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The promise of induced pluripotent stem cells in research and therapy

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Abstract

The field of stem-cell biology has been catapulted forward by the startling development of reprogramming technology. The ability to restore pluripotency to somatic cells through the ectopic co-expression of reprogramming factors has created powerful new opportunities for modelling human diseases and offers hope for personalized regenerative cell therapies. While the field is racing ahead, some researchers are pausing to evaluate whether induced pluripotent stem cells are indeed the true equivalents of embryonic stem cells and whether subtle differences between these cells might affect their research applications and therapeutic potential.

After a decade of constraints, pluripotent stem-cell biology is now a flourishing research area, following the achievement of a long-standing ambition — the successful derivation of pluripotent stem cells from a patient's cells. In a momentous contribution, in 2006 Takahashi and Yamanaka illustrated how cell fates can be altered by the ectopic co-expression of transcription factors¹. The manipulation of cell fates through reprogramming has altered fundamental ideas about the stability of cellular identity, stimulating major new directions in research into human disease modelling, tissue differentiation *in vitro* and cellular transdifferentiation. Despite heady progress, a major question remains: are the new induced pluripotent stem (iPS) cells equivalent to the classic embryonic stem (ES) cells and thus a suitable alternative for research and therapy? Whereas the initial wave of papers argued convincingly that the two cell types were functionally equivalent, a more refined analysis of how iPS cells behave *in vitro*, coupled with genome-wide genetic and epigenetic analysis, has revealed numerous subtle but substantial molecular differences, probably owing to technical limitations inherent in reprogramming. In this Review, we describe the derivation of iPS cells, outline the functional assessments of pluripotency, and then recount how global assessments of gene expression, gene copy number variation, DNA methylation and chromatin modification provide a more nuanced comparison of iPS cells and ES cells. Ultimately, we detail how these features influence the utility of each of these cell types for disease modelling and therapeutics, and we offer predictions for the evolution of the art of reprogramming somatic cells.

Pluripotent stem cells

The years since Takahashi and Yamanaka's breakthrough have seen a flood of papers touting advances in reprogramming technology, including alternative methods for reprogramming and the successful derivation of iPS cells from various cell types. Although the field has advanced at a breathtaking pace, investigators have recently taken a step back to more critically evaluate iPS cells relative to ES cells and have endeavoured to fully understand how these cell populations differ from one another in the hope of closing the gap between the two populations. Taking clues from the data, it seems that researchers should attempt to define each cell type more accurately and to understand its inherent properties rather than ask whether these two classes of pluripotent cell are identical. Although ES cells and iPS cells are arguably equivalent in all of their functions, these cells are bound to harbour subtle differences and to have distinct but complementary roles in research because of their distinct origins and modes of derivation. To appreciate the differences between ES cells and iPS cells, we must first define what it means to be pluripotent.

The term pluripotency has been assigned to a variety of cell types with a wide range of functional capacities. In its loosest sense, pluripotent describes a cell that can generate cell types from each of the three embryonic germ layers: the endoderm, mesoderm and ectoderm. At the strict end of the range of definitions, however, pluripotent describes a cell that can give rise to an entire organism, generating every cell type within that organism. The property of cell pluripotency was first exposed by Driesch in 1891, when he separated the two cells of the early sea urchin blastocyst and observed the development of two complete sea urchins². Many decades later, studies of embryo aggregation and blastocyst chimaerism by Mintz and colleagues³, Gardner⁴ and Brinster⁵, in the 1960s and 1970s, solidified the idea that the cells of the inner cell mass of the mouse blastocyst were pluripotent, and the isolation of mouse teratocarcinoma stem cells and native ES cells by Evans and Kaufman⁶ and Martin⁷, in 1981, ushered in the era of culturing pluripotent stem cells in a dish. The first successful isolation of human ES cells by Thomson and colleagues, in 1998, brought forth a surge of excitement in the scientific community and beyond⁸. The potential to understand early human development, tissue formation and differentiation *in vitro* through studying ES cells seemed to offer limitless possibilities. The opportunity to model diseases, discover disease mechanisms and, ultimately, use cell therapy for previously untreatable conditions was particularly alluring.

The derivation of ES cells from the human embryo, however, sparked controversy in the United States and led to a presidential executive order that restricted government funding⁹. The limited numbers of stem cell lines that were approved for research lacked the diversity necessary to address some of the most compelling questions, particularly those related to modelling and treating disease¹⁰. Most ES cells represented generic cells isolated from presumably normal embryos — except for those from embryos that had been tested by pre-implantation diagnostics and found to carry genetic diseases. The generic lines were not matched to a particular patient, so products derived from them for transplantation purposes would face rejection by the transplant recipient's immune system or necessitate that the recipient receive lifelong therapy with toxic immunosuppressive medication. To compound these limitations, when human ES cells are cultivated on mouse feeder cells, the human cells can incorporate mouse components that render the ES cells subject to immune rejection.

