Review

For reprint orders, please contact: reprints@futuremedicine.com

Regenerative Medicine

Pluripotent stem cells: the last 10 years

Erin A Kimbrel & Robert Lanza*,[1]

[1]Astellas Institute for Regenerative Medicine, 33 Locke Drive, Marlborough, MA 01752, USA

*Author for correspondance: robert.lanza@astellas.com

Pluripotent stem cells (PSCs) can differentiate into virtually any cell type in the body, making them attractive for both regenerative medicine and drug discovery. Over the past 10 years, technological advances and innovative platforms have yielded first-in-man PSC-based clinical trials and opened up new approaches for disease modeling and drug development. Induced PSCs have become the foremost alternative to embryonic stem cells and accelerated the development of disease-in-a-dish models. Over the years and with each new discovery, PSCs have proven to be extremely versatile. This review article highlights key advancements in PSC research, from 2006 to 2016, and how they will guide the direction of the field over the next decade.

First draft submitted: 26 August 2016; Accepted for publication: 4 October 2016; Published online: 2 December 2016

Keywords: clinical trials • disease-in-a-dish models • drug screening • embryonic stem cells • gene editing • induced pluripotent stem cells

The evolution of technologies for generating pluripotent stem cells, 2006–2016

At the start of the decade, it had been 8 years since the initial isolation of human embryonic stem cells (hESCs) [1] and incremental scientific progress was being made. However, ethical dilemmas regarding the use and/or destruction of human embryos (which occurs during the most commonly used hESC derivation method) as well as legislative barriers in several countries hindered hESC research endeavors [2]. Moreover, the need to source several hundred embryos for the creation of hESC lines to cover the diversity of HLA phenotypes made clinical translation of embryonic stem cell (ESC) based therapies seem difficult [3]. This situation precipitated major initiatives to find alternatives (Figure 1). Single blastomere technology is one such alternative; it was developed in 2006 as a nondestructive ESC derivation method and was first demonstrated for mouse ESCs [4], then adapted for human ESCs in the same year [5]. With this technique, a single cell or 'blastomere' is isolated from a morula (8-cell) stage embryo and, after culture and expansion, can give rise to an ESC line. Removal of a single cell has been shown not to interfere with the ability of the remaining embryo to grow and divide normally [4,5]; it was adapted from a single blastomere biopsy process that had been used by *in vitro* fertilization clinics for pre-implantation genetic diagnostics since the 1990s [6]. Today, pre-implantation genetic diagnostics is routinely performed using later stage embryos as it poses less risk to the developing embryo than biopsying the 8-cell stage [7]. As such, the availability of pronuclear and multicell stage embryos for nondestructive hESC derivation is rather low and despite the success of this technique by other groups [8], single blastomere technology is not widely used in research today (although it worth noting that six of 14 hESC-based clinical trials currently listed on clinicaltrials.gov involves the use of a single-blastomere-derived line, MA09).

Future Medicine part of fsg

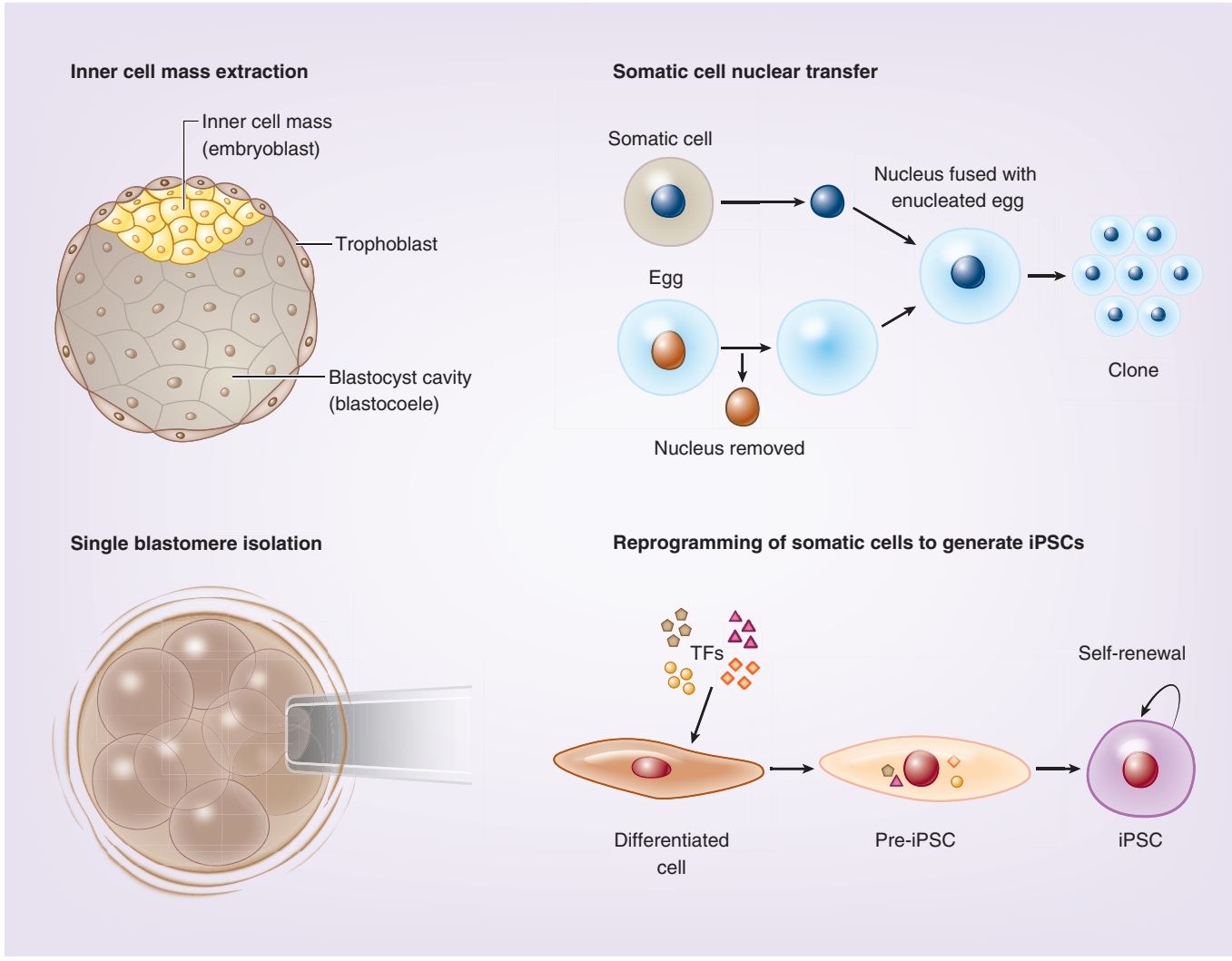


Figure 1. Four technologies for pluripotent stem cell generation.
iPSC: Induced pluripotent stem cell.

Somatic cell nuclear transfer (SCNT) is another alternative for generating hESCs without the destruction of naturally made embryos. This technique has been used successfully in other species such as calves, pigs and mice since the late 1990s and early 2000s [9–11], yet for various reasons including the availability of federal funding, institutional review board (IRB) requirements and public sentiment, it took until 2013 for it to be successfully applied to humans [12]. In SCNT, the nucleus of an unfertilized egg is removed and replaced with the nucleus from a somatic cell. Precise culture conditions coupled with maternal factors within the egg promote the reprogramming of the somatic cell nucleus back to a pluripotent state and can give rise to an ESC line. The first report of human SCNT hESCs used fetal and infant somatic cells as nuclear donors, while a second report used adult cells from 35- and 75-year-old males to successfully derive karyotypically normal SCNT hESC lines [13], thus demonstrating that reprogramming is possible irrespective of the age of the somatic cell donor. Despite these successes, SCNT has not been widely used for ESC derivation due to the need for high-quality eggs and precise microsurgical techniques. Moreover, the requirement for egg donation is a significant barrier to its widespread use. While only a few labs have been able to successfully generate karyotypically normal human SCNT hESCs to date [12–14], further attempts to derive SCNT hESCs are still underway. Just recently, in mid-2016, the Korean Government granted CHA University the right to use 600 cryopreserved eggs in SCNT research in order to generate hESCs that can be used to help find cures for incurable diseases [15]. As researchers are finding ways to improve the efficiency of SCNT [16], it may become a preferred method for the generation of hESCs in the future. Indeed, as far back as the 1960s, SCNT in other

species was shown to completely erase lineage-specific signatures in somatic nuclei and reprogram them to a totipotent state [17,18]. SCNT could be used to create banks of HLA-matched hESCs to cover the diversity of HLA types in the human population, especially in countries such as Korea or Japan, where this could be achieved for a significant proportion of the population using a small bank of HLA-homozygous cell lines.

Emergence & optimization of induced PSC technology

Arguably the most important alternative to conventional methods for hESC generation was the invention of induced PSC (iPSC) technology in 2006 [19] and its application to human cells in 2007 [20,21]. iPSC technology avoids the use and destruction of human eggs and/or embryos altogether, thereby largely circumventing ethical controversy. iPSCs are generated through the reprogramming of somatic cells back to an embryonic-like state; the addition of exogenous reprogramming factors triggers this reprogramming process. iPSC technology revolutionized the field of PSC research and led to a 2012 joint Nobel Prize for iPSC pioneer, Shinya Yamanaka, and John Gurdon, for his previous groundwork in exploring the biological principle of reprogramming [22].

