CHAPTER **22**

# Induced Pluripotent Stem Cells as Human Disease Models

**John T. Dimos, Irene Griswold-Prenner, Marica Grskovic, Stefan Irion, Charles Johnson** and **Eugeni Vaisberg**

Contents

## 1. INTRODUCTION

Recent advances in reprogramming technologies allow conversion of adult somatic cells into induced pluripotent stem cells (iPSC), permitting generation of disease- and patient-specific stem cell lines. Like embryonic

stem cells, iPSC potentially can be expanded without limits and differentiated into any somatic cell type. This technology potentially allows for any human cell type to be generated at a scale impossible to obtain from primary sources. Previously inaccessible human cell types (*e.g.*, neurons) can now be generated for investigating basic biological and pathological processes. Differentiated cells derived from patients' iPSC are being used to generate disease-specific models that can then be applied in drug discovery assays, drug development applications, toxicology screening and biomarker discovery. The iPSC technology provides human pharmacological and disease-relevant models to increase the biological and pathological context of these translational applications. We review how introducing increased human biological content and context (*i.e.*, patient-derived disease-relevant cells) may improve the probability of success in identifying and developing novel disease modifying drugs.

## 2. iPSC TECHNOLOGY

In a breakthrough advance several years ago, Shinya Yamanaka and colleagues at Kyoto University demonstrated that mouse somatic cells could be reprogrammed to an embryonic-like, pluripotent state by the enforced expression of a defined set of factors [1]. They tested ectopic expression in somatic cells of 24 genes active in embryonic stem cells and found that four factors, Oct4, Sox2, Klf4 and c-Myc together, converted a small percentage of cells to a pluripotent state. The result was cell colonies with morphology and growth characteristics of embryonic stem cells. These cells were named iPSC to reflect that pluripotency was induced in what had been differentiated somatic cells. The iPSC that were selected for the activation of essential pluripotency factors *Nanog* and *Oct4*, or based on morphology alone, were remarkably similar to embryonic stem cells yielding live chimeric mice, some with germ line contribution [2,3]. The most compelling evidence for iPSC having full developmental potential came from tetraploid complementation studies [4–6]. In these experiments, tetraploid blastocysts, which are incapable of progressing through embryonic development, are injected with iPSC to complement or rescue the defective early embryo-like structure. Fertile mice were generated entirely derived from the injected cells. These data show that iPSC can generate adult mice; thus, iPSC are functionally indistinguishable from embryonic stem cells.

The potential of iPSC was recognized immediately, and the technology was rapidly and successfully applied to human cells [7,8]. To date, iPSC derived from a number of patients with specific clinical conditions have been differentiated into disease-relevant cell types, creating ''disease in a dish'' models to facilitate drug development. The availability of nearly

limitless amounts of disease-relevant cell types from patients will likely have huge benefits for drug discovery [9].

## 3. iPSC DERIVATION AND PRODUCTION

Deriving high-quality, comprehensively characterized iPSC in a scalable process is crucial for their use in translational applications. A typical process for iPSC production starts with acquiring a small skin biopsy from an appropriately selected patient. The skin biopsy is used to generate a fibroblast cell line, into which reprogramming factors are introduced (see below). After several weeks in culture, iPSC colonies emerge, are manually selected, purged from any background cells and a cell line is derived. The process is followed by a thorough characterization of iPSC lines, their expansion and storage in a biobank.

To date, human iPSC have been generated from a number of human tissues, including keratinocytes [10,11], hepatocytes [12], adipose-derived stem cells [13,14], neural stem cells [15], astrocytes [16], cord blood [17–19] and amniotic cells [20,21], illustrating the robustness of current reprogramming methods. Dermal fibroblasts remain the chief source of human iPSC and have demonstrated reliability and relatively high efficiency of reprogramming [8,22–26]. Due to its accessibility, peripheral blood is also an attractive source of donor cells [27,28]. It is likely that practical issues (*e.g.*, cell accessibility and limited discomfort of the patient) will determine the choice of starting somatic cell types.

Due to its reliability and relatively high efficiency, retrovirus-mediated transduction remains the most widely used method for delivering reprogramming factors. Genes coding for reprogramming factors delivered in this way are randomly and stably integrated into the genome and could affect the process of reprogramming, the differentiation of iPSC into mature cell types [29] and variability between different iPSC lines from a single patient. Newer methods have been designed to overcome these problems, including excisable vectors [30–32], nonintegrating vectors [33,34], transient plasmid transfections [34,35], direct protein transduction [36], RNA-based Sendai viruses [37–39] and mRNA-based transcription factor delivery [40]. Many considerations such as availability, efficiency, reliability, cost, time and convenience will determine which method will become widely used for a particular application.

The screening of small molecule collections to find compounds that either enhance reprogramming or replace the transcription factors Oct4, Sox2, Klf4 and c-Myc has shown some success [41,42]. Some compounds that have enhanced reprogramming act on chromatin remodeling. Examples are valproic acid (HDAC inhibitor), 5-azacytidine, *N*-phthalyltryptophan (DNA methyltransferase inhibitors) and BIX-01294 (G9a histone

methyl transferase inhibitor) [43–45]. Additional reprogramming enhancers have been found and are believed to inhibit GSK3, TGF-β, Alk5, MEK or others targets [46–48]. Reprogramming transcription factors have been replaced with combinations of small molecules [49–51]. Adult human keratinocytes after transduction with only OCT4 were reprogrammed by treatment with the small molecules shown in Figure 1 [52]. Importantly, reprogramming of adult somatic cells solely with a cocktail of small molecules has not been accomplished.

