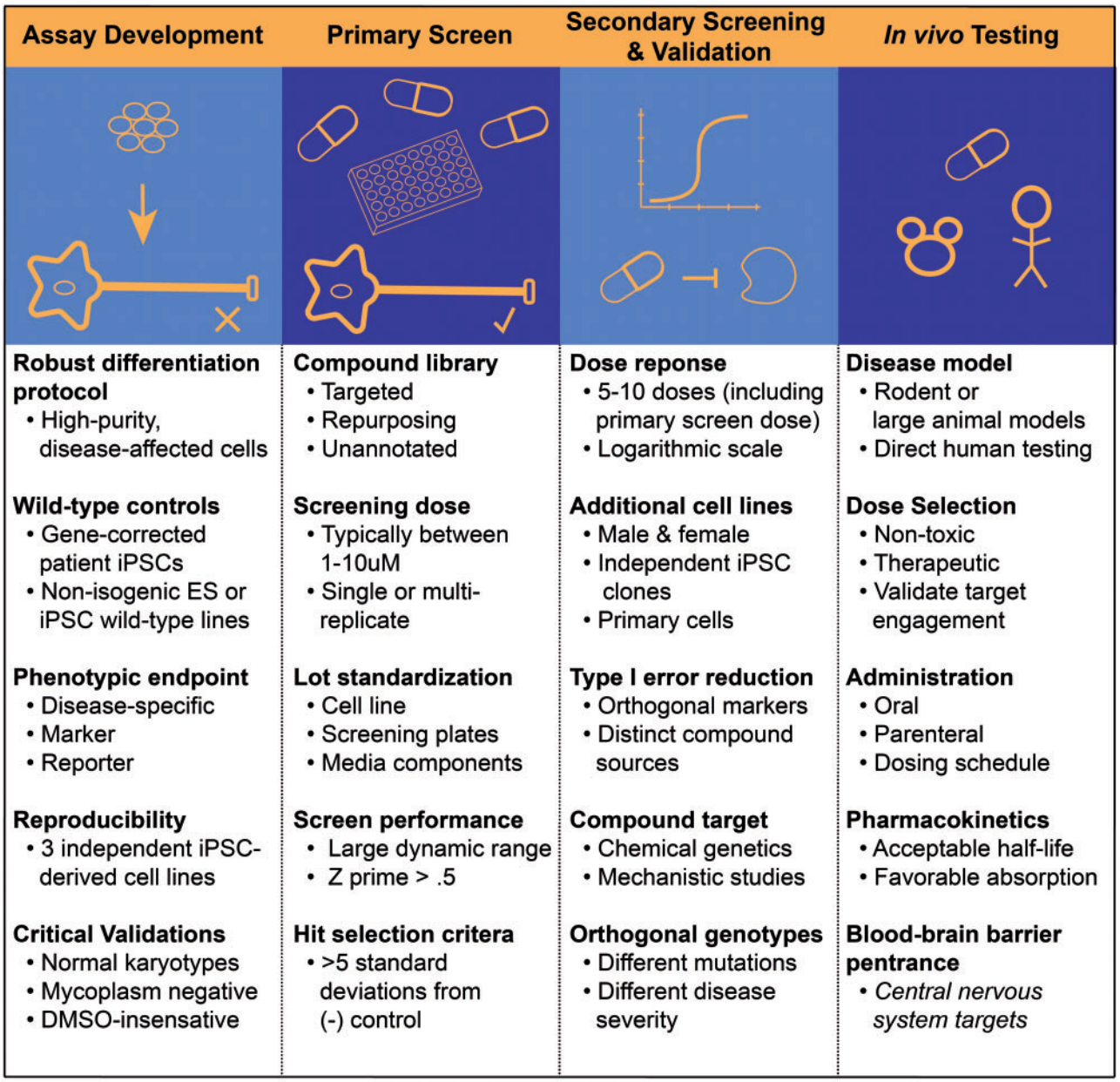


**Figure 1.** A workflow detailing the four primary components of an iPSC-based compound screen along with important considerations: (1) assay development which necessitates the establishment of the cellular model and a screen-able endpoint. (2) The primary screen which involves testing either small or large compound collections to identify putative disease-modulators. (3) Secondary screening and validation which rigorously verify the efficacy and potential molecular targets of primary screening hits compound. (4) *In vivo* testing which confirms a compound's efficacy and safety in a whole organism harboring disease-causative mutations.

consisting of a Cas9 nuclease/guide RNA duplex has enabled the efficient modification of cellular genomes (80–82) isogenic, corrected iPSC cell lines (83).

Prior to use all iPSCs should be propagated at least five passages to remove or inactivate residual reprogramming factors. Staining with canonical markers of pluripotency (including a non-reprogramming factor like Nanog) should be used to confirm line identity. As additional level of rigor a teratoma assay may be performed to prove pluripotency. Finally all selected clones should possess normal karyotypes and be tested and confirmed mycoplasma negative as abnormal karyotypes and mycoplasma infections can have profound effects on cellular phenotypes (84,85).

## Assay development

A phenotypic assay necessitates the use of disease-affected cell types. A differentiation protocol that efficiently generates the affected cell type in high-purity should be employed to produce diseased tissue in sufficient quantities without confounding, contaminating cell types. During differentiation multiple independent clones (typically 3) per genotype should be used to establish a cellular endpoint which represents a disease-relevant phenotype. This phenotype should be robust, reproducible and amendable to screening. Since screening libraries typically contain compounds dissolved in DMSO, this phenotype should be confirmed to be DMSO-insensitive.

## Primary screening

These recommendations are primarily applicable for large compound screens, however many of the underlying principles still apply to smaller, targeted screens. We encourage the appreciation of all components of chemical screening.

**Library selection.** Targeted compound testing involves the screening of a small number of compounds fall under two specific categories: (1) use of known or purported disease-modifying drugs to validate the cellular genetic disease model and (2) discovery of dysregulated pathways or targets followed by application of established compound effectors and assessment of pathologic modulation. The former approach provides mostly incremental advancements but may be critical to establish the cellular model for future unbiased screening using large compound libraries. The latter can accelerate the identification of bona fide disease-modulators and potentially supplant the need to screen a large number of compounds, however caution should be paid to prevent overinterpretation of a specific phenotype. For instance, pathological processes can often lead to broad dysregulation of cellular biology, which could confound the selection of a specific target or pathway, and may lead to an inconclusive or patently erroneous results.