To realize the full potential of ES cells, researchers foresaw that customized, personalized pluripotent stem cells specific to each patient would be generated by using somatic-cell nuclear transfer (SCNT) — the procedure that had been used successfully to clone Dolly the sheep from adult mammary cells. Nuclear-transfer-generated ES (ntES) cell lines would capture a patient's complete genome in a cell that could be induced to become any tissue,

thus allowing differentiation into disease-relevant cells for analysis or cell-replacement therapy. Despite successful proof of principle in mouse studies¹¹, and the clear distinctions between generating ntES cells for medical research and creating cloned blastocysts for reproduction, the ethical controversy driven by widespread opposition to human cloning has severely curtailed research into human SCNT. Only this year, when investigators gained access to a large number of human oocytes, was the derivation of pluripotent stem cells through human SCNT accomplished¹². However, the investigators in this study needed to leave the oocyte nucleus intact to derive pluripotent stem cells, so the resultant cells were triploid, thus affording research applications for these cells but limiting their suitability for therapeutic use¹².

Despite the many hindrances to the study and derivation of human ES and ntES cells over the past decade, great strides were being made in understanding the pathways that regulate the maintenance and pluripotency of ES cells. This progress was not lost on those seeking an alternative source of personalized patient-specific stem cells, and in 2006 Takahashi and Yamanaka announced the successful derivation of iPS cells from adult mouse fibroblasts through the ectopic co-expression of only four genes¹. In an elegant screen of 24 gene candidates selected for their links to ES-cell pluripotency, these researchers found four factors that were sufficient to reprogram adult fibroblasts into iPS cells: OCT4 (also known as POU5F1), SOX2, Krüppel-like factor 4 (KLF4) and c-MYC. This historic contribution inspired an astonishing flurry of follow-on studies, with successful reprogramming quickly translated to human fibroblasts^{13–15} and then to a wide variety of other cell types, including pancreatic β cells¹⁶, neural stem cells^{17,18}, mature β cells¹⁹, stomach and liver cells²⁰, melanocytes²¹, adipose stem cells²² and keratinocytes²³, demonstrating the seemingly universal capacity to alter cellular identity.

Mouse and human iPS cells differ in appearance, however, with mouse iPS-cell colonies appearing more dome-like and refractile than human iPS-cell colonies. Human iPS-cell colonies are flatter than those of mice and are akin to a distinct type of pluripotent stem cell that is derived from the epiblast of the early mouse embryo²⁴, a feature that indicates that mouse and human iPS cells, like mouse and human ES cells, probably reflect distinct developmental states (Fig. 1). The pluripotent state of mouse stem cells is called a 'naive' state because it closely resembles the most primitive state, or ground state, of the mouse inner cell mass; this is different from the more 'primed' state of human stem cells, which proliferate in response to different cytokines, reflecting the distinct developmental statuses of these populations²⁵. Regardless of the method of derivation, iPS cells maintain the key features of ES cells, including the ability to propagate in culture indefinitely and the capacity to generate cells from each of the three embryonic germ layers (see ref. ²⁶ for a review). Such broad similarities are not proof that iPS cells are molecularly or functionally equivalent to ES cells. Yamanaka's intention was to derive an alternative source of pluripotent stem cells with the same range of functions as ES cells but offering even greater potential for clinical use. To determine the degree of success garnered by reprogramming, we must explore the set of assays that were developed to assess the key characteristic of ES cells: pluripotency.

Assessment of pluripotency

In the past few years, consistent standards for the identification and evaluation of iPS cells and for the assessment of their functional equivalence to ES cells have become widely accepted²⁷. A variety of reprogramming methods have been developed to derive iPS cells, and each has advantages and disadvantages (Table 1). Assessing reprogramming begins with identifying compact colonies that have distinct borders and well-defined edges and comprise cells with a large nucleus, large nucleoli and scant cytoplasm. A wide range of colony morphologies result from reprogramming, and although many colonies appear

morphologically similar to ES cells, only some colonies have comparable molecular and functional features. To accurately distinguish reprogrammed, bona fide iPS cells from those that are only partially reprogrammed, investigators look for a series of molecular hallmarks. Fully reprogrammed cells express a network of pluripotency genes, including *OCT4*, *SOX2* and *NANOG*, in levels comparable to ES cells, and they reactivate telomerase gene expression, downregulate *THY1* and upregulate *SSEA1* (ref. ²⁸).

Markers of pluripotency

Positive staining for alkaline phosphatase activity has been widely used as a marker of pluripotency; however, recently published data have shown this enzyme to be insufficient as a marker of true iPS cells, because intermediately reprogrammed cells also stain positively²⁹. The same report shows that iPS cells that are generated by virus-mediated reprogramming silence proviral genes when the endogenous pluripotency genes are activated and that this event is paired with the expression of the embryonic antigens SSEA3, TRA-1-60, TRA-1-81, DNA methyltransferase 3 β (DNMT3 β) and REX1 (ref. ²⁹). Genome-wide epigenetic reprogramming is crucial for deriving fully reprogrammed cells, and the degree of success is measured, in part, by evaluating the methylation status at the promoters of the genes responsible for maintaining pluripotency, as well as at the genes important for driving differentiation³⁰. A crucial event during epigenetic reprogramming is the reactivation of the silent X chromosome, which occurs late in reprogramming and represents a hallmark of ground-state pluripotency^{28,30,31}. If iPS cells acquire all of these molecular features, they are expected to behave like ES cells and to demonstrate reprogramming-factor independence, which is marked by silencing of the proviral transgenes. Variations in epigenetic reprogramming, the extent of methylation, the persistence of expression of integrated proviruses and other known and unknown factors can alter the differentiation potential of iPS cells. Because of the potential for heterogeneity, it is essential to know as much as possible about the nature of a cell line before labelling it pluripotent.