Today, generating iPSCs takes many shapes and forms, with different reprogramming factors, different methods for introducing factors to cells, different starting cell types, among others. The technology has undergone a fascinating evolution from its first report in 2006 to the present day and it will continue to evolve in years to come. The first reports of human iPSC (hiPSC) generation by Yamanaka and colleagues used Oct4, Sox2, Klf4 and the proto-oncogene, c-myc to reprogram human dermal fibroblasts using retroviruses [20]. In considering the application of iPSCs for clinical use, these and other early iPSC studies highlighted two important safety issues that would steer iPSC research in the years that followed: any cocktail of reprogramming factors should avoid the use of a proto-oncogene such as c-myc, since it confers a risk of developing tumors if its expression is re-activated and nonintegrating reprogramming methods should be developed to avoid the mutagenic risks associated with viral insertion into the genome. A month after the first human iPSC paper was published, another group showed that indeed, the use of c-myc could be avoided when generating human iPSCs. This group still used Oct4 and Sox2, but replaced c-myc as well as Klf4 with Nanog and Lin28, thus removing the risk of using a proto-oncogene for reprogramming [23]. Several other groups followed suit and began experimenting with different combinations of reprogramming factors and different types of starting somatic cells [24–28]; it soon appeared that various combinations of factors and various starting somatic cell types could be used to generate iPSCs.

Within a couple of years, nonintegrating reprogramming methods were being reported as well. Nowadays, mRNA [29], recombinant proteins [30], episomes [31,32], mini-circles [33], PiggyBac transposons [34] and Sendai virus [35] have all been used to generate so called second-generation iPSCs. In addition, small molecules, such as methyltransferase inhibitors such as 50-azacytidine and RG108 and/or histone deacetylase inhibitors such as valproic acid have been found to enhance reprogramming efficiency when used in combination with the typical cocktails of genetic factors [36,37]. A mixture of seven small molecules alone (without any genetic factors) has also been reported to reprogram mouse somatic cells [38] suggesting that a chemical approach may also work for generating human iPSCs. These second-generation reprogramming methods not only avoid the risk of tumor formation associated with their integrating virus-based predecessors but they have also helped improve reprogramming efficiency [39].

In 2009–2011, right around the same time that various second-generation reprogramming methods were being developed, reports were starting to emerge that iPSCs were not equivalent to ESCs and that differentiation potential of iPSCs was either impaired or skewed based on the starting somatic cell type [40–43]. Epigenetic [42,44–46] and genetic [47–52] analyses showed that iPSCs display different DNA modification, histone modification and gene expression patterns than ESCs and that different iPSC lines also varied from one another in this manner. Differences in the somatic cell type used for reprogramming, the specific reprogramming method employed, as well as the extent of culturing are thought to influence the degree of disparity between various iPSC lines and/or ESCs [42,43,45,53]. Yet, in some instances, epigenetic memory can be reduced or even eliminated through subsequent passaging of iPSC clones, or alternatively by differentiation and secondary reprogramming, whereas errors that arise during reprogramming may be corrected through the addition of chromatin modifying drugs to the culture media [45,54]. Improvements and modifications made to reprogramming methods over the past decade have helped improve the safety and quality of iPSCs such that the development of iPSC-based therapies is moving forward rapidly. In years to come, the development of iPSC-based therapies may overtake conventional hESC-based ones since their generation does not involve the destruction of embryos or even the use of any unfertilized eggs. This is particularly appealing for the long-discussed genera-

tion of banks of HLA-matched PSCs to cover patient diversity on a larger scale and reduce or avoid the need for concomitant immunosuppression.

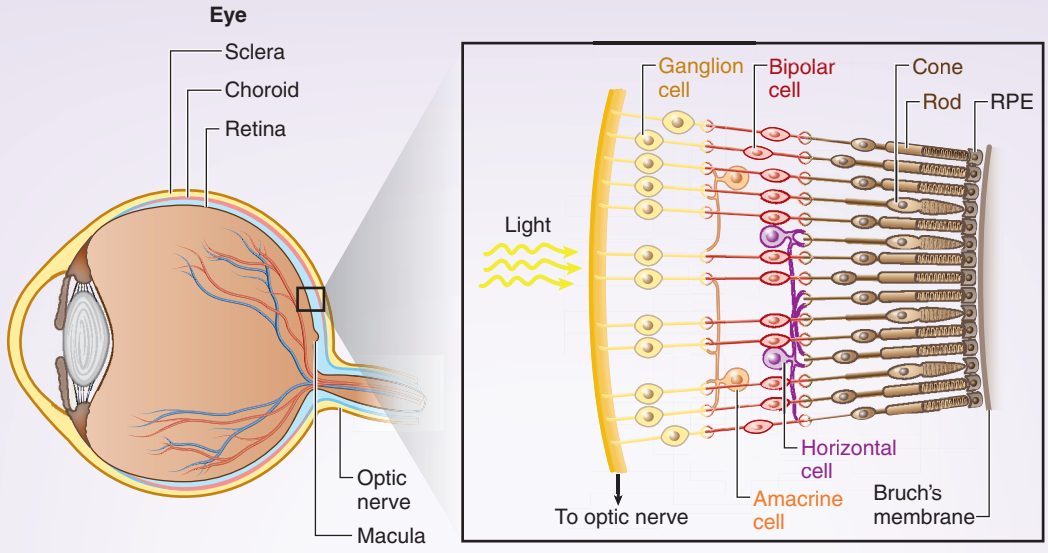
The start of clinical trials for PSC-derived therapies

PSCs may be useful for treating a wide variety of diseases given their ability to differentiate, theoretically, into every cell type in the body. The last 5–6 years have seen the PSC field begin to deliver on this promise, with a handful of clinical trials being approved in spinal cord injury, macular degeneration, diabetes and heart disease. Starting it off in 2009, Geron received investigational new drug (IND) approval to begin testing its hESC-derived oligodendrocyte precursors, GRNOPC1 (location) in a Phase I trial for spinal cord injury. This was the first trial aimed at testing the safety and potential efficacy of a PSC-derived therapy. In October 2010, Geron began transplanting GRNOPC1 into spinal cord patients and the following year, presented safety data at the American Congress of Rehabilitation Medicine suggesting that GRNOPC1 was well-tolerated and caused no serious adverse effects. However, a month later, Geron unexpectedly announced that they were stopping the study to focus on oncology drug-based therapies [55]. A total of five patients received injections of GRNOPC1 in the short-lived trial. In October 2013, Asterias Biotherapeutics, a subsidiary of Biotime, Inc. acquired Geron's hESC assets, and stated its intention to resurrect the defunct trial by rebranding the experimental therapy as AST-OPC1. Within 7 months, Asterias secured US$14.3 million in funding from the California Institute of Regenerative Medicine (CIRM) [56]. A few months later, in August 2014, the US FDA cleared Asterias for a new Phase I/IIa clinical trial (NCT02302157) to transplant AST-OPC1 into spinal cord injury patients. As of July 2016, Asterias has dosed a total of eight patients in this trial: the first three patients received a low dose of 2 million cells each (cohort 1) and the other five received 10 million cells each (cohort 2), which is predicted to be within the efficacious range [57]. Also in mid-2016, Asterias announced results of the 4–5 year follow-up of the original five patients from the Geron trial. The data suggest long-term safety of the therapy as well as reduced spinal cord cavitation (deterioration) in four of the five patients [58].

In 2010, a few months before Geron transplanted GRNOPC1 into its first patient, Advanced Cell Technology (ACT; MA, USA) received IND approval to begin testing hESC-derived retinal pigment epithelium (RPE) for age-related macular degeneration and Stargardt disease, a juvenile form of macular degeneration. ACT transplanted hESC-RPE into their first age-related macular degeneration (AMD) patient in July 2011 and continued to enroll patients over the next few years without incident, despite Geron's abandonment of its trial during this time. In 2012 and 2014, ACT published preliminary (4 months) [59] and medium to long-term (average follow-up period of 22 months) [60] safety data from its trials, which showed that subretinal injection of hESC-RPE was well-tolerated. There were no reported serious adverse events and no evidence of abnormal cell growth or tumor formation from the transplanted cells. Optical coherence tomography imaging showed the existence of subretinal pigmented patches in the eyes receiving hESC-RPE, suggesting that the cells engrafted and could survive even after perioperative immunosuppression was stopped. Vision-related quality of life was reported to improve in many patients who received the therapy. In addition, out of 18 patients (nine with AMD, nine with Stargardt), eight improved on their visual acuity tests by 15 letters or more. Three patients improved by 5–15 letters, six remained stable and one decreased. Currently, ACT (briefly called Ocata Therapeutics, but renamed the Astellas Institute for Regenerative Medicine after being acquired by Astellas Pharma Inc. in early 2016) has six Phase I and Phase II clinical trials registered on ClinicalTrials.gov evaluating the use of its hESC-derived RPE for treating dry AMD and Stargardt in the USA and UK. In addition, RPE derived from their hESCs re being used in two additional Phase I/II clinical trials in South Korea for dry AMD and Stargardt; these trials have similarly shown positive safety data, with no adverse proliferation, tumorigenicity or ectopic tissue from the treatment [61].