Large compound library screens provide the opportunity to rapidly interrogate multiple pathways and targets in an unbiased or semi-unbiased fashion. This enables the identification of compound modulators which target previously unappreciated disease mechanisms. Importantly compound libraries should contain enough structural diversity to prevent target bias, be obtained from reliable sources to guarantee reagent purity and identity, and be maintained in a manner to prevent degradation or contamination (86). It is worth noting that many iPSC-based chemical screens have opted to use repurposing libraries containing clinically approved or clinical candidate drugs with (1) well annotated targets that can be easily validated in downstream studies, (2) favorable drug-like character to enable rapid transition into pre-clinical animal models and human patients and (3) established safety profiles to preclude unforeseen toxicity events. Unfortunately the chemical redundancy (compounds in the same structural class) in these libraries limits their discovery power but may be an acceptable tradeoff for more rapid clinical translation.

**Screening dose and replicates.** Primary screen design for large compound libraries involves navigating a sea of compromises due to resource constraints (financial, time, reagents and personnel). While it is possible to perform the primary screen using multiple doses and cell lines typical screens use a single line screened at a single dose between 1 and 10 uM. It is important to note that compound effectors with low potency will likely be missed at the lower end of this range while some high-potency modulators may be toxic at elevated doses. For added rigor a low and high primary screening dose may be used to reduce the potential for these false negatives.

**Reagent standardization.** Since reagent composition can change from lot to lot, it is important to assemble validated lots prior to the initiation of a screen. These lots should set aside and utilized during the primary screen. Also as many large compound screens take substantial time to execute its critical to perform the screen prior to reagent degradation.

**Screening performance and hit selection.** To control for changes to the assay over time, it is critical to include positive (i.e. wild-type cells) and negative (vehicle treated) controls to gauge assay performance and for post-hoc normalization of inter-plate variability. Calculation of Z prime is a commonly used method to evaluate screening performance on a per-plate and global basis in biochemical assays (87) but can also be applied to cell-based screens in spite of their greater variability. Even in cases were Z prime is not ideal results may still be used but the risk for false positives increases. Finally while primary screening hits can be selected a number of ways a 5-sigma improvement over the negative control serves as a robust identification method.

## Secondary screening & validation

These intervening steps serve as valuable filters to select a lead compound which will be further characterized through molecular target identification and *in vivo* studies. This process is critical for the elimination of false positives and discovery of legitimate disease-modulators.

**Dose-response.** The demonstration of a dose response can be critical for establishing a genuine on-target effect. Furthermore these curves can be used to ascertain the half-maximal effective concentration (EC50) of a compound which can be important in prioritizing high-potency disease-modulators.

**Biological replicates.** Cells derived from unique iPSC clones, different patients with the same or distinct genotypes and different sexes (if disease appropriate) provide material to validate primary screening hits and eliminate any line specific artifacts. Primary cells from patients (if accessible) or disease-relevant animal models can provide an additional filter to demonstrate that the compound effects bona fide disease tissue and reduces the chance of potential additional iPSC-based artifact. Importantly, while the use of different genotypes or species adds an additional layer of rigor, it is possible that compound modulators are mutation- or organism-specific and so the potential exists for over-filtering hits.

**Orthogonal, non-biological reagents.** The use of alternative reagents or reagent sources is vital to prevent false positives and reagent-specific artifacts. For instance, compounds can be mislabeled or degraded in a library collection, gravely misleading the call of a screening hit. One should obtain independent sources of compound hits or their filtered, putative lead compound prior to further advancement. Additionally, orthogonal markers (antibodies, chemical labels) should be employed to reduce compound-induced, positive phenotypic readouts that are irrelevant to the underlying disease process.

**Target identification.** The lead compound's annotated mechanism of action should be evaluated. In particular two questions can be asked: (1) is the target expressed in the screened cell type(s) and (2) do structurally distinct compounds with the same annotated target also demonstrate efficacy? These preliminary data can support or refute a canonical mechanism-mediated effect. Further investigations using gene knockdowns or knockouts can provide additional verification. It is worth mentioning that while the establishment of a molecular target is important for understanding the mechanism of disease-modulation, downstream *in vivo* validations do not necessarily require this information.

## *In vivo* testing

Demonstration of *in vivo* efficacy serves as the pre-eminent validation of *in vitro* therapeutic chemical screening. As such it is

essential to appreciate unique features of *in vivo* testing. Proper dosing, in particular, is critical to evaluating *in vivo* disease-modulation as under-dosing will result in a lack of efficacy whereas overdosing may cause toxicity. While it may seem attractive use *in vitro* compound concentrations to inform *in vivo* doses, due to pharmacokinetic effects, dose should be determined empirically or be informed by previously published *in vivo* studies using the same compound. Exploratory studies using 2–3 doses with verification of target engagement in the cell type of interest is highly recommended. These studies will also provide the necessary data for an *a priori* power calculation as part of a large scale, blinded trial with randomization (treatment, genotype) (88). Finally special attention should be paid for central nervous system targets as many compounds do not cross the blood–brain barrier (89). If possible using a filter of known blood–brain barrier penetrance during secondary screening may be prudent prior to selection of the lead compound.

## Recent iPSC-Based Drug Screening Efforts

Current drug screening efforts (2016–2018) are summarized in Supplementary Material, Table S1. Of note, the majority of these reports employ targeted screening approaches using a small number of known or predicted disease-modulators. This may reflect the inherent challenges in generating disease-relevant cells types at high scale and purity for larger screening approaches (medium- and high-throughput).

## The Next Frontier: iPSC-Derived 3D Organoids

2D monolayer culture methods have traditionally been used for iPSC-based studies. While these modalities excel at rapidly modeling cell intrinsic deficits they ultimately lack the contextual elements within an organ, and therefore diseased cell types are potentially devoid of disease-relevant inputs. In particular an organ's biological architecture, endogenous signaling and intercellular interactions may directly impact disease pathogenesis (90). While it is not possible to fully recapitulate these aspects, new 3D organoid-based technologies offer greater physiological relevance relative to their 2D counterparts (91). Additionally its worth mentioned that 2D generated cultures recapitulate largely fetal or early postnatal phenotype while organoids may afford the opportunity for extended maturation to more adult (and potentially disease-relevant) stages (92,93).