Functional assays of pluripotency

When iPS cell lines are isolated and documented to carry the molecular features of fully reprogrammed cells, they are typically also assessed in functional assays. Characterization of the functional abilities of iPS cells begins with *in vitro* differentiation. The cells can be differentiated as embryoid bodies — compact balls of loosely organized tissues that resemble the gastrulating embryo — or through two-dimensional directed differentiation in a culture dish. Such cultures can then be assessed for markers of each of the three germ layers. Analysis of the pluripotency of mouse cells typically entails the development of a chimaera, which evaluates the potential of iPS cells to contribute to the normal development of adult tissues after injection into the blastocyst. Whether germline transmission occurs after blastocyst chimaerism is measured by the ability of chimaeras to produce all-iPS-cell mice in their offspring. These offspring have the genomic integrity of the injected iPS cell line, as well as the ability to form functional germ cells. The highest stringency test for mouse iPS cells — tetraploid complementation — entails the injection of iPS cells into tetraploid blastocysts to measure the ability of the iPS cells to direct the normal development of an entire organism. This test has been accomplished for only a limited subset of iPS cells^{32–34}, although the successful cells had an efficiency that paralleled tetraploid complementation carried out with ES or ntES cells^{33,35,36}.

The current functional gold standard for human iPS cells involves the evaluation of teratoma formation. In this assay, the *in vivo* differentiation potential of human iPS cells is measured after their injection subcutaneously or intramuscularly into immunodeficient mice^{37,38}. If the cells are truly pluripotent, they will form well-differentiated tumours comprising elements from each of the three germ layers. This assay provides information about the

spontaneous differentiation potential of the injected iPS cells. Although it is the most stringent assay available for human iPS cells, it is not powerful enough to assess whether iPS cells can produce all of the cell types of the human body, and it also cannot assess the contribution of iPS cells to the germ line. All of these functional assays have a caveat: the standards for iPS cells are still hotly debated, especially when anticipating the use of iPS cells for therapy³⁹. Adopting a consistent set of standards that can be applied uniformly worldwide is essential as stem-cell research and applications move forward.

Functional differences between iPS cells and ES cells

Despite the multitude of assays used to evaluate pluripotency, and although many parallels have been found between iPS cells and ES cells, there is a wide range of evidence showing that there are subtle yet substantial differences between these cell types. Disparities were first observed in the differentiation abilities of iPS cells and ES cells in both teratoma-forming and *in vitro* differentiation assays. Some mouse iPS cells showed lower efficiencies of teratoma formation than ES cells, whereas some human iPS cells showed less propensity to differentiate along haematopoietic, neuroepithelial and neuronal lineages than human ES cells^{40–41,42}. Some researchers interpreted these findings to mean that iPS cells have an intrinsically lower differentiation capacity than ES cells⁴¹, whereas other research groups have offered different explanations, including that the cell of origin might have a specific effect on the differentiation capacities of the derived iPS cells.

The results from cell-of-origin studies indicated that the parental cell can influence the differentiation capacity of the resultant iPS cells. In one study, mouse bone-marrow-derived and B-cell-derived iPS cells showed more efficient differentiation along haematopoietic lineages than did fibroblast-derived iPS cells or neural-progenitor-derived iPS cell lines. Interestingly, treatment of the neural-progenitor-derived iPS cells with trichostatin A, a potent histone-deacetylase inhibitor, plus 5-azacytidine, a methylation-resistant cytosine analogue, increased the blood-forming capacity of these cells, suggesting that their limitations were due to epigenetic modifications. Whereas the bone-marrow-derived and neural-progenitor-derived iPS cells contributed well to all tissues in the chimaera assay, including to the germ line, the fibroblast-derived iPS cells contributed only poorly⁴³. This study laid some of the early groundwork for later lines of investigation that probed the molecular differences between iPS cells and ES cells and provided an explanation for the functional differences between these cells.

One investigation found that some iPS cells derived from human retinal-pigment epithelial cells show an increased propensity to differentiate back into these cells than do ES cells or iPS cells derived from other tissues⁴⁴. More recently, Bar-Nur and colleagues showed that iPS cells generated from human pancreatic islet β cells retain open chromatin at the loci of key β -cell genes and that this correlates with a greater capacity to differentiate into insulin-producing cells both *in vivo* and *in vitro* than that of ES cells or isogenic non- β -cell-derived iPS cells⁴⁵. These functional differences extend beyond differentiation and potency to disease modelling. For example, fragile X syndrome is caused by aberrant silencing of the *FMR1* gene during human development; iPS cells that were reprogrammed from adult skin fibroblasts from an individual with fragile X syndrome failed to reactivate the *FMR1* gene, whereas ES cells derived from embryos with this syndrome, as diagnosed by pre-implantation testing, expressed *FMR1* (ref. ⁴⁶). Consequently, the potential for epigenetic memory in the fragile-X-syndrome-derived iPS cells, and substantial differences between fragile-X-syndrome-derived iPS and ES cells, must be considered when studying this condition and potentially many other conditions. To determine whether the pluripotent cells being used are appropriate to address a particular question or to use in a given application, it

is crucial to compare not only the *in vivo* and *in vitro* differentiation potentials but also the genetic and epigenetic disparities that underpin these functional differences.

iPS cells versus ES cells

Refined analyses, described in this section, have addressed whether iPS cells are suitable alternatives to classic ES cells for use in research and therapy.