## 4. DIFFERENTIATION—PROBLEMS AND PROMISE

To use iPSC in drug discovery and human disease modeling, mature differentiated cell type(s) of interest must be generated reliably and consistently [53,54]. The most successful differentiation approaches have been based on models that try to mimic normal embryonic development. A selected set of signaling pathways, including WNT [55], BMP/activin [56], FGF [57] and Notch [58,59], play a major role during this process and, their exact dosage and precise timing allow for the hundreds of individual cell types to be specified. For example, during the development of the human liver, BMP and FGF signaling from nascent cardiac mesoderm directs uncommitted endodermal progenitors into the hepatic lineage [60], while inhibition of the BMP signaling pathway directs these cells into the pancreatic lineage [61–63]. To generate populations of differentiated cell types, the natural process is mimicked as closely as possible under defined conditions. In addition, screening small molecule libraries led to the discovery of many compounds that influence lineage commitment [64–67]. While these approaches show promising results, the maturity of the cells generated has yet to be addressed. Often, differentiated cells will only show an adult phenotype when grown for extended periods of time *in vitro* [68] or *in vivo* [62], and their ability to engraft functionally into animal models is variable between cell types and based on desired maturation/integration endpoints [69,70]. For certain applications, an immature cell type might be sufficient in disease modeling, especially to investigate a developmental disease, or if the physiological phenotype manifests in immature cells. Developing methods to produce and culture differentiated cell types remains an active and productive line of basic investigation.

In principle, iPSC can give rise to all somatic cell types, but in practice, *in vitro* differentiation protocols to date have been developed for only a subset of specific cell types [23,54,71,72]. In many cases, the differentiation process is inefficient and produces cultures with mixed cell types. Developing efficient assays to evaluate such cultures may require either additional cell type sorting or selection, which is often limited by availability

butyric acid          PS48          tranylcypromine

A-83-01          PD0325901          CHIR99021
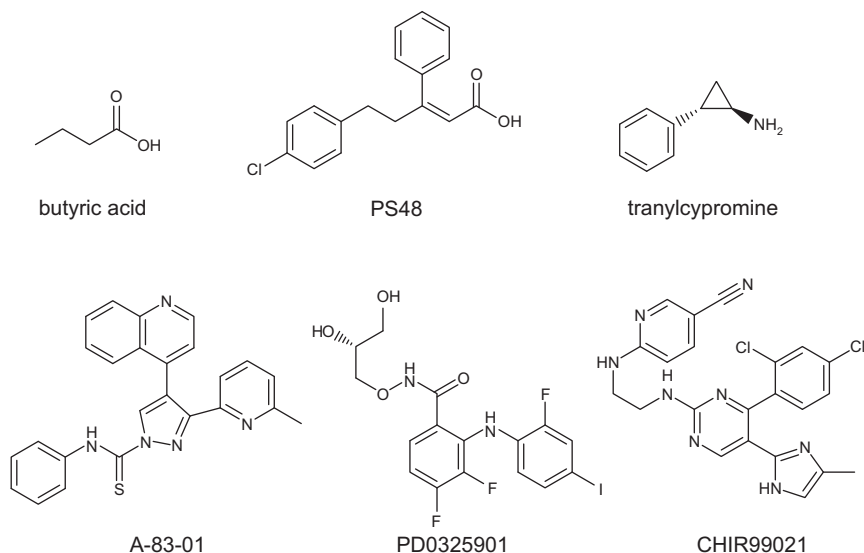
**Figure 1** Small molecules used to enhance reprogramming of human keratinocytes transduced with OCT4 [52].

of selective surface markers or by introduction of reporter systems to facilitate cell sorting or selection. The extent to which iPSC differentiated cell cultures can be or should be homogeneous remains unclear and likely highly situation dependent.

## 5. LEADS FOR DRUG DISCOVERY AND DEVELOPMENT

The identification of a relevant disease phenotype—a molecular or functional difference in the patient-derived differentiated iPSC compared to cells from healthy control individuals—remains a challenge for using iPSC-based models in drug discovery. Identification of a disease-relevant ''phenotype,'' or *in vitro* disease-correlate, provides a cellular model of the pathology in which disease mechanisms can be investigated, and in which agents with therapeutic potential can be identified and tested. These disease models can identify and validate targets for drug discovery and development.

iPSC have been derived from patients with a variety of conditions [22,23,73]. iPSC based disease phenotypes have been identified mainly for monogenic diseases, including spinal muscular atrophy (SMA) [25], fragile X syndrome [74], Hutchinson Gilford Progeria [75], familial dysautonomia (FD) [24], LEOPARD syndrome [26], Rett

syndrome (RTT) [76,77], Long-QT syndrome [78] and multiple liver diseases (familial hypercholesterolemia, glycogen storage disease type 1a and alpha1-antitrypsin deficiency) [73]. More recently, disease phenotypes have been identified in neurons from iPSC from a familial Parkinson's disease patient [79] and in patients with schizophrenia [80], demonstrating that phenotypes in multifactorial diseases may be possible to identify as well. These studies show that disease-relevant cells can be generated from iPSC. Further, these cells can manifest disease phenotypes and are beginning to demonstrate benchmark compound responsiveness under limited testing conditions (see below).