Around the same time that ACT's 2014 safety data were being published, Japan's RIKEN Institute successfully transplanted the world's first iPSC-derived therapy into humans. They too chose the eye and (wet) AMD as a first indication but decided to transplant autologous iPSC-derived RPE into patients instead of using an off-the-shelf allogeneic cellular product. The use of autologous cells is thought to avoid the risks of immune rejection [62] and has therefore been an attractive option, although it necessitates more time and labor since custom-made, individualized lots of iPSCs need to be generated for each patient. In the RIKEN trial, transplantation of autologous iPSC-RPE into the first patient went well and there were no adverse events, yet the trial was suspended after just this one patient. A genetic mutation, potentially in a known oncogene, was found in the autologous iPSCs generated for the trial's second patient. Investigators involved in this trial have since tested the concept of using HLA-matched allogeneic iPSC-RPE in nonhuman primates

and observed no rejection in the absence of immunosuppression [63]; however, this concept has not yet been tested empirically in humans. The lead investigator, Masayo Takahashi, has said that the trial will likely resume with the use of allogeneic iPSC-derived RPE presumably since a single lot of quality control (QC)-validated RPE can be used for many patients, yet so far the trial remains suspended [64]. Since 2012, a handful of other groups have received IND approval to test their own PSC-derived RPE for AMD (Figure 2). Given the risks of first-in-human PSC-based therapies, the eye is considered a logical place to begin developing therapies. First, the eye is a locally contained environment, providing a natural barrier to any potentially



Product (name)	Trial #	Indication	Trial phase	Status	Sponsor	Location
hESC-RPE (MA09-RPE)	NCT01344993	Dry AMD	1/2	Completed	AIRM	USA
hESC-RPE (MA09-RPE)	NCT01345006	Stargardt disease	1/2	Completed	AIRM	USA
hESC-RPE (MA09-RPE)	NCT01469832	Stargardt disease	1/2	Completed	AIRM	UK
hESC-RPE (MA09-RPE)	NCT01674829	Dry AMD	1/2	Unknown	CHA Biotech (licensed from AIRM)	Korea
hESC-RPE (MA09-RPE)	NCT01625559	Stargardt disease	1	Unknown	CHA Biotech (licensed from AIRM)	Korea
hESC-RPE (MA09-RPE)	NCT02563782	Dry AMD	2	Not recruiting	AIRM	USA
hESC-RPE (CPBP-RPE1)	NCT02590692	Dry AMD	1/2	Recruiting	RegenerativePatch Technologies	USA
hESC-RPE (Opregen)	NCT02286089	Dry AMD	1/2	Recruiting	Cell Cure Neurosciences	Israel
hESC-RPE (PF-05206388)	NCT01691261	Wet AMD	1	Not recruiting	Pfizer	UK
hESC-RPE	NCT02749734	AMD & Stargardt	1	Recruiting	Southwest Hospital Chongqing	China
hESC-RPE	NCT02755428	Dry AMD	0	Recruiting	Chinese Academy of Sciences	China
iPSC-RPE	NA	Wet AMD	1	On hold	RIKEN	Japan

Figure 2. Retinal degenerative diseases in the back of the eye have been the most commonly targeted indications to date for pluripotent stem cell-based therapies.
AMD: Age-related macular degeneration; RPE: Retinal pigment epithelium.
Information extracted from [67].

deleterious cells spreading systemically. Second, its immune-privileged nature may make it more accepting of transplanted allogeneic cells in the long-term. Third, the lens provides a way to noninvasively image the transplantation site over time and functional readouts such as visual acuity are easy to obtain. Indeed, CellCure Neuroscience, Pfizer, Regenerative Patch Technologies (RPT)/California Project to Cure Blindness (CPCB) and most recently two groups in China all have active trials listed on clinicaltrials.gov for evaluating hESC-derived RPE as a therapy for AMD. Pfizer and RPT/CPCB are using an immobilized membrane approach while CellCure Neuroscience is testing a cell suspension. Pfizer's trial, in collaboration with the London Project to Cure Blindness, Moorsfield Eye Hospital, the University College London Institute of Ophthalmology and the National Institute for Health Research in the UK successfully transplanted cells into its first patient in the fall of 2015 [65]. RPT utilizes a hESC-derived RPE monolayer developed by the California Project to Cure Blindness with a grant from the CIRM [66] and they are actively recruiting patients for their trial.

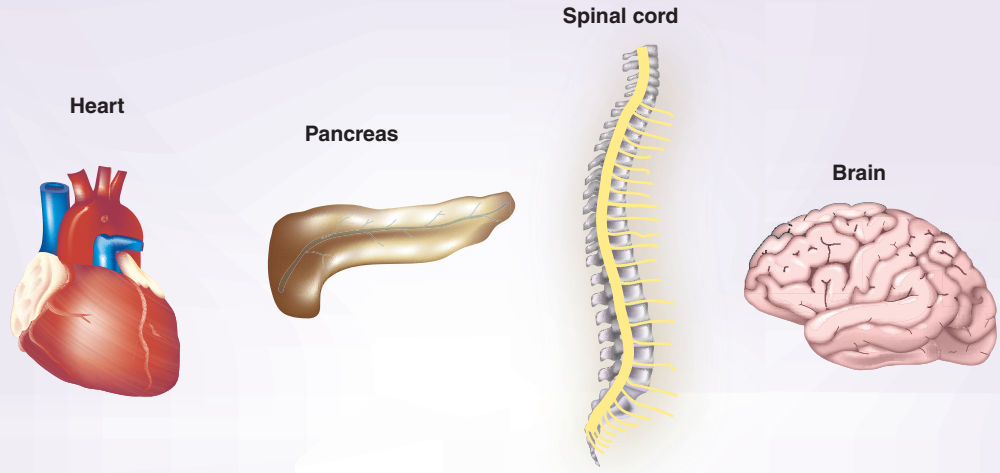
More than a decade of PSC research and development has also led to clinical trials for PSC-derived therapies in other disease areas (Figure 3). In 2014, Viacyte received IND approval to begin a Phase I/II trial to treat Type 1 diabetes. Their product, VC-01, consists of hESC-derived pancreatic endoderm cells encapsulated in a biocompatible drug delivery device that can be implanted under the skin. The semipermeable device permits the release of metabolically active factors while allowing nutrients and oxygen into the device and protecting the encapsulated cells from immune-rejection. Preclinical work has shown that once implanted, the cells differentiate and produce insulin, which is released from the device in sufficient quantities to regulate blood glucose levels in a mouse model of diabetes [68]. Viacyte's trial, NCT02239354, is now recruiting patients.

In addition to the above trials, a PSC-derived therapy was approved for an ischemic heart disease Phase I clinical trial in 2013. The Assistance Publique-Hopitaux de Paris is testing the feasibility and safety of $CD15^+$ $ISL1^+$ hESC-derived cardiac progenitors for improving heart function in patients with severe left ventricular systolic dysfunction. The hESC-derived cardiac progenitors are embedded in a fibrin patch and engrafted onto an area of epicardium during a scheduled coronary artery bypass or mitral valve surgery. An autologous flap of pericardium placed over the patch is designed to provide nutrient support to the embedded progenitors. Preclinical evidence shows the cells engrafted and differentiated into cardiomyocytes in a nonhuman primate myocardial infarction model [69]. In addition, these cells were shown to improve cardiac function in rodents even though the engrafted cells were no longer found 4 months after the surgery [70]. Transient survival of engrafted progenitors may provide paracrine signaling to recruit endogenous progenitors and/or accelerate endogenous repair mechanisms and potentially explain the sustained functional improvement despite disappearance of the engrafted cells. This trial, NCT02057900 is also recruiting patients, with six enrolled thus far.

The next wave of PSC-derived therapies destined for clinical trials

The last decade has also seen incredible progress on the development of other PSC-based therapies, some very close to beginning clinical trials. Several groups including the New York Stem Cell Consortium and Jun Takahashi's group at Kyoto University have made great progress in generating PSC-derived dopaminergic (DA) neurons for the treatment of Parkinson's disease (PD). Preclinical work has shown that both hESC- [71,72] and iPSC-derived [73] DA neurons rescue motor function in a 6-OHDA rat model of Parkinson's disease. Another study showed that transplantation of autologous iPSC-DA neurons into the putamen of cynomolgus monkeys resulted in long-term (up to 2 years) survival of the engrafted cells and improvements in motor neuron function [74]. A consortium of researchers developing stem cell-based therapies for PD called G-force PD was established in 2014 as a forum to discuss their collective progress and challenges [75]. Universal challenges include uncertainties regarding the body of preclinical evidence that regulatory agencies will require in order to demonstrate safety and efficacy of PD cell-based therapies, GMP manufacturing and scale-up issues, clinical trial design, ethics and commercialization. Despite these potential hurdles, it is likely that one or more of these groups will be able to start clinical trials in the next few years.

A long-standing goal for PSC research has been the *in vitro* generation of glucose-responsive, insulin-producing mature pancreatic β cells to treat diabetes. Many ESC/iPSC differentiation protocols have been developed for β-cell generation, yet it has been consistently challenging to fully mature them *in vitro* [76]. In 2014, a protocol developed in Doug Melton's lab was finally able to overcome this challenge and resulted in the *in vitro* generation of β cells expressing mature pancreatic β cell markers such as Pdx1 and Nkx6.1. Importantly, these cells were shown to secrete insu-



Indication	Severe heart failure	Type 1 diabetes	Spinal cord injury	Parkinson disease
Product	hESC-cardiac progenitors	Encapsulated hESC-pancreatic endoderm cells (VC-01)	hESC-oligodendrocyte progenitors (GRN-OPC1)	PSC-dopaminergic neurons (still in development)
Trial #	NCT02057900	NCT02239354	NCT01217008	NA
Trial phase	1	1/2	1	NA
Trial status	Recruiting	Recruiting	Completed	NA
Sponsor	Assistance Publique-Hospitaux de Paris	ViaCyte	Asterias Biotherapeutics (acquired Geron's technology)	Several potential sponsors (e.g., NY Stem Cell Foundation, Kyoto University, and others)
Location	France	USA	USA	TBD
Indication			**Spinal cord injury**	
Product			hESC-oligodendrocyte progenitors (AST-OPC1)	
Trial #			NCT02302157	
Phase			1/2	
Status			Recruiting	
Sponsor			Asterias Biotherapeutics	
Location			USA	

Figure 3. PSC-based therapies are also being tested in other organ systems besides the eye.
NA: Not applicable; TBD: To be determined.
Information was extracted from [67].

lin in a glucose-responsive manner and be capable of regulating hyperglycemia in preclinical models [77]. A follow-up study has shown that encapsulation of these β cells within an alginate matrix protects them from rejection in an immune-competent streptozotocin-induced diabetic mouse model without compromising their ability to reverse hyperglycemia [78]. This work is now part of the recently established Semma Therapeutics, which joins Viacyte in the quest to develop a PSC-based therapy for Type 1 diabetes.