Pluripotent-derived cells are amendable to organoid-based studies as they show capability of self-organization and have demonstrated the recapitulation of complex tissue architectures of the biliary tree (94), brain (95–98), fallopian tube (99), intestine (100), liver (101,102), kidney (103), pancreas (104,105), retina (106–109) and stomach (110). Such protocols have been applied to a host a genetic diseases including Alagille syndrome (111,112), autism (113), cystic fibrosis (94,112,114), enhanced s-cone syndrome (115), familial adenomatous polyposis (116), Huntington's disease (117), Leber congenital amaurosis (118), microcephaly (96), microlissencephaly (119), Miller–Dieker lissencephaly syndrome (120,121), polycystic kidney disease (122), retinitis pigmentosa (107–109), Rett syndrome (123,124) and Timothy syndrome (125). While these pluripotent-based organoids have yet to be heavily scaled they have been used as part of small-scale, targeted drug testing for Alagille syndrome (112), cystic fibrosis (94,112), familial adenomatous polyposis (116), polycystic liver disease (112) and Timothy syndrome (125).

While organoid-based studies offer a number of benefits over 2D cultures they also come with a number of caveats: (1) self-organization variability leading to inter-organoid differences. This necessitates the use of many individual organoids to confirm that the observed phenotypes are not a mere consequence of this natural variation. (2) Diffusion effects that manifest with increasing organoid size. Large organoid cores will often show significant necrosis whose effects may reverberate to the outer layers. Notably, solving these issues is an active area of research and as vascularization or pseudo-vascularization techniques are refined these issues may fade (102,126). (3) Assay endpoints (especially antibody-based phenotypic) are challenging to assess in floating 3D organoids. Reporter-based approaches or novel assays will need to be developed to improve the throughput of these technologies.

While organoids represent powerful new biological tools their increased complexity will likely prevent the usurpation of 2D cultures for the foreseeable future. Instead organoids will likely fulfill a role in critical validation studies as part of secondary screening pipelines using monolayer cultures.

## Concluding Remarks

While iPSC technologies may transform the treatment of genetic disorders, these scientific tools may also pay dividends for common diseases that affect large swaths of the population. In particular many recent blockbuster drugs have been used to treat rare, monogenetic diseases (1). As such the identification of drugs for genetic disorders could serve as important intersections of discovery for the benefit of potentially large numbers of patients. Such novel approaches are imperative given the spiraling costs for a single drug approval (estimated to be ~3 billion in 2018), the multi-decade stagnation of clinical approvals and a rising clinical trial failure rate (~90% in 2018) (127–130). It is worth noting that iPSC-based drugs screens are still in its infancy. As these technologies are further refined they may solve issues in drug development and will potentially fulfill a critical role in the delivery of novel therapies for patients.

## Supplementary Material

Supplementary Material is available at *HMG* online.

*Conflict of Interest statement.* P.J.T. is a co-founder and consultant for Convelo Therapeutics, which has licensed patents from Case Western Reserve University inventors (P.J.T. and M.S.E.). P.J.T. and Case Western Reserve University hold equity in Convelo Therapeutics. P.J.T. is a consultant and on the Scientific Advisory Board of Cell Line Genetics. P.J.T. is Chair of the Scientific Advisory Board (volunteer position) for the Pelizaeus-Merzbacher Disease Foundation. All other authors have no competing interests.

## Funding

Work in the Tesar lab has supported by grants from the NIH (currently R01NS093357, R01NS095280 and T32GM007250), New York Stem Cell Foundation, National Multiple Sclerosis Society, Pelizaeus Merzbacher Disease Foundation, European Leukodystrophy Association, and philanthropic support from the Peterson, Fakhouri, Long, Goodman, Geller, Galbut/Heil, Judge and Weidenthal families.