*Use of iPSC in drug discovery under development*

| | |
|---|---|
| Phenotypic screen | Identify hits that alter disease phenotype in human patient affected cells |
| Target ID and target validation | RNAi screens to identify targets that alter disease phenotype in human patient cells |
| Lead optimization | Select between compounds for whole cell activity and compound tracking with disease phenotype modulation |
| Candidate selection | Select between compounds with various mechanisms of action across a panel of patients |
| Tox screening | Test in cardiomyocytes, hepatocytes and neurons for toxic effects |
| Biomarker discovery | Identify biomarkers in human patient affected cells that track with disease and/or compound efficacy |
| Mechanism-based safety | Tests for target activity regulation in nontarget cells |
| Trial cohort selection (''*in vitro* clinical trial'') | Tests PDC in a panel of patient cells to choose potential patient responders |
| IND enabling studies | Supplement or replace animal models of efficacy |
| Personalized medicine | Companion diagnostic |

An example of the application of iPSC in neurodegeneration studies is for SMA, a motor neuron degenerative disorder and the most common cause of infant death by a heritable disease. It is caused by a decrease in survival of motor neuron 1 (SMN1) protein due to deletions in the *SMN1* gene. Although SMN protein is expressed ubiquitously, motor neurons seem most vulnerable in patients, suggesting a specific or additional role

of SMN in motor neurons. Lacking patient motor neurons, researchers have screened for compounds that elevate SMN levels in engineered cell lines and SMA patient fibroblasts. However, mechanisms that control SMN protein expression in fibroblasts may be different than in motor neurons. In the first step in disease model establishment, Ebert and colleagues created iPSC lines from SMA patients [25]. Target protein-rich structures called Gems were detected in iPSC from both healthy and SMA patients, but the number of Gems in SMA iPSC was lower than in healthy iPSC. Two compounds, valproic acid, an HDAC inhibitor, and tobramycin, an aminoglycoside antibiotic, that had both been shown to increase SMN Gems and protein levels in patient fibroblasts [81] were tested in SMA iPSC to determine if reprogramming changed their responsiveness to these compounds. Ebert *et al.* showed that both valproic acid and tobramycin increase Gem numbers and SMN levels in iPSC derived from an SMA patient. They further differentiated iPSC into motor neurons and documented that SMA-iPSC cultures have a decreased number of Gems and motor neurons, indicating that disease-relevant cellular phenotypes can be recapitulated in patient-derived motor neurons. It will be interesting to determine if valproic acid and/or tobramycin also increase SMN levels in their SMA patient-derived motor neurons or if altered mechanisms regulate SMN levels in motor neurons. This work provides additional validation that SMN deficiency is retained after reprogramming and differentiation, and that a disease-relevant phenotype can be identified in neurons from patient-derived iPSC.

Although seeing expected disease phenotypes in differentiated cells from patient-derived iPSC is encouraging, the next challenge will be discovering phenotypes in more complex or idiopathic diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's disease, or type 2 diabetes mellitus. Successful demonstration of cellular pathology *in vitro*, such as TDP-43 displacement from nucleus to cytoplasm in ALS, an increase in hyperphosphorylated microtubule-associated Tau protein in Alzheimer's disease [82], or an insulin resistance phenotype in type 2 diabetes [83], will provide novel and potentially revolutionary opportunities for drug discovery. The use of patient-derived cells is expected to be highly complementary to current drug discovery methodologies, especially where the introduction of high-throughput human pharmacology is needed [84].

## 6. STEM CELL MODULATORS
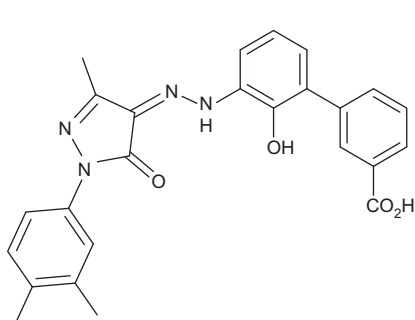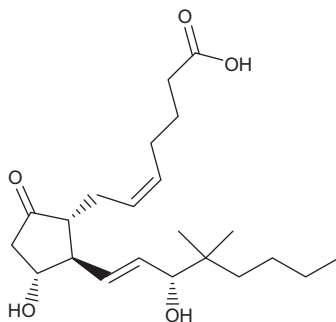


eltrombopag                16,16-dimethylprostaglandin E2

Drugs acting on endogenous stem cells already have shown great success as therapies. For example, the protein erythropoietin (Epogen®) has blockbuster sales and enhances red blood cell production *via* stimulation of blood stem cell (colony-forming unit-erythroid [CFU-E]) conversion. The use of iPSC technology has several possibilities for aiding discovery of drugs that act on endogenous stem cell populations. Such agents have been called stem cell modulators and could have a therapeutic effect by increasing or decreasing stem cell proliferation or by promoting differentiation of endogenous stem cells to a particular mature cell type [85]. Therapeutically targeting endogenous stem cells *in vivo* is greatly enabled by having the ability to produce stem cells from patient-derived iPSC. In principle, endogenous stem cells could be produced from iPSC in the same way that fully differentiated cells are produced, thereby enabling the identification of small molecules for control of stem cell fate. The discovery of eltrombopag shows that manipulating stem cells *in vivo* can be achieved by small molecules. Eltrombopag is an orally bioavailable, nonpeptide agonist of the thrombopoietin receptor [86]. It increases platelet production by stimulating proliferation and differentiation of megakaryocytes from endogenous blood stem cells leading to a therapeutic effect. Eltrombopag activity is specific to human and chimpanzee, underscoring the importance of using human cells in drug discovery.

Another stem cell modulator, 16,16-dimethylprostaglandin E2 (Ft1050), has entered clinical trials for optimizing transplantation of human hematopoietic stem cells from umbilical cord blood [87]. Adult or umbilical cord hematopoietic stem cells (HSC) are routinely transplanted into patients following myeloablative chemotherapy. The number of HSC in cord blood is low, and adults require the blood from two cords for potentially successful reconstitution of the blood system. The clinical trial underway briefly treats cord blood before transplantation with 16,16-dimethylprostaglandin E2 to activate the endogenous blood

stem cells. Since the efficiency of reconstitution is limited, improved homing, engraftment or proliferation could require less material in transplantation.