PSCs are being developed for therapeutic use in various other diseases as well. For example, autologous iPSCs are being generated for patients with the blistering skin disorder, epidermolysis bullosa as part of a cell replacement strategy. Patches of skin with spontaneous revertant mosaicism, in which the disease-causing gene

has spontaneously corrected itself [79] or diseased skin samples that have undergone gene editing [80] are being used to produce iPSCs, which in turn are differentiated into normal keratinocytes to use in skin grafts for these patients. In the eye, retinal progenitors are being developed from both ESCs [81–83] and iPSCs [81,84] to use as a cell replacement therapy for retinal degenerative diseases, such as retinitis pigmentosa (RP), whereby transplantation of the progenitors would lead to *in vivo* differentiation and functional engraftment by mature photoreceptors. PSCs are also being developed to provide trophic support and/or to maintain the health of endogenous cells at risk for degeneration in various diseases. For example, iPSC-derived macrophages are being manipulated for therapeutic use in Alzheimer's disease (AD) patients. These macrophages have been engineered to express high levels of the β-amyloid-degrading enzyme, neprilysin 2, in an effort to reduce the burden of disease-associated plaques and spare the health of existing neurons in AD [85]. Similarly, in amyotrophic lateral sclerosis (ALS), iPSC-derived neural stem cells may provide therapeutically useful trophic support to endogenous neurons, as shown in a SOD1G93A ALS mouse model [86].

Researchers have also made some progress in combining PSCs and tissue engineering for transplantation. For example, iPSC-derived endodermal progenitors were combined with human endothelial cells and mesenchymal cells to generate 3D liver buds. Upon transplantation, these buds established vascular connections within host animals, differentiated into mature liver cell types and rescued chemically induced lethal liver failure [87]. PSC-based 3D tissues are under development for other organs such as eye, heart, lung, kidney and brain. These may be used in the future as a source of cells/tissue for transplantation, or as discussed below, for disease modeling and/or drug screening efforts.

PSCs as tools for 'disease-in-a-dish' models and drug screening platforms

In addition to direct therapeutic uses for PSC-derived cell types, both hESCs and iPSCs have been used in nonclinical applications (Table 1). This includes the establishment of 'disease-in-a-dish' models for various ailments, although the ease of generating iPSCs has made them a more attractive option than sourcing suitable ESCs for this purpose [88]. Major initiatives have been developed to provide central resources for disease-specific iPSCs, including the Coriell CIRM iPSC Biorepository. As of June 2016, this searchable collection had roughly 3000 iPSC lines for diseases

Table 1. Nonclinical applications for pluripotent stem cells.

Application	PSC type	Purpose	Selected examples [Ref.]
Disease-in-a-dish models (reviewed in [88,91])	Both hESCs and iPSCs (although more for iPSC)	*In vitro* models for various disease areas including: cardiovascular, neurologic, ocular, musculoskeletal, pulmonary, hematologic, skin, digestive, metabolic, endocrine and others	Neurodegenerative: Huntington's [92], Alzheimer's [93,94] and Parkinson's diseases [95] Pulmonary: cystic fibrosis, α-1 antitrypsin deficiency and emphysema [96], idiopathic pulmonary fibrosis [97] Digestive/metabolic/endocrine: enteric anendocrinosis [98], kidney disease [99], hypothyroidism [100]
Drug development and screening (reviewed in [91,101])	Both hESCs and iPSCs (although more for iPSC)	Candidate drug testing Library screening Toxicity screening	Use of ALS specific iPSC-derived motor neurons to test anacardic acid's ability to rescue cellular disease phenotype [102] Neural crest progenitors derived from familial dysautonomia specific iPSC were used in a 7000 small-molecule library screen to find ones that could be used therapeutically [103] Normal [104] and disease-specific [105] iPSC-derived cardiomyocytes, iPSC-derived hepatocytes [106]
Gene editing (e.g., with ZFN, TALENS, Crispr/Cas9 or AAV; (reviewed in [107])	Both hESCs and iPSCs	Study role of genes in disease To create isogenic controls for disease modeling To minimize or eliminate immunogenicity of transplanted cells	Introduction of specific APP and presenilin 1 mutations in iPSC to study their role in Alzheimer's disease [108] Isogenic controls for study of long QT syndrome in iPSC-derived cardiomyocytes [109] Knocking out genes for HLA expression [110–113]

AAV: Adeno-associated virus; ALS: Amyotrophic lateral sclerosis; APP: Apolipoprotein; hESC: Human embryonic stem cell; iPSC: Induced pluripotent stem cell; QT: Electrical depolarization/repolarization interval for ventricles on an EKG; TALEN: Transcription activator-like effector DNA-binding domain nuclease; ZFN: Zinc finger nuclease.

ranging from childhood neurodevelopmental disorders to hepatic conditions, eye disorders and respiratory diseases [89]. Similarly, the NYSCF has a repository of over a thousand iPSC lines covering a broad range of indications and a searchable database to help researchers identify relevant disease-specific iPSC lines for their specific interests [90].

Over the past several years, numerous publications have described the differentiation of disease-specific PSCs into relevant cell types for disease modeling purposes (reviewed in [91]). Monogenic diseases such as familial hypercholesterolemia, spinal muscular atrophy, Huntington's disease or the pulmonary disorders α-1 antitrypsin deficiency and cystic fibrosis are among the most straightforward to model since alterations in a single gene are largely responsible for the disease phenotype. For example, the Huntington's disease consortium established a small collection of iPSC lines from Huntington's disease patients and nondiseased controls; differentiation of these lines toward a neuronal phenotype has allowed consortium investigators to examine HD-specific alterations in cell metabolism, stress responses, adhesion properties, among others. [92]. In another example, over 100 iPSC lines from patients with various lung diseases were including α-1 antitrypsin deficiency, cystic fibrosis and emphysema were generated to study their specific disease phenotypes and are also being used in the development of gene-correction strategies for many inherited pulmonary disorders [96]. In addition, iPSCs from patients with *NKX2-1* haploinsufficiency have been used to model hypothyroidism and to determine the signaling pathways governing thyroid lineage differentiation and maturation with the goal of developing cell replacement strategies to treat this disease [100].

Complex diseases, which may involve multiple genes, environmental influence, or interplay between multiple cell types are more challenging to model *in vitro* but progress has been made in using PSCs for this purpose as well. As an example, iPSCs have been generated from both familial and sporadic AD patients to examine differential stress responses from AD iPSCs upon their differentiation into neurons [114]. Sporadic AD iPSC-derived neurons have also been used to show that variants in the *SORL-1* gene can lead to increased risk of developing AD due to reduced responsiveness to BDNF and its resulting effects on apolipoprotein processing [93]. In many other examples, the modeling of complex diseases is facilitated by the development of 3D organoid systems. 3D cultures involving stratified layers of the retina have been created to study retinal degenerative diseases [94], while intestinal organoids complete with epithelial derived villus-like structures, Goblet and paneth cells have been created to study intestinal development and diseases such as enteric anendocrinosis [98]. iPSC-based kidney organoids are also under development. In a recent study, kidney organoids were shown to contain nephrons which descend into distal and proximal tubules, an early loop of Henle and vascularized glomeruli, similar to kidneys during the first trimester of embryonic development [99]. Such organoids can be used to study nephrogenesis and various kidney diseases. 3D iPSC-based lung organoids have been created to study diseases such as idiopathic pulmonary fibrosis and may also be applied to development of organ transplant strategies [97]. Lastly, self-organizing midbrain organoids derived from iPSCs are being used to generate DA neurons and neuromelanin-producing cells for the study of Parkinson's disease and other neurologic diseases [95].

Over the last decade, there has been great interest in using PSCs for drug discovery, drug screening and evaluation of potential drug toxicities. Differentiation protocols have been improved in terms of efficiency, maturation and yield such that now, a variety of different PSC-based platforms are being used. For example, neural crest progenitors differentiated from familial dysautonomia patient-specific iPSCs were used to screen an approximately 7000 small molecule library for those that could rescue expression of *IKBKAP,* the inadequate transcription of which causes the fatal neurological disease. The screen led to the discovery that α2 adrenergic receptor activity can regulate IKBKAP expression; drugs that increase this receptor's activity may be useful therapeutic agents for familial dysautonomia [103]. In another study, cardiac progenitor cells derived from iPSCs were used in a screen for compounds that would enhance the proliferation and differentiation of the progenitors, which could help facilitate cardiac tissue repair. The screen led to the discovery that inhibitors of TGF-β type 1 receptor kinase stimulate cardiac progenitor differentiation into cardiomyocytes [115]. In another proof of concept experiment, ALS patient-specific iPSC-derived motor neurons contained insoluble protein aggregates in the cytosol and had short neurites, mirroring the phenotype of ALS patient motor neurons. Gene expression analysis of these cells led to proof of concept testing of chemical compounds for correcting these abnormalities. The histone acetyltransferase inhibitor, anacardic acid, was found to reverse the deleterious ALS motor neuron phenotype. The authors suggest that this iPSC motor neuron based system should therefore be a useful drug screening platform to develop novel drugs for treating ALS [102].