Genetics and epigenetics

Global gene-expression analysis and bisulphite genomic sequencing accompanying early derivations of iPS cells provided the initial evidence for differences between iPS cells and ES cells at the epigenetic level¹⁴. Further exploration of these differences led to the identification of only a few, seemingly consistent, differences in global gene expression that were more pronounced in earlier passages of iPS cells⁴⁷. Many of the differentially expressed genes were imprinted in ES cells⁴⁸.

Looking beyond the expression patterns to the DNA sequence itself has revealed genetic variation between iPS cells and ES cells. A recent publication suggested that chromosomal aberrations are a common feature of stem-cell populations that are propagated *in vitro*, with each type of stem cell — whether ES cells, iPS cells or multipotent stem cells — being prone to distinct abnormalities⁴⁹. Both human iPS and ES cells showed a tendency for gains at chromosomes 12 and 17. Whereas iPS cells had additional gains at chromosomes 1 and 9, ES cells had additional gains at chromosomes 3 and 20. Other work identified an accumulation of point mutations in reprogrammed cells, particularly occurring in oncogenic pathways⁵⁰, whereas another study noted an increase in copy number variants (CNVs) in early-passage human iPS cells relative to intermediate-passage iPS cells or ES cells. The number and size of these CNVs were negatively correlated with the passage number in iPS cells, suggesting that a selective disadvantage is conferred by these aberrations. A comparison of iPS cells and their parental cell of origin showed that the majority of CNVs were created *de novo* in fragile regions of the genome⁵⁰. A comprehensive study by Laurent and colleagues found a higher frequency of subchromosomal CNVs in pluripotent cell samples than in non-pluripotent cell samples⁵¹. This work uncovered variation between genomic regions enriched for CNVs in human ES cells and iPS cells. A small subset of samples of ES cells harboured a large number of duplications, whereas several iPS-cell samples contained moderate numbers of deletions. Reprogramming was associated with deletions in tumour-suppressor genes, whereas extended time in culture led to duplications of oncogenes in human iPS cells.

The finding that human iPS cells derived from a variety of tissues have residual, persistent donor-cell-specific gene-expression patterns sparks the question of whether the current measure of a fully reprogrammed cell is sufficient⁵² or whether iPS cells retain some type of ‘somatic memory’ from their past identity. To understand this observation better, a more detailed analysis at the epigenetic level is required.

Reprogramming cells to a pluripotent state entails global epigenetic remodelling and introduces epigenetic changes, some of which are necessary for reprogramming to occur and others of which are inadvertently introduced during the process. A failure to demethylate pluripotency genes is associated with partial reprogramming in iPS cells. Whole genome profiling of the DNA methylomes of multiple human iPS and ES cell lines, as well as somatic and progenitor cell lines, from different laboratories using different reprogramming techniques and with a variety of cells derived from distinct germ layers has shown that — although overall iPS-cell DNA methylomes closely resemble human ES-cell DNA methylomes — iPS cells have significant variability in their somatic memory, as well as

aberrant iPS-cell-specific differential methylation. Some studies have suggested that this occurs in a passage-dependent manner, but others have shown that differentially methylated regions (DMRs) in iPS cells are transmitted to differentiated progeny at a high frequency and cannot be erased through passaging^{29,53-55}. Overall, there are remarkable global similarities between the DNA methylomes of generic iPS and ES cells; however, a core set of DMRs that seems to represent hotspots of failed epigenetic reprogramming has been identified⁵⁵. These DMRs are enriched for genes that are important for developmental processes^{58,55}. The high incidence of unique DMRs in iPS cells compared with progenitor somatic cells or ES cells suggests that these patterns are stochastic and arise during reprogramming. In the most exhaustive comparison so far, Kim and colleagues reported that more DMRs were present in mouse iPS cells than in ntES or embryo-derived ES cells⁴³. However, these DMRs did not pertain to specific loci and thus do not represent consistent differences between iPS cells and ES cells. This lack of consistency suggests that aberrant DMRs in mouse iPS cells reflect the technical limitations inherent in reprogramming, rather than indicating loci that can reliably distinguish ES cells from iPS cells⁴³.

In addition, the residual iPS-cell-specific methylation in many iPS-cell isolates links these cells to their tissue of origin and, ultimately, affects their differentiation propensity^{43,55}. Residual signatures can be distinct enough to enable the myeloid and lymphoid origins of blood-derived iPS cells to be discerned⁴³. In iPS cells derived from non-haematopoietic cells, such as fibroblasts and neural progenitors, there can be residual repressive methylation at loci that are required for haematopoietic fates, reducing the blood-forming potential *in vitro*. Exogenous supplementation of neural-progenitor-derived iPS cells with the cytokine WNT3A can increase the blood-forming potential of these cells, supporting the idea that incomplete reprogramming owing to epigenetic marks can be overcome by manipulating the culture conditions. Treatment of cultures with demethylating agents or knockdown of *DNMT1* expression has been shown to convert intermediately reprogrammed cells into fully pluripotent cells, further supporting this idea⁴³.