## 7. PREDICTIVE TOXICOLOGY WITH iPSC

The use of *in vitro* generated, patient-derived mature cells offers the opportunity to establish predictive models for cardio-, hepato- and neurotoxicology [88–90]. Using iPSC technology, cardiomyocytes, hepatocytes and neurons can be produced at scale from distinct patient populations and used in standard toxicology assays. Using such a renewable source should result in lower variability than preparations derived from human cadavers [91,92]. The increased use of human cells in discovery and development is expected to predict toxic effects and efficacy early in the discovery process. For example, human pooled primary liver microsomes are used for metabolic stability prediction [93]. Primary cells are difficult to obtain; however, iPSC-derived hepatic cells are a renewable source that will accelerate evaluation in human cells. Human cell toxicology is more predictive of clinical responses than transformed or primary animal cells [94]. An unlimited source of cells from various tissues with known genotypes will likely accelerate human cell-based assays. Now that iPSC-derived cardiomyocytes have become commercially available, the performance of these cells can be compared to current standards. In addition, the opportunity to obtain cardiomyocytes, hepatocytes, and neuronal cell types from the same individual offers a new angle on drug toxicity evaluation. The pharmaceutical industry must fully vet this technology before it can be reviewed by regulatory agencies as a complement or alternative to long-used and established *in vitro* toxicology systems [95].

## 8. *IN VITRO* CLINICAL TRIAL

Drug candidates are identified and optimized using nonclinical models, which generally encompass a small number of human or animal cell lines, animal efficacy and toxicology studies. This paradigm, for all its success, poses a significant liability: drug candidates remain isolated from the diversity and heterogeneity of human systems and populations until tested in a traditional clinical trial. In other words, potential drugs and human pharmacology do not collide until a clinical trial. This pipeline structure, regardless of thoughtful milestones, allows a large number of drug candidates to fail after many millions of dollars have been spent on their optimization, development and clinical testing. Most trials that fail do so for one of two main reasons: (1) lack of efficacy in the selected

patient cohort or (2) adverse effects and safety concerns. A preclinical, disease-relevant human pharmacology model that identifies, optimizes, and selects drug candidates could mitigate these risks. The iPSC technology may enable ''*in vitro* clinical trials.''

An *in vitro* clinical trial would allow a development candidate to be tested across a broad and diverse cohort of patient samples for activity. Cell-based assays would assess a compound's ability to modify a disease phenotype *in vitro* in patient-derived disease-relevant cells. This *in vitro* clinical trial could test a single or small number of candidate compounds at multiple concentrations, including vehicle only, across a panel of cell lines designed to represent potential patient cohorts for an eventual clinical trial. This design reduces the number of samples needed as all testing is internally controlled and longitudinal, and has potential to predict clinical efficacy years before costly actual clinical trials are conducted. As importantly, this design would allow for refinement of the target patient population, and clinical trials could be better focused on patients with disease subtypes more likely to respond to treatment.

Patient populations differ not only with respect to disease subtype but also in general genetic background. Estimates from the international Haplotype Mapping Project suggest that there are about 10 million single nucleotide polymorphisms in the human population, in addition to copy number variations, deletions, insertions, inversions and epigenetic differences. Regulatory agency-approved nonclinical toxicology provides good prediction of possible clinical toxicity, but iPSC technology may provide an expanded toxicology evaluation using a human pharmacological model. In addition to testing a compound's activity on a broad sampling of patient-derived disease-relevant cells, cells important for drug metabolism could also be generated. For example, a carefully selected cohort of patients representing the major p450 isotypes could be used to generate hepatocytes for metabolic and toxicity profiling. As with testing compound activity for disease modification, this iPSC patient focused approach allows for testing on a broad range of patient cells for toxicological problems from drug–drug interactions years before they would normally be uncovered in a costly clinical trial.

## 9. PERSONALIZED MEDICINE: PATIENT PROFILING FOR OPTIMAL DRUG EFFICACY

Widespread industrialization of iPSC could enable this technology in a personalized medicine context. Specific differentiated cells could be used to screen for drug toxicity, efficacy and drug–drug interaction on that patient's own cells. If performed routinely, especially for drugs that show fatal adverse reactions, this *in vitro* prescreening might mitigate these

risks. Additionally, the most effective or least toxic drug could be selected from a panel of available treatments prior to trial-and-error testing in patients. This scenario requires a dramatic reduction in the time it takes to generate such a screening panel. In addition, the cost of such an individualized approach might be overwhelming and a panel of iPSC lines that covers the majority of a given patient population might be a suitable alternative. Regardless of how this eventually might be implemented, iPSC technology potentially can allow for the discovery, development and selection of the right drug for the right patient.