Cardio- and hepatotoxicity are major safety concerns for any new drug. It is estimated that the development of one third of drugs has been discontinued because

of suspected cardiotoxic effects [104]. Many of these failures come late in drug development; therefore implementing toxicity screening early in development could save considerable time and costs. Over the past 10 years, researchers have begun to use PSC derivatives to screen for potentially toxic effects of drugs earlier in their development (reviewed in [101]). For example, a panel of iPSC-derived cardiomyocytes was generated from patients with inherited cardiac diseases such as long QT syndrome, familial hypertrophic or familial dilated cardiomyopathy as well as from normal healthy controls. These were used to model disease-specific cardiotoxicity profiles and to evaluate differences in susceptibility to known cardiotoxic drugs [105]. In another study, iPSC-derived cardiomyocytes were used in a system that was designed to mimic the organization and physiological responsiveness of cardiac tissue better than individual cardiomyocytes. The system can evaluate electrophysiological, physiological and biological properties of cardiac tissue in response to different pharmacological agents and therefore may be quite useful in evaluating potential cardiotoxicities of new drug candidates [104].

Hepatotoxicity is another major safety concern for drugs in development and PSC-based systems are also being used to evaluate the potentially toxic effects of novel drugs to the liver. For example, in a proof of principle experiment, iPSC-derived hepatocytes from a variety of individuals were used to screen a library of 240 heterogeneous compounds with known hepatotoxic effects. The iPSC-hepatocytes were assessed for drug-induced effects on viability, apoptosis, mitochondrial membrane potential, phospholipid accumulation, cytoskeletal alterations and other properties [106]. Such a multiparametric system can provide information on potential mechanisms of toxicity through a comparison to compounds with well-characterized toxicities and also be used to address potential hepatotoxic effects of drugs on specific patient populations, including the elderly or those with a specific type of disease. Pushing PSC-derivatives toward mature, adult cellular phenotypes should help improve the accuracy and reliable of PSC-based toxicity screening platforms. Toward this end, 3D scaffolds supplemented with primary cardiomyocytes are being used to drive the maturation of iPSC-cardiomyocytes [116] and 3D liver buds are being used to functionally mature iPSC-derived hepatocytes [117].

Gene editing & PSCs: approaches

Gene editing technologies have been developed to correct disease-causing genetic mutations, functionally replace and/or knock-out expression of dysfunctional genes. Nuclease-based methodologies for editing the genome dominate the field of gene editing and major classes include natural homing endonucleases-zinc finger nucleases (ZFNs) as well as transcription activator-like effector DNA-binding domain nucleases (TALENs), and clustered regularly-interspaced short palindromic repeats (CRISPR) technology driven by Cas9 nuclease activity (aka 'Crispr/Cas9'). These systems were first applied to PSCs back in 2009 when two different groups utilized ZFNs to edit genomic sequences at a variety of discrete loci within hESCs and iPSCs [118,119]. Since then, both TALEN-based gene editing and CRISPR/Cas technology have been applied to PSCs by various groups (reviewed in [107]). Multiplexing capability as well as an observed increased targeting efficiency of CRISPR/Cas over TALENs capability has made CRISPR technology perhaps the most popular choice for gene editing [120]. In addition, the combination of TALENS and CRISPR technologies has been used to create an inducible multiplex gene targeting system called 'iCrispr' for temporal control over gene editing at discrete stages of differentiation in iPSC-based disease modeling [121].

An alternative to nuclease-based gene editing is the use of adeno-associated viruses (AAVs); various serotypes, strains and recombinant AAVs have been developed to facilitate gene therapy as well as gene editing endeavors in various cell types, including PSCs [122]. Although AAV use in PSCs has not been as popular as that of TALENS or CRISPR/Cas, they appear to have an excellent safety record with more than 100 clinical trial testing AAV variants for therapeutic purposes ([123,124]).

Gene editing & PSCs: goals

Regardless of the editing system employed, the objectives of PSC-based gene editing endeavors fall into two major categories: improving disease models and drug screening systems through the creation of isogenic controls, and gene editing for cell-based therapies. For the former, isogenic controls created through gene editing technology can facilitate the study of mutations and complex diseases in a properly controlled and isolated manner. For example, ZFN technology was used to create isogenic controls for the study of long QT syndrome in iPSC-derived cardiomyocytes [125]. In another study, hetero- and homozygous mutations in the genes for apolipoprotein and presenilin 1 were generated in iPSCs with Crispr/Cas technology and their differentiation into cortical neurons has proven to recapitulate specific features of AD [108]. On a larger scale, the UK-based company, Horizon Discovery has created a genome-editing platform for the generation of several hundred pairs of isogenic cell lines using their HAP1 cell line [126]. A similar large scale plat-

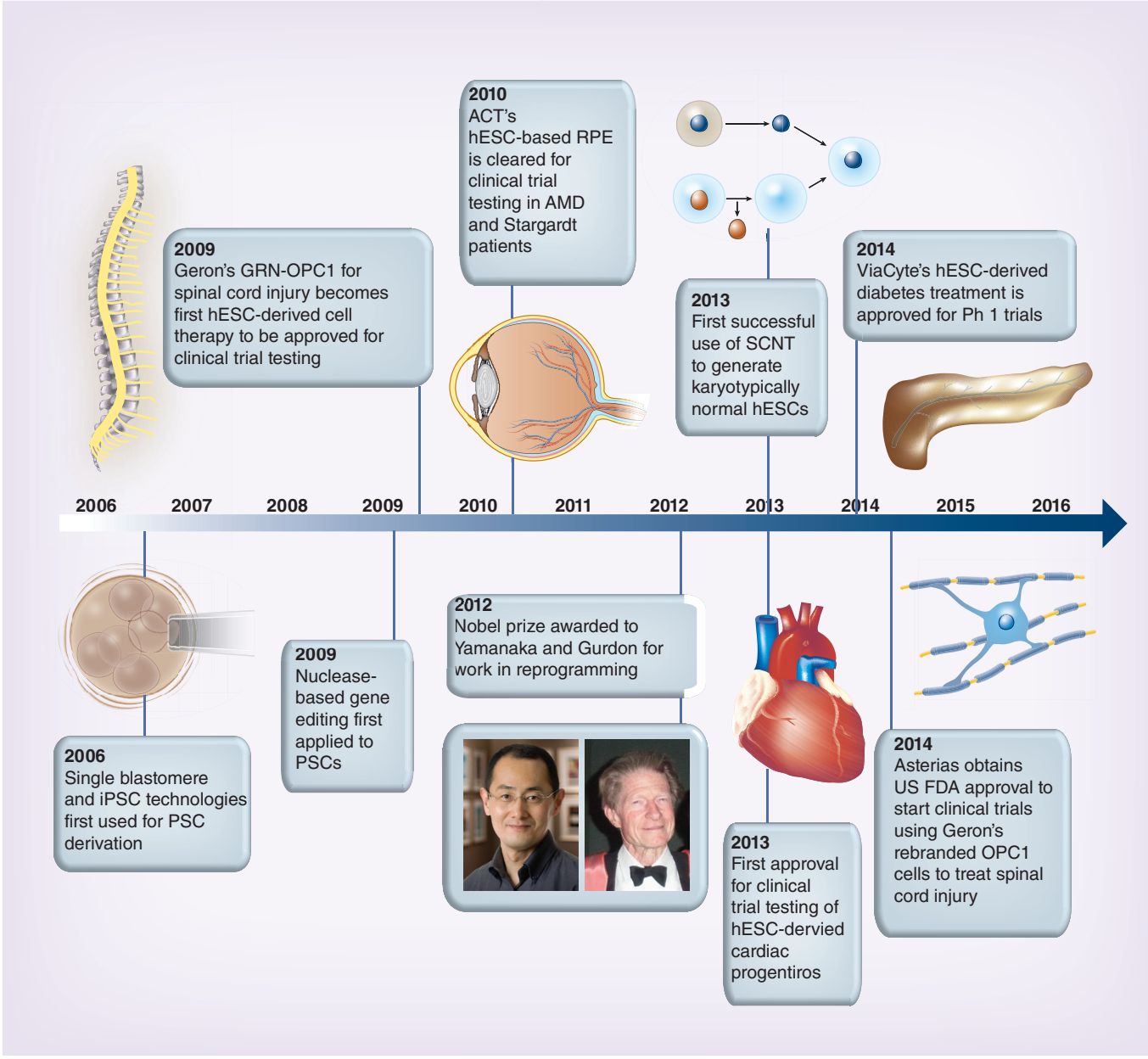


Figure 4. Timeline of key events in pluripotent stem cell research from 2006–2016.
ACT: Advanced Cell Technology; AMD: Age-related macular degeneration; hESC: Human embryonic stem cell; iPSC: Induced pluripotent stem cell; PSC: Pluripotent stem cell; RPE: Retinal pigment epithelium.

form could also be applied for PSC-based isogenic controls.

The second major undertaking for gene editing in PSCs involves cell-based therapies, particularly for monogenic diseases. Proof of principle studies includes a report where Crispr/Cas gene editing was used to correct the mutation of the β-globin gene in iPSCs from a β-thalassemia patient [109]. These corrected iPSCs displayed improved differentiation capacity into various types of hematopoietic progenitors and may be one day used as a source of autologous hematopoietic stem cells for transplantation and repopulation of the hematopoietic system. Similarly, Crispr/Cas9 was used to correct a mutation in the gene encoding the RP GTPase regulator in iPSCs derived from a patient with X-linked RP [127]. These corrected cells could in principle be differentiated into photoreceptors or their progenitors and used in cell replacement strategies for RP patients.