## References


1. Brinkman, R.R., Dube, M.P., Rouleau, G.A., Orr, A.C. and Samuels, M.E. (2006) Human monogenic disorders - a source of novel drug targets. *Nat. Rev. Genet.*, **7**, 249–260.
2. Braun, M.M., Farag-El-Massah, S., Xu, K. and Cote, T.R. (2010) Emergence of orphan drugs in the United States: a quantitative assessment of the first 25 years. *Nat. Rev. Drug Discov.*, **9**, 519–522.
3. Ramsey, B.W., Davies, J., McElvaney, N.G., Tullis, E., Bell, S.C., Dřevínek, P., Griese, M., McKone, E.F., Wainwright, C.E. and Konstan, M.W. (2011) A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N. Engl. J. Med.*, **365**, 1663–1672.
4. Wainwright, C.E., Elborn, J.S., Ramsey, B.W., Marigowda, G., Huang, X., Cipolli, M., Colombo, C., Davies, J.C., De Boeck, K., Flume, P.A. *et al.* (2015) Lumacaftor-Ivacaftor in patients with cystic fibrosis homozygous for Phe508del CFTR. *N. Engl. J. Med.*, **373**, 220–231.
5. Finkel, R.S., Mercuri, E., Darras, B.T., Connolly, A.M., Kuntz, N.L., Kirschner, J., Chiriboga, C.A., Saito, K., Servais, L., Tizzano, E. *et al.* (2017) Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N. Engl. J. Med.*, **377**, 1723–1732.
6. Cirak, S., Arechavala-Gomeza, V., Guglieri, M., Feng, L., Torelli, S., Anthony, K., Abbs, S., Garralda, M.E., Bourke, J., Wells, D.J. *et al.* (2011) Exon skipping and dystrophin restoration in patients with Duchenne muscular dystrophy after systemic phosphorodiamidate morpholino oligomer treatment: an open-label, phase 2, dose-escalation study. *Lancet*, **378**, 595–605.
7. Maqsood, M.I., Matin, M.M., Bahrami, A.R. and Ghasroldasht, M.M. (2013) Immortality of cell lines: challenges and advantages of establishment. *Cell Biol. Int.*, **37**, 1038–1045.
8. Uhl, E.W. and Warner, N.J. (2015) Mouse models as predictors of human responses: evolutionary medicine. *Curr. Pathobiol. Rep.*, **3**, 219–223.
9. Lin, J.H. (2008) Applications and limitations of genetically modified mouse models in drug discovery and development. *Curr. Drug Metab.*, **9**, 419–438.
10. Yamanaka, S. (2012) Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell*, **10**, 678–684.
11. Dutta, D., Heo, I. and Clevers, H. (2017) Disease modeling in stem cell-derived 3D organoid systems. *Trends Mol. Med.*, **23**, 393–410.
12. Yazawa, M., Hsueh, B., Jia, X., Pasca, A.M., Bernstein, J.A., Hallmayer, J. and Dolmetsch, R.E. (2011) Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature*, **471**, 230–234.
13. Yahata, N., Asai, M., Kitaoka, S., Takahashi, K., Asaka, I., Hioki, H., Kaneko, T., Maruyama, K., Saido, T.C., Nakahata, T. *et al.* (2011) Anti-Aβ drug screening platform using human iPS cell-derived neurons for the treatment of Alzheimer's disease. *PLoS One*, **6**, e25788.
14. Moretti, A., Bellin, M., Welling, A., Jung, C.B., Lam, J.T., Bott-Flugel, L., Dorn, T., Goedel, A., Hohnke, C., Hofmann, F. *et al.* (2010) Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N. Engl. J. Med.*, **363**, 1397–1409.
15. Marchetto, M.C., Carromeu, C., Acab, A., Yu, D., Yeo, G.W., Mu, Y., Chen, G., Gage, F.H. and Muotri, A.R. (2010) A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell*, **143**, 527–539.
16. Lee, G., Papapetrou, E.P., Kim, H., Chambers, S.M., Tomishima, M.J., Fasano, C.A., Ganat, Y.M., Menon, J., Shimizu, F., Viale, A. *et al.* (2009) Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. *Nature*, **461**, 402–406.
17. Itzhaki, I., Maizels, L., Huber, I., Zwi-Dantsis, L., Caspi, O., Winterstern, A., Feldman, O., Gepstein, A., Arbel, G., Hammerman, H. *et al.* (2011) Modelling the long QT syndrome with induced pluripotent stem cells. *Nature*, **471**, 225–229.
18. Ebert, A.D., Yu, J., Rose, F.F., Jr, Mattis, V.B., Lorson, C.L., Thomson, J.A. and Svendsen, C.N. (2009) Induced pluripotent stem cells from a spinal muscular atrophy patient. *Nature*, **457**, 277–280.
19. Brennand, K.J., Simone, A., Jou, J., Gelboin-Burkhart, C., Tran, N., Sangar, S., Li, Y., Mu, Y., Chen, G., Yu, D. *et al.* (2011) Modelling schizophrenia using human induced pluripotent stem cells. *Nature*, **473**, 221–225.
20. Lee, G., Ramirez, C.N., Kim, H., Zeltner, N., Liu, B., Radu, C., Bhinder, B., Kim, Y.J., Choi, I.Y., Mukherjee-Clavin, B. *et al.* (2012) Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue IKBKAP expression. *Nature Biotechnol.*, **30**, 1244–1248.
21. McNeish, J., Gardner, J.P., Wainger, B.J., Woolf, C.J. and Eggan, K. (2015) From dish to bedside: lessons learned while translating findings from a stem cell model of disease to a clinical trial. *Cell Stem Cell*, **17**, 8–10.
22. Kahan, B.W. and Ephrussi, B. (1970) Developmental potentialities of clonal in vitro cultures of mouse testicular teratoma. *J. Natl. Cancer Inst.*, **44**, 1015–1036.
23. Pierce, G.B., Jr and Verney, E.L. (1961) An in vitro and in vivo study of differentiation in teratocarcinomas. *Cancer*, **14**, 1017–1029.
24. Rosenthal, M.D., Wishnow, R.M. and Sato, G.H. (1970) In vitro growth and differetiation of clonal populations of multipotential mouse clls derived from a transplantable testicular teratocarcinoma. *J. Natl. Cancer Inst.*, **44**, 1001–1014.
25. Evans, M.J. (1972) The isolation and properties of a clonal tissue culture strain of pluripotent mouse teratoma cells. *J. Embryol. Exp. Morphol.*, **28**, 163–176.
26. Jami, J. and Ritz, E. (1974) Multipotentiality of single cells of transplantable teratocarcinomas derived from mouse embryo grafts. *J. Natl. Cancer Inst.*, **52**, 1547–1552.
27. Jakob, H., Boon, T., Gaillard, J., Nicolas, J. and Jacob, F. (1973) [Teratocarcinoma of the mouse: isolation, culture and properties of pluripotential cells]. *Ann. Microbiol. (Paris)*, **124**, 269–282.
28. Brinster, R.L. (1974) The effect of cells transferred into the mouse blastocyst on subsequent development. *J. Exp. Med.*, **140**, 1049–1056.
29. Papaioannou, V.E., McBurney, M.W., Gardner, R.L. and Evans, M.J. (1975) Fate of teratocarcinoma cells injected into early mouse embryos. *Nature*, **258**, 70–73.
30. Martin, G.R. (1975) Teratocarcinomas as a model system for the study of embryogenesis and neoplasia. *Cell*, **5**, 229–243.
31. Martin, G.R. (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. U S A*, **78**, 7634–7638.
32. Evans, M.J. and Kaufman, M.H. (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature*, **292**, 154–156.