## 10. CONCLUSION AND THE ROLE OF SMALL MOLECULE CHEMISTRY

Small molecule medicinal chemistry will likely be impacted and perhaps be changed by iPSC technology. The search is already underway to find small molecules to enhance or replace genetic-based factor delivery for cellular reprogramming. Chemically reprogrammed cells could be used with fewer concerns of genomic integration. These cells may be safe for human transplantation and useful for regenerative medicine applications. Development of small molecule compounds for reprogramming, stabilization of the pluripotent state and differentiation will benefit iPSC technology. For differentiation of iPSC, the stability, cost and specificity of small molecules have an advantage. Many cell types have yet to be produced by *in vitro* differentiation: small molecules could have a large impact in expanding access to more cell types. For traditional small molecule drug discovery, the ability to execute HTS in disease-specific human cells could be transformative. With sufficient development, iPSC-derived panels of patient cells should improve selection of efficacious compounds for clinical trials, as well as selecting those patients who would benefit from specific drugs. For toxicology screening, human cardiomyocytes and other cell types involved in safety assessment of new compounds are available today. In the near future, large panels of many types of cells from many types of patients could assist in more rapid toxicology assessment of new chemical entities. The promise of iPSC technology is to use human cell phenotypic assays to conduct more efficient and reliable small molecule medicinal chemistry due to increased biological and disease input.

## ACKNOWLEDGMENT

## REFERENCES

[1] K. Takahashi and S. Yamanaka, *Cell*, 2006, **126**, 663.

[2] K. Okita, T. Ichisaka and S. Yamanaka, *Nature*, 2007, **448**, 313.

[3] A. Meissner, M. Wernig and R. Jaenisch, *Nat. Biotechnol.*, 2007, **25**, 1177.

[4] M. J. Boland, J. L. Hazen, K. L. Nazor, A. R. Rodriguez, W. Gifford, G. Martin, S. Kupriyanov and K. K. Baldwin, *Nature*, 2009, **461**, 91.

[5] L. Kang, J. Wang, Y. Zhang, Z. Kou and S. Gao, *Cell Stem Cell*, 2009, **5**, 135.

[6] X. Y. Zhao, W. Li, Z. Lv, L. Liu, M. Tong, T. Hai, J. Hao, C. L. Guo, Q. W. Ma, L. Wang, F. Zeng and Q. Zhou, *Nature*, 2009, **461**, 86.

[7] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda and S. Yamanaka, *Cell*, 2007, **131**, 861.

[8] J. Yu, M. A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J. L. Frane, S. Tian, J. Nie, G. A. Jonsdottir, V. Ruotti, R. Stewart, I. I. Slukvin and J. A. Thomson, *Science*, 2007, **318**, 1917.

[9] G. P. Nolan, *Nat. Chem. Biol.*, 2007, **3**, 187.

[10] T. Aasen, A. Raya, M. J. Barrero, E. Garreta, A. Consiglio, F. Gonzalez, R. Vassena, J. Bilic, V. Pekarik, G. Tiscornia, M. Edel, S. Boue and J. C. Izpisua Belmonte, *Nat. Biotechnol.*, 2008, **26**, 1276.

[11] B. W. Carey, S. Markoulaki, J. Hanna, K. Saha, Q. Gao, M. Mitalipova and R. Jaenisch, *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 157.

[12] H. Liu, Z. Ye, Y. Kim, S. Sharkis and Y. Y. Jang, *Hepatology*, 2010, **51**, 1810.

[13] N. Sun, N. J. Panetta, D. M. Gupta, K. D. Wilson, A. Lee, F. Jia, S. Hu, A. M. Cherry, R. C. Robbins, M. T. Longaker and J. C. Wu, *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 15720.

[14] T. Aoki, H. Ohnishi, Y. Oda, M. Tadokoro, M. Sasao, H. Kato, K. Hattori and H. Ohgushi, *Tissue Eng. A*, 2010, **16**, 2197.

[15] J. B. Kim, B. Greber, M. J. Arauzo-Bravo, J. Meyer, K. I. Park, H. Zaehres and H. R. Scholer, *Nature*, 2009, **461**, 649.

[16] S. Ruiz, K. Brennand, A. D. Panopoulos, A. Herrerias, F. H. Gage and J. C. Izpisua-Belmonte, *PLoS One*, 2010, **5**, e15526.

[17] A. Haase, R. Olmer, K. Schwanke, S. Wunderlich, S. Merkert, C. Hess, R. Zweigerdt, I. Gruh, J. Meyer, S. Wagner, L. S. Maier, D. W. Han, S. Glage, K. Miller, P. Fischer, H. R. Scholer and U. Martin, *Cell Stem Cell*, 2009, **5**, 434.

[18] A. Giorgetti, N. Montserrat, T. Aasen, F. Gonzalez, I. Rodriguez-Piza, R. Vassena, A. Raya, S. Boue, M. J. Barrero, B. A. Corbella, M. Torrabadella, A. Veiga and J. C. Izpisua Belmonte, *Cell Stem Cell*, 2009, **5**, 353.

[19] S. Eminli, A. Foudi, M. Stadtfeld, N. Maherali, T. Ahfeldt, G. Mostoslavsky, H. Hock and K. Hochedlinger, *Nat. Genet.*, 2009, **41**, 968.

[20] C. Li, J. Zhou, G. Shi, Y. Ma, Y. Yang, J. Gu, H. Yu, S. Jin, Z. Wei, F. Chen and Y. Jin, *Hum. Mol. Genet.*, 2009, **18**, 4340.

[21] H. X. Zhao, Y. Li, H. F. Jin, L. Xie, C. Liu, F. Jiang, Y. N. Luo, G. W. Yin, Y. Li, J. Wang, L. S. Li, Y. Q. Yao and X. H. Wang, *Differentiation*, 2010, **80**, 123.

[22] I. H. Park, N. Arora, H. Huo, N. Maherali, T. Ahfeldt, A. Shimamura, M. W. Lensch, C. Cowan, K. Hochedlinger and G. Q. Daley, *Cell*, 2008, **134**, 877.