The concept of generating PSC banks to match the diversity of HLA phenotypes has been discussed for several years as a logical way to avoid immunogenicity of PSC-based therapies. Yet, gene editing is now being used to create 'universal' PSC lines and their subsequent differentiation into nonimmunogenic

cell types. In proof of principle experiments, knocking out expression of β-2-microglobulin, which is the common light chain molecule to class I A, B, C molecules, can reduce immunogenicity in hESCs [110,111]. This approach has also been used to generate universal platelets from human iPSCs, which could be used as a potential strategy for the management of platelet refractoriness [112].

Various efforts are underway to reduce or eliminate immunogenicity of cells in a more comprehensive manner. Chad Cowan's group has stated they are utilizing Crispr/Cas technology to generate universal donor PSCs by eliminating expression of genes involved in immunogenicity in PSCs and enhancing expression of genes promoting immune tolerance such as *PD-L1* or *HLA G*, which can help avoid natural killer cell-mediated lysis [113]. The company Universal Cells, whose work is based on intellectual property generated at the University of Washington, is using a recombinant adeno-assciated virus (rAAV)-based gene editing strategy to knock-out both HLA class I and II expression, while at the same time knocking in expression of the tolerance-inducing HLA E or G and a suicide gene to safeguard against uncontrolled proliferation or other potential untoward effects of cells after transplantation [128]. Creating universal PSCs would obviate the need and expense of generating HLA-matched PSC banks for regenerative medicine. If successful, it could help reduce or avoid the need for harsh immunosuppression and improve the engraftment or persistence of PSC-derived therapeutic cells, particularly for those indications where large numbers of cells are needed.

Common challenges to the use of PSCs

Regardless of the cell source (hESC or iPSC) or type of cell being developed for clinical use, disease modeling or drug discovery, it should be noted that a common challenge for all applications of PSCs is maintaining genomic stability through the culture and/or differentiation process. Various sources of stress (e.g, culture conditions, enzymatic passaging, among others) can lead to the acquisition of chromosomal abnormalities and the potential for tumorigenicity or distortions of cell-based models. Whole genome screening methods such as comparative genomic hybridization are now being used to supplement conventional cytogenetic detection methods (such as karyotype analysis) for identifying genetic abnormalities (reviewed in [129]) and will help safeguard PSC endeavors.

Future perspective

The dramatic progress made over the past decade will almost certainly translate into exciting new advancements in decades to come (Figure 4). First-in-man PSC-based clinical trials have thus far shown that PSC-derivatives are safe to use in humans, and provide the impetus for continued clinical trial testing. To date, trials have almost exclusively employed hESCs, yet that is likely to change in the future. Improvements in iPSC quality should enable these ethically sound alternatives to hESCs to catch up or even pass hESC usage in clinical trials. As differentiation procedures and 3D technologies improve, PSCs will become ever more integral to drug screening efforts and disease modeling, although it is unlikely they will ever fully replace the

Executive summary

- Technologies for generating pluripotent stem cells (PSCs) 2006–2016: various technologies (e.g., single blastomere technology, somatic cell nuclear transfer and induced PSC [iPSC] technology), have been developed as alternatives to conventional human embryonic stem cells and each method has its own advantages and disadvantages.
- iPSC reprogramming technology: this technology has rapidly evolved since its inception 10 years ago, and the development of safer, nonintegrating reprogramming methods will accelerate the clinical development of iPSC-derivatives and broaden their utility in years to come.
- The start of clinical trials: for the first time in history, PSC-based cell therapies are being tested in human clinical trials- almost exclusively with the use of human embryonic stem cell derivatives and focused heavily on treating eye-related disorders, although PSC-derivatives are also in clinical trials for treating myocardial infarction, diabetes and spinal cord injury and several other cell types are in development.
- PSCs for 'disease-in-a-dish' models and drug screening platforms: large repositories of disease-specific iPSCs have been generated to facilitate disease modeling and derivatives of these are being used to model a wide range of diseases including neurodegenerative, intestinal, metabolic, dermal, ocular, hematopoietic, pulmonary and others. PSC-derived cardiomyocytes and hepatocytes are among those being used to evaluate potential toxicities from drugs early in their development.
- Gene editing and PSCs: gene editing in PSCs is being pursued for two major purposes: correction or insertion of disease-causing mutations which will enable generation of much needed isogenic controls for the study of disease processes (and correction strategies may also one day lead to potential cell-based therapies), and to create universal cells for the generation of nonimmunogenic PSCs to mitigate risks of immune rejection and facilitate engraftment of therapeutic derivatives.

use of *in vivo* disease models. Another major advancement that will likely drive PSC research in years to come involves the marriage of gene editing technology with PSCs. The ability to precisely correct disease-causing mutations, create isogenic controls and potentially eliminate immunogenicity of PSC derivatives make gene editing in PSCs an incredibly important endeavor. The PSC field will likely produce additional exciting breakthroughs in the coming decade – advancements that could one day make incurable diseases curable.

Financial & competing interests disclosure

EA Kimbrel and R Lanza are employees of the Astellas Institute for Regenerative Medicine (AIRM), a company in the field of stem cells and regenerative medicine. This review article was funded by AIRM and written by the authors, EA Kimbrel and R Lanza. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Thomson JA, Itskovitz-Eldor J, Shapiro SS *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* 282(5391), 1145–1147 (1998).

•• **Describes the initial isolation of human embryonic stem cells from blastocyst stage embryos and is the starting point for the field of pluripotent stem cells (PSCs).**

2 Little M, Hall W, Orlandi A. Delivering on the promise of human stem-cell research. What are the real barriers? *EMBO Rep.* 7(12), 1188–1192 (2006).

3 Taylor CJ, Bolton EM, Pocock S, Sharples LD, Pedersen RA, Bradley JA. Banking on human embryonic stem cells: estimating the number of donor cell lines needed for HLA matching. *Lancet* 366(9502), 2019–2025 (2005).

4 Chung Y, Klimanskaya I, Becker S *et al.* Embryonic and extraembryonic stem cell lines derived from single mouse blastomeres. *Nature* 439(7073), 216–219 (2006).

5 Klimanskaya I, Chung Y, Becker S, Lu S-J, Lanza R. Human embryonic stem-cell lines derived from single blastomeres. *Nature* 444, 481–485 (2006).

6 Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 344(6268), 768–770 (1990).

7 Mcarthur SJ, Leigh D, Marshall JT, Gee AJ, De Boer KA, Jansen RP. Blastocyst trophectoderm biopsy and preimplantation genetic diagnosis for familial monogenic disorders and chromosomal translocations. *Prenat. Diagn.* 28(5), 434–442 (2008).

8 Ilic D, Giritharan G, Zdravkovic T *et al.* Derivation of human embryonic stem cell lines from biopsied blastomeres on human feeders with minimal exposure to xenomaterials. *Stem Cells Dev.* 18(9), 1343–1350 (2009).

9 Cibelli JB, Stice SL, Golueke PJ *et al.* Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* 280(5367), 1256–1258 (1998).

10 Onishi A, Iwamoto M, Akita T *et al.* Pig cloning by microinjection of fetal fibroblast nuclei. *Science* 289(5482), 1188–1190 (2000).

11 Wakayama S, Kohda T, Obokata H *et al.* Successful serial recloning in the mouse over multiple generations. *Cell Stem Cell* 12(3), 293–297 (2013).

12 Tachibana M, Amato P, Sparman M *et al.* Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* 153(6), 1228–1238 (2013).

13 Chung YG, Eum JH, Lee JE *et al.* Human somatic cell nuclear transfer using adult cells. *Cell Stem Cell* 14(6), 777–780 (2014).

14 Yamada M, Johannesson B, Sagi I *et al.* Human oocytes reprogram adult somatic nuclei of a type 1 diabetic to diploid pluripotent stem cells. *Nature* 510(7506), 533–536 (2014).

15 Times TK. Resumed stem cell study (2016). www.koreatimes.co.kr/

16 Chung YG, Matoba S, Liu Y *et al.* Histone demethylase expression enhances human somatic cell nuclear transfer efficiency and promotes derivation of pluripotent stem cells. *Cell Stem Cell* 17(6), 758–766 (2015).

17 Gurdon JB, Wilmut I. Nuclear transfer to eggs and oocytes. *Cold Spring Harb. Perspect. Biol.* 3(6), pii: a002659 (2011).

18 Jullien J, Pasque V, Halley-Stott RP, Miyamoto K, Gurdon JB. Mechanisms of nuclear reprogramming by eggs and oocytes: a deterministic process? *Nat. Rev. Mol. Cell Biol.* 12(7), 453–459 (2011).

19 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4), 663–676 (2006).

20 Takahashi K, Tanabe K, Ohnuki M *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5), 861–872 (2007).

•• **This is the first description of the generation of human induced pluripotent stem cells (iPSCs), for which the Nobel Prize was awarded, in part to Shinya Yamanaka.**

21 Yu J, Vodyanik MA, Smuga-Otto K *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318(5858), 1917–1920 (2007).

22 The Nobel Prize in Physiology or Medicine 2012: Sir John B. Gurdon and Shinya Yamanaka. www.nobelprize.org/

23 Yu J, Vodyanik MA, Smuga-Otto K *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318(5858), 1917–1920 (2007).

24 Aoki T, Ohnishi H, Oda Y *et al.* Generation of induced pluripotent stem cells from human adipose-derived stem cells without c-MYC. *Tissue Eng.* 16(7), 2197–2206 (2010).