When iPS cells are forced to differentiate along a particular lineage, they become more amenable to generating cells of that lineage after a further round of reprogramming. This finding shows that the differentiation propensity and DNA methylation profile can be reset, and it suggests that the 'epigenetic memory' of the donor cell can be exploited, especially in cases in which directed differentiation is particularly challenging⁴³. Another important feature of epigenetic reprogramming is the reactivation of the inactivated X chromosome. During normal development in eutherian mammals (those with a placenta), one X chromosome is randomly inactivated in each cell in females. Whether this epigenetic silencing event is reset in iPS cells remains an area of controversy, in part because of the poor fidelity of X-inactivation markers in pluripotent cells⁵⁶. Some studies have shown that the majority of female human iPS clones retain an inactivated X chromosome (which is transcriptionally silent)⁵⁷, whereas others have indicated that some human iPS clones lose immunostaining for trimethylated H3K27 on the X chromosome (a marker of epigenetic silencing), indicating X reactivation⁵⁸. In addition, some of the earliest studies of iPS cells showed X reactivation in reprogrammed female mouse fibroblasts^{31,58}. However, recently published data support the finding that X reactivation does not occur in human iPS cells and, interestingly, reprogramming was found to favour expression of a particular X chromosome when induced from a mixed X-inactivated population of fibroblasts⁵⁹. Finally, epigenetic reprogramming sometimes fails to properly restore bivalent domains, which mark developmental loci with active and repressive histone modifications⁶⁰.

Although many of the studies cited here have generated data suggesting that there are epigenetic differences between iPS cells and ES cells, there are several limitations on extending these data to all iPS cells in a more general (and more useful) sense. The

published comparisons were often made using iPS cells derived from a multitude of laboratories by a variety of methodologies, and reanalysis of the gene-expression microarray data using an unsupervised clustering algorithm shows a strong correlation between transcriptional signatures and specific laboratories for both iPS cells and ES cells. This finding indicates that specific culture protocols and laboratory environments can affect the transcriptional profile of iPS and ES cells. Therefore, the data produced in a particular laboratory might be specific to the cells derived there⁶¹.

In addition, most iPS colonies are clones derived from a single reprogrammed cell, whereas ES cells used for analysis are typically non-clonal. The subcloning of ES cells has revealed genetic and epigenetic anomalies that would probably have otherwise gone undetected in the heterogeneous ES-cell population⁶². With regard to somatic memory, there is poor overlap between the gene sets that have been reported to be characteristic of a particular cell type of origin, suggesting that the retention of somatic memory is stochastic and is a reflection of the technical failure of reprogramming to fully erase the somatic epigenome. To exacerbate the issue, the iPS cells used for comparison often have different genetic backgrounds and have frequently been derived from fibroblasts that were already heterogeneous in their make-up, affecting both the gene-expression patterns and the functionality of the iPS cells.

Throughout the literature, there is also a lack of correlation between the gene-expression patterns and the epigenetic patterns observed. An additional consideration is the presence of different viral insertions in individual iPS cell lines, which can also affect the functionality of the derived cells¹. Evidence to support this idea is provided by the reduced number of differences observed among iPS cells and between iPS and ES cells when transgenes are removed⁶³. Many of the aforementioned studies have focused on differences in either transcriptional profiles or changes in epigenetic marks; however, the most recent studies have evaluated iPS cells and ES cells from both of these angles in parallel, together with their *in vitro* differentiation potential, generating the most comprehensive and compelling data that have been published so far.

Holistic analysis

Stadtfeld and colleagues explored the epigenetic and functional discrepancies between iPS cells and ES cells using a novel reprogramming strategy that allowed direct comparison of genetically matched cells derived from the same source³². These authors derived iPS and ES cells from mice carrying an integrated doxycycline-inducible reprogramming cassette in every cell, a strategy that sidesteps the confounding effects of variable genetic backgrounds and viral integration that have been observed in other studies. The overall messenger RNA and microRNA expression patterns of iPS cells and ES cells were indistinguishable except for the aberrant silencing of a few transcripts localized to the imprinted *Dlk1-Dio3* gene cluster on chromosome 12qF1, a region that is important for development. A failure to reactivate this locus meant that iPS cells contributed poorly to chimaeras and were unable to generate all-iPS-cell mice. By contrast, iPS cells with normal *Dlk1-Dio3* expression contributed to high-grade chimaeras and supported the development of viable all-iPS-cell mice. The treatment of iPS cells that failed to reactivate *Dlk1-Dio3* with a histone-deacetylase inhibitor rescued the ability of these clones to support the development of all-iPS-cell mice by relieving this region of aberrant hypermethylation. However, recent data from iPS and ES cells derived from a mouse strain carrying a distinct reprogramming cassette suggest that different levels of expression of reprogramming factors, rather than aberrant silencing of *Dlk1-Dio3*, account for the different behaviour of the cell lines⁶⁴. The disparate results from these studies highlight that iPS cells can behave differently based on subtle variations in the expression of only a few loci.

To systematically compare human iPS cells derived from different somatic cell types and ES cells, Ohi and colleagues compared ES cells with iPS cells reprogrammed from somatic cells representative of the three embryonic germ layers⁷⁴. Transcriptional and epigenetic profiling of these cells showed transcriptional differences, owing, in part, to incomplete promoter methylation, which enabled iPS cells to be discerned on the basis of their cell of origin. The differential methylation between iPS cells and ES cells did not correlate with varying levels in DNA methyltransferases; however, the authors found a nonrandom pattern of incompletely silenced genes in genetic regions that are isolated from other genes that undergo silencing during reprogramming. This finding could be explained by inefficient or delayed recruitment of the silencing machinery and DNA methyltransferases to particular somatic genes because of the isolated location of these genes⁶⁵.