[23] J. T. Dimos, K. T. Rodolfa, K. K. Niakan, L. M. Weisenthal, H. Mitsumoto, W. Chung, G. F. Croft, G. Saphier, R. Leibel, R. Goland, H. Wichterle, C. E. Henderson and K. Eggan, *Science*, 2008, **321**, 1218.

[24] G. Lee, E. P. Papapetrou, H. Kim, S. M. Chambers, M. J. Tomishima, C. A. Fasano, Y. M. Ganat, J. Menon, F. Shimizu, A. Viale, V. Tabar, M. Sadelain and L. Studer, *Nature*, 2009, **461**, 402.

[25] A. D. Ebert, J. Yu, F. F. Rose Jr., V. B. Mattis, C. L. Lorson, J. A. Thomson and C. N. Svendsen, *Nature*, 2009, **457**, 277.

[26] X. Carvajal-Vergara, A. Sevilla, S. L. D'Souza, Y. S. Ang, C. Schaniel, D. F. Lee, L. Yang, A. D. Kaplan, E. D. Adler, R. Rozov, Y. Ge, N. Cohen, L. J. Edelmann, B. Chang, A. Waghray, J. Su, S. Pardo, K. D. Lichtenbelt, M. Tartaglia, B. D. Gelb and I. R. Lemischka, *Nature*, 2010, **465**, 808.
[27] Y. H. Loh, S. Agarwal, I. H. Park, A. Urbach, H. Huo, G. C. Heffner, K. Kim, J. D. Miller, K. Ng and G. Q. Daley, *Blood*, 2009, **113**, 5476.
[28] Y. H. Loh, O. Hartung, H. Li, C. Guo, J. M. Sahalie, P. D. Manos, A. Urbach, G. C. Heffner, M. Grskovic, F. Vigneault, M. W. Lensch, I. H. Park, S. Agarwal, G. M. Church, J. J. Collins, S. Irion and G. Q. Daley, *Cell Stem Cell*, 2010, **7**, 15.
[29] C. A. Sommer, A. G. Sommer, T. A. Longmire, C. Christodoulou, D. D. Thomas, M. Gostissa, F. W. Alt, G. J. Murphy, D. N. Kotton and G. Mostoslavsky, *Stem Cells*, 2010, **28**, 64.
[30] K. Kaji, K. Norrby, A. Paca, M. Mileikovsky, P. Mohseni and K. Woltjen, *Nature*, 2009, **458**, 771.
[31] A. Lacoste, F. Berenshteyn and A. H. Brivanlou, *Cell Stem Cell*, 2009, **5**, 332.
[32] K. Woltjen, I. P. Michael, P. Mohseni, R. Desai, M. Mileikovsky, R. Hamalainen, R. Cowling, W. Wang, P. Liu, M. Gertsenstein, K. Kaji, H. K. Sung and A. Nagy, *Nature*, 2009, **458**, 766.
[33] M. Stadtfeld, M. Nagaya, J. Utikal, G. Weir and K. Hochedlinger, *Science*, 2008, **322**, 945.
[34] J. Yu, K. Hu, K. Smuga-Otto, S. Tian, R. Stewart, I. I. Slukvin and J. A. Thomson, *Science*, 2009, **324**, 797.
[35] F. Jia, K. D. Wilson, N. Sun, D. M. Gupta, M. Huang, Z. Li, N. J. Panetta, Z. Y. Chen, R. C. Robbins, M. A. Kay, M. T. Longaker and J. C. Wu, *Nat. Methods*, 2010, **7**, 197.
[36] D. Kim, C. H. Kim, J. I. Moon, Y. G. Chung, M. Y. Chang, B. S. Han, S. Ko, E. Yang, K. Y. Cha, R. Lanza and K. S. Kim, *Cell Stem Cell*, 2009, **4**, 472.
[37] N. Fusaki, H. Ban, A. Nishiyama, K. Saeki and M. Hasegawa, *Proc. Jpn. Acad. Ser. B Phys. Biol. Sci.*, 2009, **85**, 348.
[38] T. Seki, S. Yuasa, M. Oda, T. Egashira, K. Yae, D. Kusumoto, H. Nakata, S. Tohyama, H. Hashimoto, M. Kodaira, Y. Okada, H. Seimiya, N. Fusaki, M. Hasegawa and K. Fukuda, *Cell Stem Cell*, 2010, **7**, 11.
[39] K. Nishimura, M. Sano, M. Ohtaka, B. Furuta, Y. Umemura, Y. Nakajima, Y. Ikehara, T. Kobayashi, H. Segawa, S. Takayasu, H. Sato, K. Motomura, E. Uchida, T. Kanayasu-Toyoda, M. Asashima, H. Nakauchi, T. Yamaguchi and M. Nakanishi, *J. Biol. Chem.*, 2011, **286**, 4760.
[40] L. Warren, P. D. Manos, T. Ahfeldt, Y. H. Loh, H. Li, F. Lau, W. Ebina, P. K. Mandal, Z. D. Smith, A. Meissner, G. Q. Daley, A. S. Brack, J. J. Collins, C. Cowan, T. M. Schlaeger and D. J. Rossi, *Cell Stem Cell*, 2010, **7**, 618.
[41] M. Stadtfeld and K. Hochedlinger, *Genes Dev.*, 2010, **24**, 2239.
[42] A. Kochegarov, *Expert. Opin. Ther. Pat*, 2009, **19**, 275.
[43] T. S. Mikkelsen, J. Hanna, X. Zhang, M. Ku, M. Wernig, P. Schorderet, B. E. Bernstein, R. Jaenisch, E. S. Lander and A. Meissner, *Nature*, 2008, **454**, 49.
[44] W. Li, H. Zhou, R. Abujarour, S. Zhu, J. J. Young, T. Lin, E. Hao, H. R. Scholer, A. Hayek and S. Ding, *Stem Cells*, 2009, **27**, 2992.
[45] Y. Shi, J. T. Do, C. Desponts, H. S. Hahm, H. R. Scholer and S. Ding, *Cell Stem Cell*, 2008, **2**, 525.
[46] J. Yu, K. F. Chau, M. A. Vodyanik, J. Jiang and Y. Jiang, *PLoS One*, 2011, **6**, e17557.
[47] J. K. Ichida, J. Blanchard, K. Lam, E. Y. Son, J. E. Chung, D. Egli, K. M. Loh, A. C. Carter, F. P. Di Giorgio, K. Koszka, D. Huangfu, H. Akutsu, D. R. Liu, L. L. Rubin and K. Eggan, *Cell Stem Cell*, 2009, **5**, 491.
[48] T. Lin, R. Ambasudhan, X. Yuan, W. Li, S. Hilcove, R. Abujarour, X. Lin, H. S. Hahm, E. Hao, A. Hayek and S. Ding, *Nat. Methods*, 2009, **6**, 805.