33. Robertson, J.A. (2001) Human embryonic stem cell research: ethical and legal issues. *Nat. Rev. Genet.*, **2**, 74–78.
34. Tesar, P.J., Chenoweth, J.G., Brook, F.A., Davies, T.J., Evans, E.P., Mack, D.L., Gardner, R.L. and McKay, R.D. (2007) New cell lines from mouse epiblast share defining features with human embryonic stem cells. *Nature*, **448**, 196–199.
35. Brons, I.G., Smithers, L.E., Trotter, M.W., Rugg-Gunn, P., Sun, B., Chuva de Sousa Lopes, S.M., Howlett, S.K., Clarkson, A., Ahrlund-Richter, L., Pedersen, R.A. *et al.* (2007) Derivation of pluripotent epiblast stem cells from mammalian embryos. *Nature*, **448**, 191–195.
36. Factor, D.C., Corradin, O., Zentner, G.E., Saiakhova, A., Song, L., Chenoweth, J.G., McKay, R.D., Crawford, G.E., Scacheri, P.C. and Tesar, P.J. (2014) Epigenomic comparison reveals activation of "seed" enhancers during transition from naive to primed pluripotency. *Cell Stem Cell*, **14**, 854–863.
37. Bao, S., Tang, F., Li, X., Hayashi, K., Gillich, A., Lao, K. and Surani, M.A. (2009) Epigenetic reversion of post-implantation epiblast to pluripotent embryonic stem cells. *Nature*, **461**, 1292–1295.
38. Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S. and Jones, J.M. (1998) Embryonic stem cell lines derived from human blastocysts. *Science*, **282**, 1145–1147.
39. Eiges, R., Urbach, A., Malcov, M., Frumkin, T., Schwartz, T., Amit, A., Yaron, Y., Eden, A., Yanuka, O., Benvenisty, N. *et al.* (2007) Developmental study of fragile X syndrome using human embryonic stem cells derived from preimplantation genetically diagnosed embryos. *Cell Stem Cell*, **1**, 568–577.
40. Verlinsky, Y., Strelchenko, N., Kukharenko, V., Rechitsky, S., Verlinsky, O., Galat, V. and Kuliev, A. (2005) Human embryonic stem cell lines with genetic disorders. *Reprod. Biomed. Online*, **10**, 105–110.
41. Pickering, S.J., Minger, S.L., Patel, M., Taylor, H., Black, C., Burns, C.J., Ekonomou, A. and Braude, P.R. (2005) Generation of a human embryonic stem cell line encoding the cystic fibrosis mutation deltaF508, using preimplantation genetic diagnosis. *Reprod. Biomed. Online*, **10**, 390–397.
42. Turetsky, T., Aizenman, E., Gil, Y., Weinberg, N., Shufaro, Y., Revel, A., Laufer, N., Simon, A., Abeliovich, D. and Reubinoff, B.E. (2007) Laser-assisted derivation of human embryonic stem cell lines from IVF embryos after preimplantation genetic diagnosis. *Hum. Reprod.*, **23**, 46–53.
43. Niclis, J., Trounson, A.O., Dottori, M., Ellisdon, A., Bottomley, S.P., Verlinsky, Y. and Cram, D. (2009) Human embryonic stem cell models of Huntington disease. *Reprod. Biomed. Online*, **19**, 106–113.
44. Gurdon, J.B. (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. *J. Embryol. Exp. Morphol.*, **10**, 622–640.
45. Gurdon, J.B., Elsdale, T.R. and Fischberg, M. (1958) Sexually mature individuals of Xenopus laevis from the transplantation of single somatic nuclei. *Nature*, **182**, 64–65.
46. Gurdon, J.B. (1962) Adult frogs derived from the nuclei of single somatic cells. *Dev. Biol.*, **4**, 256–273.
47. Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J. and Campbell, K.H. (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature*, **385**, 810–813.
48. Tachibana, M., Amato, P., Sparman, M., Gutierrez, N.M., Tippner-Hedges, R., Ma, H., Kang, E., Fulati, A., Lee, H.S., Sritanaudomchai, H. *et al.* (2013) Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell*, **153**, 1228–1238.
49. Cowan, C.A., Atienza, J., Melton, D.A. and Eggan, K. (2005) Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science*, **309**, 1369–1373.
50. Tada, M., Takahama, Y., Abe, K., Nakatsuji, N. and Tada, T. (2001) Nuclear reprogramming of somatic cells by in vitro hybridization with ES cells. *Curr. Biol.*, **11**, 1553–1558.
51. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, **126**, 663–676.
52. Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R. *et al.* (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science*, **318**, 1917–1920.
53. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. and Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, **131**, 861–872.
54. Ohi, Y., Qin, H., Hong, C., Blouin, L., Polo, J.M., Guo, T., Qi, Z., Downey, S.L., Manos, P.D., Rossi, D.J. *et al.* (2011) Incomplete DNA methylation underlies a transcriptional memory of somatic cells in human iPS cells. *Nat. Cell Biol.*, **13**, 541–549.
55. Lister, R., Pelizzola, M., Kida, Y.S., Hawkins, R.D., Nery, J.R., Hon, G., Antosiewicz-Bourget, J., O'Malley, R., Castanon, R., Klugman, S. *et al.* (2011) Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*, **471**, 68–73.
56. Kim, K., Zhao, R., Doi, A., Ng, K., Unternaehrer, J., Cahan, P., Hongguang, H., Loh, Y.-H., Aryee, M.J., Lensch, M.W. *et al.* (2011) Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat. Biotechnol.*, **29**, 1117–1119.
57. Bock, C., Kiskinis, E., Verstappen, G., Gu, H., Boulting, G., Smith, Z.D., Ziller, M., Croft, G.F., Amoroso, M.W., Oakley, D.H. *et al.* (2011) Reference maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell*, **144**, 439–452.
58. Polo, J.M., Liu, S., Figueroa, M.E., Kulalert, W., Eminli, S., Tan, K.Y., Apostolou, E., Stadtfeld, M., Li, Y., Shioda, T. *et al.* (2010) Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat. Biotechnol.*, **28**, 848–855.
59. Kim, K., Doi, A., Wen, B., Ng, K., Zhao, R., Cahan, P., Kim, J., Aryee, M.J., Ji, H., Ehrlich, L.I.R. *et al.* (2010) Epigenetic memory in induced pluripotent stem cells. *Nature*, **467**, 285–290.
60. Hawkins, R.D., Hon, G.C., Lee, L.K., Ngo, Q., Lister, R., Pelizzola, M., Edsall, L.E., Kuan, S., Luu, Y., Klugman, S. *et al.* (2010) Distinct epigenomic landscapes of pluripotent and lineage-committed human cells. *Cell Stem Cell*, **6**, 479–491.
61. Hanna, J., Saha, K., Pando, B., van Zon, J., Lengner, C.J., Creyghton, M.P., van Oudenaarden, A. and Jaenisch, R. (2009) Direct cell reprogramming is a stochastic process amenable to acceleration. *Nature*, **462**, 595–601.
62. Doi, A., Park, I.-H., Wen, B., Murakami, P., Aryee, M.J., Irizarry, R., Herb, B., Ladd-Acosta, C., Rho, J., Loewer, S. *et al.* (2009) Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat. Genet.*, **41**, 1350–1353.
63. Chin, M.H., Mason, M.J., Xie, W., Volinia, S., Singer, M., Peterson, C., Ambartsumyan, G., Aimiuwu, O., Richter, L., Zhang, J. *et al.* (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell*, **5**, 111–123.