In another comprehensive study, Polo and colleagues evaluated the effect of cellular origin on the gene-expression pattern, epigenetic properties and functional abilities of genetically matched mouse iPS cells⁶⁶. Using the same 'secondary' reprogramming strategy used by Wernig and colleagues (whereby reprogramming is assessed using tissues from a mouse generated from iPS cells carrying integrated, doxycycline-inducible reprogramming factors), Polo and colleagues generated iPS cells from tail-tip fibroblasts, splenic B cells, bone-marrow-derived granulocytes and skeletal muscle precursors, and they showed that each iPS cell line retained a transcriptional memory of its cell of origin. This memory was evident in that markers for each respective cell of origin remained actively expressed and was supported by the finding that iPS cell lines derived from the same cell of origin clustered together on the basis of global transcriptional data. A similar correlation was found on evaluation of methylation patterns, which showed subtle but substantial differences, reflecting the consequences of different histone marks. The effects of somatic memory extended beyond the genetic and epigenetic levels to functional significance, affecting the autonomous differentiation potential of the different iPS cell lines after embryoid-body formation. A clear bias that reflected the cells of origin was observed in the iPS cell lines. Notably, the transcriptional, epigenetic and functional effects evaluated in early-passage iPS cell lines became less significant with continued passaging. This finding indicated that complete reprogramming is a gradual process that extends beyond the time frame necessary to observe the activation of endogenous pluripotency genes, transgene-free growth and differentiation into cell types from each of the three germ layers.

Exploring equivalence

Having considered data from a multitude of published studies, generated by the painstaking efforts of many research groups, we return to our earlier question: are iPS cells equivalent to ES cells? The answer is not straightforward. Rather, there is an emerging consensus that iPS cells and ES cells are neither identical nor distinct populations. Instead, they are overlapping, with greater variability inherent within each population than between the populations. The heterogeneity and behaviour of each class of cell line is more complex than has previously been thought. The two pluripotent stem-cell types are, in theory, functionally equivalent; however, in practice, they harbour genetic and epigenetic differences that reflect their different histories. It remains to be seen whether there are any consistent molecular distinctions between iPS cells and ES cells.

It is also important to consider that, in contrast to long-standing belief, ES cells themselves have considerable epigenetic heterogeneity and have differing propensities for differentiation — much like those found in iPS cells^{67,68}. These observations, paired with those discussed in this Review, are a call for researchers to take a step back from the direct comparison of iPS cells and ES cells, and they highlight the need to redefine what it means to belong to either of these cell classes. Some researchers have already taken heed of this message and generated a bioinformatics assay for pluripotency⁷², whereas others have

produced a 'scorecard' to evaluate the character of both iPS and ES cell lines and predict the quality and utility of any pluripotent cell line in a high-throughput manner^{47,69}. Using DNA methylation mapping, gene-expression profiling and a quantitative differentiation assay, Bock and colleagues made a systematic comparison of 20 established ES cell lines and 12 iPS cell lines⁵³. They confirmed that, despite overall similarities, transcriptional and epigenetic variation is common between iPS cell lines, between ES cell lines, and between iPS cell and ES cell populations. These data provide a reference for the variation among human pluripotent cell lines, which assists in predicting the functional consequences of these differences. We can conclude from these studies that any given iPS cell line generated by today's technology might not be completely equivalent to the ideal ES cell.

The differences between iPS cells and ES cells, as well as those among iPS cells, clearly affect the utility of these cells in research, disease modelling and therapeutics, providing an impetus for investigators to evaluate their cell populations carefully and precisely. The differences do not diminish the potential of iPS cells, given that iPS cells have considerable advantages over ES cells. Rather than replacing ES cells with iPS cells, it is becoming clear that these two cell types complement one another. Researchers are still in the process of developing the necessary protocols to harness the potential of iPS cells; however, as it becomes clear how to evaluate the genetic, epigenetic and functional status of different iPS cell lines, further applications of these cells will be uncovered, and progress will be made in creating iPS cell lines and designing protocols to accomplish the ambitious goals of the field.

Medical applications of iPS cells

Generating patient-specific stem cells has been a long-standing goal in the field of regenerative medicine. Despite considerable challenges, generating disease-specific and patient-specific iPS cells through reprogramming has become almost routine. These cells provide a unique platform from which to gain mechanistic insight into a variety of diseases, to carry out *in vitro* drug screening, to evaluate potential therapeutics and to explore gene repair coupled with cell-replacement therapy (Fig. 2). In the past few years, the number of reports on applications of iPS cells has steadily increased, testifying to the broad influence of this breakthrough technology (Table 2). Despite the continued presence of substantial hurdles, the pace of this work is such that no review can capture the current state of the field; thus, we point to a few publications that highlight the promising medical applications of iPS cells but also indicate their key limitations.

In 2009, Lee and colleagues harnessed iPS cells to demonstrate disease modelling and drug screening for familial dysautonomia, a rare genetic disorder of the peripheral nervous system⁷⁰. In almost all cases, familial dysautonomia is caused by a single point mutation in the gene encoding the inhibitor of nuclear factor- κ B (I κ B)-kinase-complex-associated protein (*IKBKAP*) that manifests as an extensive autonomic nervous system deficit and dysfunction in small-fibre sensory neurons. Although many traditional cell-based models have been used to study the pathogenesis of familial dysautonomia and screen for candidate drugs, none has used symptom-relevant human cell types. With the successful derivation of iPS cells from patients with familial dysautonomia, investigators produced central and peripheral nervous system precursors and subsequently found three disease-related phenotypes, thus providing validation that disease-relevant cell types could accurately reflect disease pathogenesis *in vitro*. After screening with multiple compounds, the authors showed that the disease phenotype could be partially normalized by kinetin, a plant hormone. This initial report demonstrated how iPS cells can facilitate the discovery of therapeutic compounds and described how these cells provided a platform for modelling

different severities of familial dysautonomia and for generating predictive tests to determine differences in the clinical manifestation of the disorder.