25 Nakagawa M, Koyanagi M, Tanabe K *et al.* Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* 26(1), 101–106 (2008).

26 Zhao HX, Li Y, Jin HF *et al.* Rapid and efficient reprogramming of human amnion-derived cells into pluripotency by three factors OCT4/SOX2/NANOG. *Differentiation* 80(2–3), 123–129 (2010).

27 Giorgetti A, Montserrat N, Aasen T *et al.* Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. *Cell Stem Cell* 5(4), 353–357 (2009).

28 Kim JB, Greber B, Arauzo-Bravo MJ *et al.* Direct reprogramming of human neural stem cells by OCT4. *Nature* 461(7264), 649–643 (2009).

29 Warren L, Manos PD, Ahfeldt T *et al.* Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 7(5), 618–630 (2010).

30 Kim D, Kim CH, Moon JI *et al.* Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* 4(6), 472–476 (2009).

31 Yu J, Hu K, Smuga-Otto K *et al.* Human induced pluripotent stem cells free of vector and transgene sequences. *Science* 324(5928), 797–801 (2009).

32 Okita K, Matsumura Y, Sato Y *et al.* A more efficient method to generate integration-free human iPS cells. *Nat. Methods* 8(5), 409–412 (2011).

33 Jia F, Wilson KD, Sun N *et al.* A nonviral minicircle vector for deriving human iPS cells. *Nat. Methods* 7(3), 197–199 (2010).

34 Woltjen K, Michael IP, Mohseni P *et al.* piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* 458(7239), 766–770 (2009).

35 Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc. Jpn Acad. Ser. B Phys. Biol. Sci.* 85(8), 348–362 (2009).

36 Shi Y, Do JT, Desponts C, Hahm HS, Scholer HR, Ding S. A combined chemical and genetic approach for the generation of induced pluripotent stem cells. *Cell Stem Cell* 2(6), 525–528 (2008).

37 Huangfu D, Maehr R, Guo W *et al.* Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. *Nat. Biotechnol.* 26(7), 795–797 (2008).

38 Hou P, Li Y, Zhang X *et al.* Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science* 341(6146), 651–654 (2013).

39 Yamanaka S. A fresh look at iPS cells. *Cell* 137(1), 13–17 (2009).

40 Hu BY, Weick JP, Yu J *et al.* Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc. Natl Acad. Sci. USA* 107(9), 4335–4340 (2010).

41 Feng Q, Lu SJ, Klimanskaya I *et al.* Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. *Stem Cells* 28(4), 704–712 (2010).

42 Kim K, Zhao R, Doi A *et al.* Donor cell type can influence the epigenome and differentiation potential of human induced pluripotent stem cells. *Nat. Biotechnol.* 29(12), 1117–1119 (2011).

43 Bar-Nur O, Russ HA, Efrat S, Benvenisty N. Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells. *Cell Stem Cell* 9(1), 17–23 (2011).

44 Lister R, Pelizzola M, Kida YS *et al.* Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature* 471(7336), 68–73 (2011).

45 Polo JM, Liu S, Figueroa ME *et al.* Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat. Biotechnol.* 28(8), 848–855 (2010).

46 Kim K, Doi A, Wen B *et al.* Epigenetic memory in induced pluripotent stem cells. *Nature* 467(7313), 285–290 (2010).

47 Ghosh Z, Wilson KD, Wu Y, Hu S, Quertermous T, Wu JC. Persistent donor cell gene expression among human induced pluripotent stem cells contributes to differences with human embryonic stem cells. *PLoS ONE* 5(2), e8975 (2010).

48 Chin MH, Mason MJ, Xie W *et al.* Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* 5(1), 111–123 (2009).

49 Marchetto MC, Yeo GW, Kainohana O, Marsala M, Gage FH, Muotri AR. Transcriptional signature and memory retention of human-induced pluripotent stem cells. *PLoS ONE* 4(9), e7076 (2009).

50 Chin MH, Pellegrini M, Plath K, Lowry WE. Molecular analyses of human induced pluripotent stem cells and embryonic stem cells. *Cell Stem Cell* 7(2), 263–269 (2010).

51 Guenther MG, Frampton GM, Soldner F *et al.* Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. *Cell Stem Cell* 7(2), 249–257 (2010).

52 Wang A, Huang K, Shen Y *et al.* Functional modules distinguish human induced pluripotent stem cells from embryonic stem cells. *Stem Cells Dev.* 20(11), 1937–1950 (2011).

53 Yamanaka S. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 10(6), 678–684 (2012).

54 Stadtfeld M, Apostolou E, Ferrari F *et al.* Ascorbic acid prevents loss of Dlk1-Dio3 imprinting and facilitates generation of all-iPS cell mice from terminally differentiated B cells. *Nat. Genet.* 44(4), 398–405 (2012).

55 Varughese A. Abandoning the stem cell clinical trial ship. *Biotechniques* (2011).www.biotechniques.com/

56 Treatment for spinal cord Injury to start clinical trial funded by California's stem cell agency. www.cirm.ca.gov/

57 Asterias Biotherapeutics Completes Enrollment and Dosing for the First Efficacy Cohort of the AST-OPC1 SCiSTAR Phase 1/2a Clinical Trial in Complete Cervical Spinal Cord Injury. http://asteriasbiotherapeutics.com/

58 Biotherapeutics A. Asterias Biotherapeutics Announces Positive New Long-Term Follow-Up Results for AST-OPC1. (2016). http://asteriasbiotherapeutics.com/

59 Schwartz SD, Hubschman JP, Heilwell G *et al.* Embryonic stem cell trials for macular degeneration: a preliminary report. *Lancet* 379(9817), 713–720 (2012).

60 Schwartz SD, Regillo CD, Lam BL *et al.* Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt's macular dystrophy: follow-up of two open-label Phase 1/2 studies. *Lancet* 385(9967), 509–516 (2015).

• **This is the first report of mid- to long-term safety data for any PSC-based therapy in clinical trials. It importantly showed the therapy, human embryonic stem cell-derived retinal pigment epithelium was well tolerated and not associated with any deleterious effects.**

61 Song WK, Park KM, Kim HJ *et al.* Treatment of macular degeneration using embryonic stem cell-derived retinal pigment epithelium: preliminary results in asian patients. *Stem Cell Rep.* 4(5), 860–872 (2015).

62 Morizane A, Doi D, Kikuchi T *et al.* Direct comparison of autologous and allogeneic transplantation of iPSC-derived neural cells in the brain of a non-human primate. *Stem Cell Rep.* 1(4), 283–292 (2013).

63 Sugita S, Iwasaki Y, Makabe K *et al.* Successful transplantation of retinal pigment epithelial cells from MHC Homozygote iPSCs in MHC-matched models. *Stem Cell Rep.* 7(4), 635–648 (2016).

64 Garber K. RIKEN suspends first clinical trial involving induced pluripotent stem cells. *Nat. Biotechnol.* 33(9), 890–891 (2015).

65 The London Project to Cure Blindness. www.thelondonproject.org/

66 California's Stem Cell Agency. www.cirm.ca.gov

67 ClinicalTrials.gov. http://clinicaltrials.gov

68 Kroon E, Martinson LA, Kadoya K *et al.* Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nat. Biotechnol.* 26(4), 443–452 (2008).

69 Blin G, Nury D, Stefanovic S *et al.* A purified population of multipotent cardiovascular progenitors derived from primate pluripotent stem cells engrafts in postmyocardial infarcted nonhuman primates. *J. Clin. Invest.* 120(4), 1125–1139 (2010).

70 Bellamy V, Vanneaux V, Bel A *et al.* Long term functional benefits of human embryonic stem cell-derived cardiac progenitors embedded into a fibrin scaffold. *J. Heart Lung Transplant.* 34(9), 1198–1207 (2015).

71 Grealish SE, Diguet A, Kirkeby B *et al.* Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of parkinson's disease. *Cell Stem Cell* 15, 653–665 (2014).

72 Kriks S, Shim JW, Piao J *et al.* Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* 480(7378), 547–551 (2011).

73 Doi D, Samata B, Katsukawa M *et al.* Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. *Stem Cell Rep.* 2(3), 337–350 (2014).

74 Hallett PJ, Deleidi M, Astradsson A *et al.* Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. *Cell Stem Cell* 16(3), 269–274 (2015).

75 Barker RA, Studer L, Cattaneo E, Takahashi J. G-Force PD: a global initiative in coordinating stem cell-based dopamine treatments for Parkinson's disease. *NPJ Parkinson's Dis.* 1, 15017 (2015).

76 Abdelalim EM, Emara MM. Advances and challenges in the differentiation of pluripotent stem cells into pancreatic beta cells. *World J. Stem Cells* 7(1), 174–181 (2015).

77 Pagliuca FW, Millman JR, Gurtler M *et al.* Generation of functional human pancreatic beta cells *in vitro*. *Cell* 159(2), 428–439 (2014).

78 Vegas AJ, Veiseh O, Gurtler M *et al.* Long-term glycemic control using polymer-encapsulated human stem cell-derived beta cells in immune-competent mice. *Nat. Med.* 22(3), 306–311 (2016).

79 Umegaki-Arao N, Pasmooij AM, Itoh M *et al.* Induced pluripotent stem cells from human revertant keratinocytes for the treatment of epidermolysis bullosa. *Sci. Transl. Med.* 6(264), 264ra164 (2014).

80 Sebastiano V, Zhen HH, Haddad B *et al.* Human COL7A1-corrected induced pluripotent stem cells for the treatment of recessive dystrophic epidermolysis bullosa. *Sci. Transl. Med.* 6(264), 264ra163 (2014).