64. Ma, H., Morey, R., O'Neil, R.C., He, Y., Daughtry, B., Schultz, M.D., Hariharan, M., Nery, J.R., Castanon, R., Sabatini, K. *et al.* (2014) Abnormalities in human pluripotent cells due to reprogramming mechanisms. *Nature*, **511**, 177–183.
65. Paşca, S.P., Portmann, T., Voineagu, I., Yazawa, M., Shcheglovitov, A., Paşca, A.M., Cord, B., Palmer, T.D., Chikahisa, S., Nishino, S. *et al.* (2011) Using iPSC-derived neurons to uncover cellular phenotypes associated with Timothy syndrome. *Nat. Med.*, **17**, 1657–1662.
66. Nguyen, H.N., Byers, B., Cord, B., Shcheglovitov, A., Byrne, J., Gujar, P., Kee, K., Schule, B., Dolmetsch, R.E., Langston, W. *et al.* (2011) LRRK2 mutant iPSC-derived DA neurons demonstrate increased susceptibility to oxidative stress. *Cell Stem Cell*, **8**, 267–280.
67. Dimos, J.T., Rodolfa, K.T., Niakan, K.K., Weisenthal, L.M., Mitsumoto, H., Chung, W., Croft, G.F., Saphier, G., Leibel, R., Goland, R. *et al.* (2008) Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science*, **321**, 1218–1221.
68. Carvajal-Vergara, X., Sevilla, A., D'Souza, S.L., Ang, Y.-S., Schaniel, C., Lee, D.-F., Yang, L., Kaplan, A.D., Adler, E.D., Rozov, R. *et al.* (2010) Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome. *Nature*, **465**, 808–812.
69. Urbach, A., Bar-Nur, O., Daley, G.Q. and Benvenisty, N. (2010) Differential modeling of fragile X syndrome by human embryonic stem cells and induced pluripotent stem cells. *Cell Stem Cell*, **6**, 407–411.
70. Mazzulli, J.R., Xu, Y.H., Sun, Y., Knight, A.L., McLean, P.J., Caldwell, G.A., Sidransky, E., Grabowski, G.A. and Krainc, D. (2011) Gaucher disease glucocerebrosidase and alpha-synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell*, **146**, 37–52.
71. Liu, G.H., Barkho, B.Z., Ruiz, S., Diep, D., Qu, J., Yang, S.L., Panopoulos, A.D., Suzuki, K., Kurian, L., Walsh, C. *et al.* (2011) Recapitulation of premature ageing with iPSCs from Hutchinson-Gilford progeria syndrome. *Nature*, **472**, 221–225.
72. Baek, K.H., Zaslavsky, A., Lynch, R.C., Britt, C., Okada, Y., Siarey, R.J., Lensch, M.W., Park, I.H., Yoon, S.S., Minami, T. *et al.* (2009) Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. *Nature*, **459**, 1126–1130.
73. Agarwal, S., Loh, Y.H., McLoughlin, E.M., Huang, J., Park, I.H., Miller, J.D., Huo, H., Okuka, M., Dos Reis, R.M., Loewer, S. *et al.* (2010) Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients. *Nature*, **464**, 292–296.
74. Staerk, J., Dawlaty, M.M., Gao, Q., Maetzel, D., Hanna, J., Sommer, C.A., Mostoslavsky, G. and Jaenisch, R. (2010) Reprogramming of human peripheral blood cells to induced pluripotent stem cells. *Cell Stem Cell*, **7**, 20–24.
75. Yan, X., Qin, H., Qu, C., Tuan, R.S., Shi, S. and Huang, G.T. (2010) iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. *Stem Cells Dev.*, **19**, 469–480.
76. Xue, Y., Cai, X., Wang, L., Liao, B., Zhang, H., Shan, Y., Chen, Q., Zhou, T., Li, X., Hou, J. *et al.* (2013) Generating a non-integrating human induced pluripotent stem cell bank from urine-derived cells. *PLoS One*, **8**, e70573.
77. Yu, J., Hu, K., Smuga-Otto, K., Tian, S., Stewart, R., Slukvin, I.I. and Thomson, J.A. (2009) Human induced pluripotent stem cells free of vector and transgene sequences. *Science*, **324**, 797–801.
78. Seki, T., Yuasa, S. and Fukuda, K. (2012) Generation of induced pluripotent stem cells from a small amount of human peripheral blood using a combination of activated T cells and Sendai virus. *Nat. Protoc.*, **7**, 718–728.
79. Levine, A.D. (2011) Access to human embryonic stem cell lines. *Nat. Biotechnol.*, **29**, 1079–1081.
80. Jinek, M., East, A., Cheng, A., Lin, S., Ma, E. and Doudna, J. (2013) RNA-programmed genome editing in human cells. *Elife*, **2**, e00471.
81. Cong, L., Ran, F.A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P.D., Wu, X., Jiang, W., Marraffini, L.A. *et al.* (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science*, **339**, 819–823.
82. Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A. and Charpentier, E. (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, **337**, 816–821.
83. Paquet, D., Kwart, D., Chen, A., Sproul, A., Jacob, S., Teo, S., Olsen, K.M., Gregg, A., Noggle, S. and Tessier-Lavigne, M. (2016) Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. *Nature*, **533**, 125–129.
84. Rottem, S. and Naot, Y. (1998) Subversion and exploitation of host cells by mycoplasmas. *Trends Microbiol.*, **6**, 436–440.
85. Liang, G. and Zhang, Y. (2013) Genetic and epigenetic variations in iPSCs: potential causes and implications for application. *Cell Stem Cell*, **13**, 149–159.
86. Dandapani, S., Rosse, G., Southall, N., Salvino, J.M. and Thomas, C.J. (2012) Selecting, acquiring, and using small molecule libraries for high-throughput screening. *Curr. Protoc. Chem. Biol.*, **4**, 177–191.
87. Zhang, J.H., Chung, T.D. and Oldenburg, K.R. (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen*, **4**, 67–73.
88. Landis, S.C., Amara, S.G., Asadullah, K., Austin, C.P., Blumenstein, R., Bradley, E.W., Crystal, R.G., Darnell, R.B., Ferrante, R.J., Fillit, H. *et al.* (2012) A call for transparent reporting to optimize the predictive value of preclinical research. *Nature*, **490**, 187–191.
89. Patel, M.M. and Patel, B.M. (2017) Crossing the blood-brain barrier: recent advances in drug delivery to the brain. *CNS Drugs*, **31**, 109–133.
90. Liddelow, S.A., Guttenplan, K.A., Clarke, L.E., Bennett, F.C., Bohlen, C.J., Schirmer, L., Bennett, M.L., Munch, A.E., Chung, W.S., Peterson, T.C. *et al.* (2017) Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*, **541**, 481–487.
91. Yin, X., Mead, B.E., Safaee, H., Langer, R., Karp, J.M. and Levy, O. (2016) Engineering stem cell organoids. *Cell Stem Cell*, **18**, 25–38.
92. Sloan, S.A., Darmanis, S., Huber, N., Khan, T.A., Birey, F., Caneda, C., Reimer, R., Quake, S.R., Barres, B.A. and Paşca, S.P. (2017) Human astrocyte maturation captured in 3D cerebral cortical spheroids derived from pluripotent stem cells. *Neuron*, **95**, 779–790 e776.
93. Watson, C.L., Mahe, M.M., Múnera, J., Howell, J.C., Sundaram, N., Poling, H.M., Schweitzer, J.I., Vallance, J.E., Mayhew, C.N., Sun, Y. *et al.* (2014) An in vivo model of human small intestine using pluripotent stem cells. *Nat. Med.*, **20**, 1310–1314.
94. Ogawa, M., Ogawa, S., Bear, C.E., Ahmadi, S., Chin, S., Li, B., Grompe, M., Keller, G., Kamath, B.M. and Ghanekar, A. (2015) Directed differentiation of cholangiocytes from human pluripotent stem cells. *Nat. Biotechnol.*, **33**, 853–861.