Such applications of iPS cells in drug screening and discovery are destined to expand to encompass numerous disease conditions. Several research groups have generated models of long QT syndrome, a congenital disease with 12 types, each of which is associated with abnormal ion-channel function, a prolonged QT interval on an electrocardiogram and a high risk of sudden cardiac death owing to ventricular tachyarrhythmia. Much work has been carried out in animal models to probe the underlying mechanisms of this syndrome, but cardiomyocytes have distinct and complex electrophysiological properties that differ between species. In addition, the lack of *in vitro* sources of human cardiomyocytes and the inability to model patient-specific variations of this disease has impeded studies.

In a proof-of-principle study for using iPS cells to capture the physiological mechanisms of genetic variation, Moretti and colleagues differentiated iPS cells from individuals with type 1 long QT syndrome into cardiomyocytes and, as predicted, observed prolonged action potentials in the ventricular and atrial cells⁷¹. Using this model system, these investigators uncovered a dominant-negative trafficking defect associated with the particular mutation that causes this variant of long QT syndrome. Further investigation of long QT syndrome iPS-cell-derived cardiomyocytes showed that these cells had an increased susceptibility to catecholamine-induced tachyarrhythmia, and compounds that exacerbated the condition (including isoprenaline) were identified. Treatment of these cardiomyocytes with β -adrenergic receptor blockers attenuated the long QT phenotype.

Type 2 long QT syndrome has also been modelled in cardiomyocytes, by Itzhaki and colleagues⁷². The authors derived type 2 long QT syndrome iPS cells to evaluate the potency of existing and novel pharmacological agents that might exacerbate or ameliorate the condition. Their studies show that the long QT syndrome phenotype was aggravated by blockers of ERG-type potassium channels, whereas nifedipine, a calcium-channel blocker, and pinacidil, an agonist of ATP-sensitive potassium channels, both ameliorated the long QT syndrome phenotype, as shown by the decreased duration of action potentials in long QT syndrome cardiomyocytes, as well as the elimination of early after-depolarizations and the abolishment of all triggered arrhythmias. A possible limitation of these beneficial drugs is excessive shortening of the action-potential duration, leading to short QT syndrome.

Importantly, these studies established that the iPS-cell model can be used to identify complex cardiotoxic effects of drugs, as well as to define protective pharmacological agents, including optimal drug dosages. Given the number of drugs that have notoriously been withdrawn from the market owing to their tendency to induce arrhythmias, it is highly likely that the current inadequate approaches for assessing cardiotoxicity will be complemented by iPS-cell-based assessments of drug effects.

A study from our laboratory explored dyskeratosis congenita, a disorder of telomere maintenance, and provided an unanticipated insight into the basic biology of telomerase that has therapeutic implications⁷³. In its most severe form, dyskeratosis congenita is caused by a mutation in the dyskerin gene (*DKC1*), which is X linked, leading to shortened telomeres and premature senescence in cells and ultimately manifesting as the degeneration of multiple tissues. Because the reprogramming of cells to an induced pluripotent state is accompanied by the induction of the gene encoding telomerase reverse transcriptase (*TERT*), we investigated whether the telomerase defect would limit the derivation and maintenance of iPS cells from individuals with dyskeratosis congenita. Although the efficiency of iPS-cell derivation was poor, we were able to successfully reprogram patient fibroblasts. Surprisingly, whereas the mean telomere length immediately after reprogramming was

shorter than that of the parental fibroblast population, continued passage of some iPS cell lines led to telomere elongation over time. This process was accompanied by upregulation of the expression of *TERC*, which encodes the RNA subunit of telomerase.

Further analysis established that TERT and TERC, as well as DKC1, were expressed at higher levels in dyskeratosis-congenita-derived iPS cells than in the parental fibroblasts. We determined that the genes encoding these components of the telomerase pathway — including a *cis* element in the 3′ region of the *TERC* locus that is essential for a transcriptionally active chromatin structure — were direct binding targets of the pluripotency-associated transcription factors. Further analysis indicated that transcriptional silencing owing to a 3′ deletion in the *TERC* locus leads to the autosomal dominant form of dyskeratosis congenita by diminishing *TERC* transcription. Although telomere length is restored in dyskeratosis-congenita-derived iPS cells, differentiation into somatic cells is accompanied by a return to pathogenesis with low *TERC* expression and a decay in telomere length. This finding showed that TERC RNA levels are dynamically regulated and that the pluripotent state of the cells is reversible, suggesting that drugs that elevate or stabilize TERC expression might rescue defective telomerase activity and provide a therapeutic benefit. Although we set out to understand the pathogenesis of dyskeratosis congenita with this study, we showed that a high expression level of multiple telomerase components was characteristic of the pluripotent state more generally, illustrating how iPS cells can reveal fundamental aspects of cell biology.