[49] C. A. Lyssiotis, R. K. Foreman, J. Staerk, M. Garcia, D. Mathur, S. Markoulaki, J. Hanna, L. L. Lairson, B. D. Charette, L. C. Bouchez, M. Bollong, C. Kunick, A. Brinker, C. Y. Cho, P. G. Schultz and R. Jaenisch, *Proc. Natl. Acad. Sci. USA*, 2009, **106**, 8912.

[50] W. Li, W. Wei, S. Zhu, J. Zhu, Y. Shi, T. Lin, E. Hao, A. Hayek, H. Deng and S. Ding, *Cell Stem Cell*, 2009, **4**, 16.

[51] Y. Shi, C. Desponts, J. T. Do, H. S. Hahm, H. R. Scholer and S. Ding, *Cell Stem Cell*, 2008, **3**, 568.

[52] S. Zhu, W. Li, H. Zhou, W. Wei, R. Ambasudhan, T. Lin, J. Kim, K. Zhang and S. Ding, *Cell Stem Cell*, 2010, **7**, 651.

[53] S. Irion, M. C. Nostro, S. J. Kattman and G. M. Keller, *Cold Spring Harb. Symp. Quant. Biol.*, 2008, **73**, 101.

[54] G. Keller, *Genes Dev.*, 2005, **19**, 1129.

[55] C. Y. Logan and R. Nusse, *Annu. Rev. Cell Dev. Biol.*, 2004, **20**, 781.

[56] A. F. Schier, *Annu. Rev. Cell Dev. Biol.*, 2003, **19**, 589.

[57] L. Niswander and G. R. Martin, *Development*, 1992, **114**, 755.

[58] A. Aulehla and O. Pourquie, *Curr. Opin. Cell Biol.*, 2008, **20**, 632.

[59] J. Liu, C. Sato, M. Cerletti and A. Wagers, *Curr. Top. Dev. Biol.*, 2010, **92**, 367.

[60] V. Gouon-Evans, L. Boussemart, P. Gadue, D. Nierhoff, C. I. Koehler, A. Kubo, D. A. Shafritz and G. Keller, *Nat. Biotechnol.*, 2006, **24**, 1402.

[61] K. S. Zaret and M. Grompe, *Science*, 2008, **322**, 1490.

[62] E. Kroon, L. A. Martinson, K. Kadoya, A. G. Bang, O. G. Kelly, S. Eliazer, H. Young, M. Richardson, N. G. Smart, J. Cunningham, A. D. Agulnick, K. A. D'Amour, M. K. Carpenter and E. E. Baetge, *Nat. Biotechnol.*, 2008, **26**, 443.

[63] M. C. Nostro, F. Sarangi, S. Ogawa, A. Holtzinger, G. Corneo, X. Li, S. J. Micallef, I. H. Park, C. Basford, M. B. Wheeler, G. Q. Daley, A. G. Elefanty, E. G. Stanley and G. Keller, *Development*, 2011, **138**, 861.

[64] A. I. Lukaszewicz, M. K. McMillan and M. Kahn, *J. Med. Chem.*, 2010, **53**, 3439.

[65] Y. Xu, Y. Shi and S. Ding, *Nature*, 2008, **453**, 338.

[66] C. A. Lyssiotis, L. L. Lairson, A. E. Boitano, H. Wurdak, S. Zhu and P. G. Schultz, *Angew. Chem. Int. Ed. Engl.*, 2011, **50**, 200.

[67] A. J. Firestone and J. K. Chen, *ACS Chem. Biol.*, 2010, **5**, 15.

[68] C. Qiu, E. N. Olivier, M. Velho and E. E. Bouhassira, *Blood*, 2008, **111**, 2400.

[69] M. Rubart and L. J. Field, *Nat. Biotechnol.*, 2007, **25**, 993.

[70] D. James, H. S. Nam, M. Seandel, D. Nolan, T. Janovitz, M. Tomishima, L. Studer, G. Lee, D. Lyden, R. Benezra, N. Zaninovic, Z. Rosenwaks, S. Y. Rabbany and S. Rafii, *Nat. Biotechnol.*, 2010, **28**, 161.

[71] S. M. Chambers, C. A. Fasano, E. P. Papapetrou, M. Tomishima, M. Sadelain and L. Studer, *Nat. Biotechnol.*, 2009, **27**, 275.