95. Paşca, A.M., Sloan, S.A., Clarke, L.E., Tian, Y., Makinson, C.D., Huber, N., Kim, C.H., Park, J.-Y., O'Rourke, N.A., Nguyen, K.D. *et al.* (2015) Functional cortical neurons and astrocytes from human pluripotent stem cells in 3D culture. *Nat. Methods*, **12**, 671–678.
96. Lancaster, M.A., Renner, M., Martin, C.A., Wenzel, D., Bicknell, L.S., Hurles, M.E., Homfray, T., Penninger, J.M., Jackson, A.P. and Knoblich, J.A. (2013) Cerebral organoids model human brain development and microcephaly. *Nature*, **501**, 373–379.
97. Bagley, J.A., Reumann, D., Bian, S., Levi-Strauss, J. and Knoblich, J.A. (2017) Fused cerebral organoids model interactions between brain regions. *Nat. Methods*, **14**, 743–751.
98. Qian, X., Nguyen, H.N., Song, M.M., Hadiono, C., Ogden, S.C., Hammack, C., Yao, B., Hamersky, G.R., Jacob, F., Zhong, C. *et al.* (2016) Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell*, **165**, 1238–1254.
99. Kessler, M., Hoffmann, K., Brinkmann, V., Thieck, O., Jackisch, S., Toelle, B., Berger, H., Mollenkopf, H.J., Mangler, M., Sehouli, J. *et al.* (2015) The Notch and Wnt pathways regulate stemness and differentiation in human fallopian tube organoids. *Nat. Commun.*, **6**, 8989.
100. Spence, J.R., Mayhew, C.N., Rankin, S.A., Kuhar, M.F., Vallance, J.E., Tolle, K., Hoskins, E.E., Kalinichenko, V.V., Wells, S.I., Zorn, A.M. *et al.* (2011) Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature*, **470**, 105–109.
101. Huch, M., Dorrell, C., Boj, S.F., van Es, J.H., Li, V.S., van de Wetering, M., Sato, T., Hamer, K., Sasaki, N., Finegold, M.J. *et al.* (2013) In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. *Nature*, **494**, 247–250.
102. Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., Zhang, R.R., Ueno, Y., Zheng, Y.W., Koike, N. *et al.* (2013) Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*, **499**, 481–484.
103. Takasato, M., Er, P.X., Becroft, M., Vanslambrouck, J.M., Stanley, E.G., Elefanty, A.G. and Little, M.H. (2014) Directing human embryonic stem cell differentiation towards a renal lineage generates a self-organizing kidney. *Nat. Cell Biol.*, **16**, 118–126.
104. Karthaus, W.R., Iaquinta, P.J., Drost, J., Gracanin, A., van Boxtel, R., Wongvipat, J., Dowling, C.M., Gao, D., Begthel, H., Sachs, N. *et al.* (2014) Identification of multipotent luminal progenitor cells in human prostate organoid cultures. *Cell*, **159**, 163–175.
105. Huch, M., Bonfanti, P., Boj, S.F., Sato, T., Loomans, C.J., van de Wetering, M., Sojoodi, M., Li, V.S., Schuijers, J., Gracanin, A. *et al.* (2013) Unlimited in vitro expansion of adult bi-potent pancreas progenitors through the Lgr5/R-spondin axis. *EMBO J.*, **32**, 2708–2721.
106. Eiraku, M., Takata, N., Ishibashi, H., Kawada, M., Sakakura, E., Okuda, S., Sekiguchi, K., Adachi, T. and Sasai, Y. (2011) Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature*, **472**, 51–56.
107. Takahashi, Y., Sato, S., Kurashima, Y., Yamamoto, T., Kurokawa, S., Yuki, Y., Takemura, N., Uematsu, S., Lai, C.Y., Otsu, M. *et al.* (2018) A refined culture system for human induced pluripotent stem cell-derived intestinal epithelial organoids. *Stem Cell Reports*, **10**, 314–328.
108. Deng, W.L., Gao, M.L., Lei, X.L., Lv, J.N., Zhao, H., He, K.W., Xia, X.X., Li, L.Y., Chen, Y.C., Li, Y.P. *et al.* (2018) Gene correction reverses ciliopathy and photoreceptor loss in iPSC-derived retinal organoids from retinitis pigmentosa patients. *Stem Cell Reports*, **10**, 1267–1281.
109. Sharma, T.P., Wiley, L.A., Whitmore, S.S., Anfinson, K.R., Cranston, C.M., Oppedal, D.J., Daggett, H.T., Mullins, R.F., Tucker, B.A. and Stone, E.M. (2017) Patient-specific induced pluripotent stem cells to evaluate the pathophysiology of TRNT1-associated Retinitis pigmentosa. *Stem Cell Res.*, **21**, 58–70.
110. Bartfeld, S., Bayram, T., van de Wetering, M., Huch, M., Begthel, H., Kujala, P., Vries, R., Peters, P.J. and Clevers, H. (2015) In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology*, **148**, 126–136 e126.
111. Guan, Y., Xu, D., Garfin, P.M., Ehmer, U., Hurwitz, M., Enns, G., Michie, S., Wu, M., Zheng, M., Nishimura, T. *et al.* (2017) Human hepatic organoids for the analysis of human genetic diseases. *JCI Insight*, **2**, e94954.
112. Sampaziotis, F., de Brito, M.C., Madrigal, P., Bertero, A., Saeb-Parsy, K., Soares, F.A.C., Schrumpf, E., Melum, E., Karlsen, T.H., Bradley, J.A. *et al.* (2015) Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation. *Nat. Biotechnol.*, **33**, 845–852.
113. Mariani, J., Coppola, G., Zhang, P., Abyzov, A., Provini, L., Tomasini, L., Amenduni, M., Szekely, A., Palejev, D., Wilson, M. *et al.* (2015) FOXG1-dependent dysregulation of GABA/glutamate neuron differentiation in autism spectrum disorders. *Cell*, **162**, 375–390.
114. Hohwieler, M., Illing, A., Hermann, P.C., Mayer, T., Stockmann, M., Perkhofer, L., Eiseler, T., Antony, J.S., Muller, M., Renz, S. *et al.* (2017) Human pluripotent stem cell-derived acinar/ductal organoids generate human pancreas upon orthotopic transplantation and allow disease modelling. *Gut*, **66**, 473–486.
115. Wiley, L.A., Burnight, E.R., DeLuca, A.P., Anfinson, K.R., Cranston, C.M., Kaalberg, E.E., Penticoff, J.A., Affatigato, L.M., Mullins, R.F., Stone, E.M. *et al.* (2016) cGMP production of patient-specific iPSCs and photoreceptor precursor cells to treat retinal degenerative blindness. *Sci. Rep.*, **6**, 30742.
116. Crespo, M., Vilar, E., Tsai, S.Y., Chang, K., Amin, S., Srinivasan, T., Zhang, T., Pipalia, N.H., Chen, H.J., Witherspoon, M. *et al.* (2017) Colonic organoids derived from human induced pluripotent stem cells for modeling colorectal cancer and drug testing. *Nat. Med.*, **23**, 878–884.
117. Adil, M.M., Gaj, T., Rao, A.T., Kulkarni, R.U., Fuentes, C.M., Ramadoss, G.N., Ekman, F.K., Miller, E.W. and Schaffer, D.V. (2018) hPSC-derived striatal cells generated using a scalable 3D hydrogel promote recovery in a Huntington disease mouse model. *Stem Cell Reports*, **10**, 1481–1491.
118. Parfitt, D.A., Lane, A., Ramsden, C.M., Carr, A.J., Munro, P.M., Jovanovic, K., Schwarz, N., Kanuga, N., Muthiah, M.N., Hull, S. *et al.* (2016) Identification and correction of mechanisms underlying inherited blindness in human iPSC-derived optic cups. *Cell Stem Cell*, **18**, 769–781.
119. Jin, M., Pomp, O., Shinoda, T., Toba, S., Torisawa, T., Furuta, K., Oiwa, K., Yasunaga, T., Kitagawa, D., Matsumura, S. *et al.* (2017) Katanin p80, NuMA and cytoplasmic dynein cooperate to control microtubule dynamics. *Sci. Rep.*, **7**, 39902.
120. Bershteyn, M., Nowakowski, T.J., Pollen, A.A., Di Lullo, E., Nene, A., Wynshaw-Boris, A. and Kriegstein, A.R. (2017) Human iPSC-derived cerebral organoids model cellular features of lissencephaly and reveal prolonged mitosis of outer radial glia. *Cell Stem Cell*, **20**, 435–449 e434.