81 Barnea-Cramer AO, Wang W, Lu S-J *et al.* Function of human pluripotent stem cell-derived photoreceptor progenitors in blind mice. *Sci. Rep.* 6, 29784 (2016).

82 Lamba DA, Karl MO, Ware CB, Reh TA. Efficient generation of retinal progenitor cells from human embryonic stem cells. *Proc. Natl Acad. Sci. USA* 103(34), 12769–12774 (2006).

83 Osakada F, Ikeda H, Mandai M *et al.* Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. *Nat.Biotechnol.* 26(2), 215–224 (2008).

84 Osakada F, Jin ZB, Hirami Y *et al. In vitro* differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. *J. Cell Sci.* 122(Pt 17), 3169–3179 (2009).

85 Takamatsu K, Ikeda T, Haruta M *et al.* Degradation of amyloid beta by human induced pluripotent stem cell-derived macrophages expressing Neprilysin-2. *Stem Cell Res.* 13(3PA), 442–453 (2014).

86 Nizzardo M, Bucchia M, Ramirez A *et al.* iPSC-derived LewisX+CXCR4+beta1-integrin+ neural stem cells improve the amyotrophic lateral sclerosis phenotype by preserving motor neurons and muscle innervation in human and rodent models. *Hum. Mol. Genet.* doi:10.1093/hmg/ddw163 (2016) (Epub ahead of print).

87 Takebe T, Sekine K, Enomura M *et al.* Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* 499(7459), 481–484 (2013).

• **Represents an important step in the use of PSCs for tissue engineering and possible organ transplantation as these liver buds were able to establish vascular connections with the host and provide metabolic function in a model of liver failure.**

88 Halevy T, Urbach A. Comparing ESC and iPSC-based models for human genetic disorders. *J. Clin. Med.* 3(4), 1146–1162 (2014).

89 Research CIFM. CIRM human pluripotent stem cell repository (2016). www.cirm.ca.gov/

• **This is the largest searchable database of disease-specific iPSCs to date and shows the wide range of resources available for PSC-based disease models.**

90 Foundation NYSC. Stem cell search (2016). http://nyscf.org/

91 Avior Y, Sagi I, Benvenisty N. Pluripotent stem cells in disease modelling and drug discovery. *Nat. Rev. Mol. Cell Biol.* 17(3), 170–182 (2016).

92 Consortium HDI. Induced pluripotent stem cells from patients with Huntington's disease show CAG-repeat-expansion-associated phenotypes. *Cell Stem Cell* 11(2), 264–278 (2012).

93 Young JE, Boulanger-Weill J, Williams DA *et al.* Elucidating molecular phenotypes caused by the SORL1 Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. *Cell Stem Cell* 16(4), 373–385 (2015).

94 Zhong X, Gutierrez C, Xue T *et al.* Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. *Nat. Commun.* 5, 4047 (2014).

95 Jo J, Xiao Y, Sun AX *et al.* Midbrain-like organoids from human pluripotent stem cells contain functional dopaminergic and neuromelanin-producing neurons. *Cell Stem Cell* 19(2), 248–257 (2016).

96 Somers A, Jean JC, Sommer CA *et al.* Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. *Stem Cells* 28(10), 1728–1740 (2010).

97 Wilkinson DC, Alva-Ornelas JA, Sucre JM *et al.* Development of a three-dimensional bioengineering technology to generate lung tissue for personalized disease modeling. *Stem Cells Transl. Med.* doi:10.5966/sctm.2016-0192 (2016) (Epub ahead of print).

98 Spence JR, Mayhew CN, Rankin SA *et al.* Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. *Nature* 470(7332), 105–109 (2011).

99 Takasato M, Er PX, Chiu HS *et al.* Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 526(7574), 564–568 (2015).

100 Kurmann AA, Serra M, Hawkins F *et al.* Regeneration of thyroid function by transplantation of differentiated pluripotent stem cells. *Cell Stem Cell* 17(5), 527–542 (2015).

101 Scott CW, Peters MF, Dragan YP. Human induced pluripotent stem cells and their use in drug discovery for toxicity testing. *Toxicol. Lett.* 219(1), 49–58 (2013).

102 Egawa N, Kitaoka S, Tsukita K *et al.* Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci. Transl. Med.* 4(145), 145ra104 (2012).

103 Lee G, Ramirez CN, Kim H *et al.* Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue IKBKAP expression. *Nat. Biotechnol.* 30(12), 1244–1248 (2012).

104 Mathur A, Loskill P, Shao K *et al.* Human iPSC-based cardiac microphysiological system for drug screening applications. *Sci. Rep.* 5, 8883 (2015).

105 Liang P, Lan F, Lee AS *et al.* Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 127(16), 1677–1691 (2013).

106 Sirenko O, Hesley J, Rusyn I, Cromwell EF. High-content assays for hepatotoxicity using induced pluripotent stem cell-derived cells. *Assay Drug Dev. Technol.* 12(1), 43–54 (2014).

107 Hendriks WT, Warren CR, Cowan CA. Genome editing in human pluripotent stem cells: approaches, pitfalls, and solutions. *Cell Stem Cell* 18(1), 53–65 (2016).

108 Paquet D, Kwart D, Chen A *et al.* Efficient introduction of specific homozygous and heterozygous mutations using CRISPR/Cas9. *Nature* 533(7601), 125–129 (2016).

109 Song B, Fan Y, He W *et al.* Improved hematopoietic differentiation efficiency of gene-corrected beta-thalassemia induced pluripotent stem cells by CRISPR/Cas9 system. *Stem Cells Dev.* 24(9), 1053–1065 (2015).

110 Wang D, Quan Y, Yan Q, Morales JE, Wetsel RA. Targeted disruption of the beta2-microglobulin gene minimizes the immunogenicity of human embryonic stem cells. *Stem Cells Transl. Med.* 4(10), 1234–1245 (2015).

111 Riolobos L, Hirata RK, Turtle CJ *et al.* HLA engineering of human pluripotent stem cells. *Mol. Ther.* 21(6), 1232–1241 (2013).

112 Feng Q, Shabrani N, Thon JN *et al.* Scalable generation of universal platelets from human induced pluripotent stem cells. *Stem Cell Rep.* 3(5), 817–831 (2014).

113 Meissner T, Strominger J, Cowan C. The universal donor stem cell: removing the immune barrier to transplantation using CRISPR/Cas9 (TRAN1P.946). *J. Immunol.* 194(Suppl. 1), 140.28 (2015).

114 Kondo T, Asai M, Tsukita K *et al.* Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Abeta and differential drug responsiveness. *Cell Stem Cell* 12(4), 487–496 (2013).

115 Drowley L, Koonce C, Peel S *et al.* Human induced pluripotent stem cell-derived cardiac progenitor cells in phenotypic screening: a transforming growth factor-beta type 1 receptor kinase inhibitor induces efficient cardiac differentiation. *Stem Cells Transl. Med.* 5(2), 164–174 (2016).

116 Fong AH, Romero-Lopez M, Heylman CM *et al.* Three-dimensional adult cardiac extracellular matrix promotes maturation of human induced pluripotent stem cell-derived cardiomyocytes. *Tissue Eng.* 22(15–16), 1016–1025 (2016).

117 Gieseck RL 3rd, Hannan NR, Bort R *et al.* Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture. *PLoS ONE* 9(1), e86372 (2014).

118 Zou J, Maeder ML, Mali P *et al.* Gene targeting of a disease-related gene in human induced pluripotent stem and embryonic stem cells. *Cell Stem Cell* 5(1), 97–110 (2009).

119 Hockemeyer D, Soldner F, Beard C *et al.* Efficient targeting of expressed and silent genes in human ESCs and iPSCs using zinc-finger nucleases. *Nat. Biotechnol.* 27(9), 851–857 (2009).

120 Smith C, Abalde-Atristain L, He C *et al.* Efficient and allele-specific genome editing of disease loci in human iPSCs. *Mol. Ther.* 23(3), 570–577 (2015).

• **This is a comparative study evaluating the merits of two competing gene editing technologies-transcription activator-like effector DNA-binding domain nuclease and Crisp/Cas9 in disease-specific iPSCs. The use of such technology is likely to drive many PSC-based research endeavors in the years to come.**

121 Gonzalez F, Zhu Z, Shi ZD *et al.* An iCRISPR platform for rapid, multiplexable, and inducible genome editing in human pluripotent stem cells. *Cell Stem Cell* 15(2), 215–226 (2014).

122 Khan IF, Hirata RK, Wang PR *et al.* Engineering of human pluripotent stem cells by AAV-mediated gene targeting. *Mol. Ther.* 18(6), 1192–1199 (2010).

123 ClincalTrials.gov. www.clinicaltrials.gov

124 Edelstein M. Gene therapy clinical trials worldwide. (2016). www.abedia.com/wiley/vectors.php

125 Wang Y, Liang P, Lan F *et al.* Genome editing of isogenic human induced pluripotent stem cells recapitulates long QT phenotype for drug testing. *J. Am. Coll. Cardiol.* 64(5), 451–459 (2014).

126 Horizon. www.horizondiscovery.com

127 Bassuk AG, Zheng A, Li Y, Tsang SH, Mahajan VB. Precision medicine: genetic repair of retinitis pigmentosa in patient-derived stem cells. *Sci. Rep.* 6, 19969 (2016).

128 Universal Cells. www.universalcells.com

129 Weissbein U, Benvenisty N, Ben-David U. Quality control: genome maintenance in pluripotent stem cells. *J. Cell Biol.* 204(2), 153–163 (2014).