[72] F. Soldner, D. Hockemeyer, C. Beard, Q. Gao, G. W. Bell, E. G. Cook, G. Hargus, A. Blak, O. Cooper, M. Mitalipova, O. Isacson and R. Jaenisch, *Cell*, 2009, **136**, 964.

[73] S. T. Rashid, S. Corbineau, N. Hannan, S. J. Marciniak, E. Miranda, G. Alexander, I. Huang-Doran, J. Griffin, L. Ahrlund-Richter, J. Skepper, R. Semple, A. Weber, D. A. Lomas and L. Vallier, *J. Clin. Invest.*, 2010, **120**, 3127.

[74] A. Urbach, O. Bar-Nur, G. Q. Daley and N. Benvenisty, *Cell Stem Cell*, 2010, **6**, 407.

[75] J. Zhang, Q. Lian, G. Zhu, F. Zhou, L. Sui, C. Tan, R. A. Mutalif, R. Navasankari, Y. Zhang, H. F. Tse, C. L. Stewart and A. Colman, *Cell Stem Cell*, 2011, **8**, 31.

[76] M. C. Marchetto, C. Carromeu, A. Acab, D. Yu, G. W. Yeo, Y. Mu, G. Chen, F. H. Gage and A. R. Muotri, *Cell*, 2010, **143**, 527.

[77] A. R. Muotri, M. C. Marchetto, N. G. Coufal, R. Oefner, G. Yeo, K. Nakashima and F. H. Gage, *Nature*, 2010, **468**, 443.

[78] A. Moretti, M. Bellin, A. Welling, C. B. Jung, J. T. Lam, L. Bott-Flugel, T. Dorn, A. Goedel, C. Hohnke, F. Hofmann, M. Seyfarth, D. Sinnecker, A. Schomig and K. L. Laugwitz, *N. Engl. J. Med.*, 2010, **363**, 1397.

[79] H. N. Nguyen, B. Byers, B. Cord, A. Shcheglovitov, J. Byrne, P. Gujar, K. Kee, B. Schule, R. E. Dolmetsch, W. Langston, T. D. Palmer and R. R. Pera, *Cell Stem Cell*, 2011, **8**, 267.

[80] K. J. Brennand, A. Simone, J. Jou, C. Gelboin-Burkhart, N. Tran, S. Sangar, Y. Li, Y. Mu, G. Chen, D. Yu, S. McCarthy, J. Sebat and F. H. Gage, *Nature*, 2011, **473**, 221.

[81] E. C. Wolstencroft, V. Mattis, A. A. Bajer, P. J. Young and C. L. Lorson, *Hum. Mol. Genet.*, 2005, **14**, 1199.

[82] I. Grundke-Iqbal, K. Iqbal, Y. C. Tung, M. Quinlan, H. M. Wisniewski and L. I. Binder, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 4913.

[83] K. F. Petersen, S. Dufour, D. B. Savage, S. Bilz, G. Solomon, S. Yonemitsu, G. W. Cline, D. Befroy, L. Zemany, B. B. Kahn, X. Papademetris, D. L. Rothman and G. I. Shulman, *Proc. Natl. Acad. Sci. USA*, 2007, **104**, 12587.

[84] S. M. Paul, D. S. Mytelka, C. T. Dunwiddie, C. C. Persinger, B. H. Munos, S. R. Lindborg and A. L. Schacht, *Nat. Rev. Drug Discov.*, 2010, **9**, 203.

[85] P. Grayson, J. Mendlein, S. Thies and J. Yingling, *Drug Discov. Today*, 2009, **6**, 141.

[86] C. L. Erickson-Miller, E. Delorme, S. S. Tian, C. B. Hopson, A. J. Landis, E. I. Valoret, T. S. Sellers, J. Rosen, S. G. Miller, J. I. Luengo, K. J. Duffy and J. M. Jenkins, *Stem Cells*, 2009, **27**, 424.

[87] T. E. North, W. Goessling, C. R. Walkley, C. Lengerke, K. R. Kopani, A. M. Lord, G. J. Weber, T. V. Bowman, I. H. Jang, T. Grosser, G. A. FitzGerald, G. Q. Daley, S. H. Orkin and L. I. Zon, *Nature*, 2007, **447**, 1007.

[88] S. J. Kattman, C. H. Koonce, B. J. Swanson and B. D. Anson, *J. Cardiovasc. Transl. Res.*, 2011, **4**, 66.

[89] R. Pal, M. K. Mamidi, D. A. Kumar and R. Bhonde, *J. Cell Physiol.*, 2011, **226**, 1583.

[90] S. Greenhough, C. N. Medine and D. C. Hay, *Toxicology*, 2010, **278**, 250.

[91] A. Guillouzo, *Environ. Health Perspect.*, 1998, **106**(Suppl. 2), 511.

[92] R. Gebhardt, J. G. Hengstler, D. Muller, R. Glockner, P. Buenning, B. Laube, E. Schmelzer, M. Ullrich, D. Utesch, N. Hewitt, M. Ringel, B. R. Hilz, A. Bader, A. Langsch, T. Koose, H. J. Burger, J. Maas and F. Oesch, *Drug Metab. Rev.*, 2003, **35**, 145.

[93] D. Dalvie, R. S. Obach, P. Kang, C. Prakash, C. M. Loi, S. Hurst, A. Nedderman, L. Goulet, E. Smith, H. Z. Bu and D. A. Smith, *Chem. Res. Toxicol.*, 2009, **22**, 357.

[94] A. Trevisan, A. Nicolli and F. Chiara, *Expert Opin. Drug Metab Toxicol.*, 2010, **6**, 1451.

[95] K. Vojnits and S. Bremer, *Toxicology*, 2010, **270**, 10.