121. Iefremova, V., Manikakis, G., Krefft, O., Jabali, A., Weynans, K., Wilkens, R., Marsoner, F., Brandl, B., Muller, F.J., Koch, P. *et al.* (2017) An organoid-based model of cortical development identifies non-cell-autonomous defects in Wnt signaling contributing to Miller-Dieker syndrome. *Cell Rep.*, **19**, 50–59.
122. Freedman, B.S., Brooks, C.R., Lam, A.Q., Fu, H., Morizane, R., Agrawal, V., Saad, A.F., Li, M.K., Hughes, M.R., Werff, R.V. *et al.* (2015) Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat. Commun.*, **6**, 8715.
123. Mellios, N., Feldman, D.A., Sheridan, S.D., Ip, J.P.K., Kwok, S., Amoah, S.K., Rosen, B., Rodriguez, B.A., Crawford, B., Swaminathan, R. *et al.* (2018) MeCP2-regulated miRNAs control early human neurogenesis through differential effects on ERK and AKT signaling. *Mol. Psychiatry*, **23**, 1051–1065.
124. Feldman, D., Banerjee, A. and Sur, M. (2016) Developmental dynamics of Rett syndrome. *Neural Plast.*, **2016**, 6154080.
125. Birey, F., Andersen, J., Makinson, C.D., Islam, S., Wei, W., Huber, N., Fan, H.C., Metzler, K.R.C., Panagiotakos, G., Thom, N. *et al.* (2017) Assembly of functionally integrated human forebrain spheroids. *Nature*, **545**, 54–59.
126. Mansour, A.A., Goncalves, J.T., Bloyd, C.W., Li, H., Fernandes, S., Quang, D., Johnston, S., Parylak, S.L., Jin, X. and Gage, F.H. (2018) An in vivo model of functional and vascularized human brain organoids. *Nat. Biotechnol.*, **36**, 432–441.
127. DiMasi, J.A., Grabowski, H.G. and Hansen, R.W. (2016) Innovation in the pharmaceutical industry: new estimates of R&D costs. *J. Health Econ.*, **47**, 20–33.
128. Ledford, H. (2011) Translational research: 4 ways to fix the clinical trial. *Nature*, **477**, 526–528.
129. Scannell, J.W., Blanckley, A., Boldon, H. and Warrington, B. (2012) Diagnosing the decline in pharmaceutical R&D efficiency. *Nat. Rev. Drug Discov.*, **11**, 191–200.
130. Scannell, J.W. and Bosley, J. (2016) When quality beats quantity: decision theory, drug discovery, and the reproducibility crisis. *PLoS One*, **11**, e0147215.