An independent study of the reprogramming of cells from patients with dyskeratosis congenita confirmed the general transcriptional upregulation of multiple telomerase components and the maintenance of telomere lengths in many clones⁷⁴; however, in this study, no clones with elongated telomeres were identified. The different outcomes of these studies highlight the limitations of iPS-cell-based disease models that are imposed by clonal variation as a result of the inherent technical infidelity of reprogramming⁷⁵. This point also introduces an additional important consideration. Before a given iPS-cell disease model can be claimed to be truly representative of the disease, how many patients must be involved, and how many iPS cell lines must be derived from each patient? Although the answers to these questions are unclear, it is crucial to keep these issues in mind when generating disease models and making claims based on these models.

Although iPS cells are an invaluable tool for modelling diseases *in vitro*, the goal of developing patient-specific stem cells has also been motivated by the prospect of generating a ready supply of immune-compatible cells and tissues for autologous transplantation. At present, the clinical translation of iPS-cell-based cell therapies seems more futuristic than the *in vitro* use of iPS cells for research and drug development, but two groundbreaking studies have provided the proof of principle in mouse models that the dream might one day be realized. Hanna, Jaenisch and colleagues used homologous recombination to repair the genetic defect in iPS cells derived from a humanized mouse model of sickle-cell anaemia⁷⁶. Directed differentiation of the repaired iPS cells into haematopoietic progenitors followed by transplantation of these cells into the affected mice led to the rescue of the disease phenotype. The gene-corrected iPS-cell-derived haematopoietic progenitors showed stable engraftment and correction of the disease phenotype — a significant finding as proof of principle for gene repair coupled to cell-replacement therapy.

In another landmark study from Jaenisch’s research group, Wernig and colleagues derived dopaminergic neurons from iPS cells that, when implanted into the brain, became functionally integrated and improved the condition of a rat model of Parkinson’s disease⁷⁷. The successful implantation and functional recovery in this model is evidence of the

therapeutic value of pluripotent stem cells for cell-replacement therapy in the brain — one of the most promising areas for the future of iPS-cell applications.

Together, these findings provide proof of principle for using reprogramming with gene repair and cell-replacement therapy for treating diseases. Using iPS cells in cell-replacement therapy offers the promise of therapeutic intervention that is not compounded by the use of immunosuppressive drugs to prevent tissue rejection, while harnessing targeted gene-repair strategies, such as homologous recombination and zinc-finger nucleases, to repair genetic defects. These strategies provide the opportunity for generating an unlimited population of stem cells that can be differentiated into the desired cell type for studying disease mechanisms, for screening and developing drugs or for developing a suitable cell-replacement therapy. There have been considerable advances and successes to this end; however, selecting an appropriate disease target, directing the differentiation of iPS cells into phenotype-relevant cell populations and identifying disease-relevant phenotypes remain major hurdles. It is unclear whether iPS cells used for cell-replacement therapy would completely evade an immune response when returned to the patient, because a recent study has shown the immune rejection of teratomas formed from iPS cells, even in syngeneic mice⁷⁸. Nevertheless, iPS cells provide a promising model with which to study disease mechanisms, discover new therapies and develop truly personalized treatments.

Predictions for the evolution of the art

Few fields have enjoyed the remarkable upsurge in activity and excitement that followed the initial report of the reprogramming of somatic cells into iPS cells in 2006. Despite heady progress, crucial challenges must be met for the field to realize its full potential. There is as yet no consensus on the most consistent protocol or the optimal protocol for deriving the most reliable and, ultimately, the safest iPS cells. Increasing the reprogramming efficiency and effecting reprogramming without genetically modifying the cells are goals that have been achieved. However, using more-uniform protocols and more-rigorous controls would facilitate experimental consistency, and potentially therapeutic consistency, between laboratories and would yield standardized cell lines that could be used with confidence in both basic and applied studies.

Barring that, researchers must agree on standards of molecular analysis to ensure that the reprogrammed cells that most closely approximate the generic state of the naive genome can be identified. Because iPS cells are subject to the same type of culture adaptations that affect karyotypic integrity as human ES cells⁷⁹, it is important to define protocols that minimize the time in culture. In addition, cell lines used in clinical applications will need to be evaluated frequently for aberrant culture-induced changes at all stages: from the somatic cells to the reprogrammed and differentiated cells⁸⁰. Understanding the genomic alterations that take place during the reprogramming, culture and differentiation of iPS cells will be crucial for designing experiments and ensuring that the derived cells are functional, pure and appropriate for use in research and therapy. Minimizing any aberrations is important, but as long as researchers understand that aberrations will arise — and can describe and control their effects — even the imperfect cells can be used, and preferential differentiation can be taken advantage of whenever possible. Characteristics of iPS cells that were initially perceived as flaws, including varying differentiation propensities, might prove useful in clinical settings, to generate cell types that have been difficult to obtain thus far.

Generating more stringent markers of pluripotency and assays to distinguish the abilities of a given iPS cell line are key priorities. Building on the progress that has already been made using ES cells⁸¹, researchers must continue to improve the understanding of directed differentiation and to develop new protocols. With refined differentiation protocols,

researchers will be able to investigate the pathophysiological basis of genetic diseases and carry out drug screening on affected cell types. In addition, these protocols will bring the field a step closer to patient-matched cells and tissues for clinical transplantation, a long-standing ambition of the stem-cell field that might be its ultimate measure of success.

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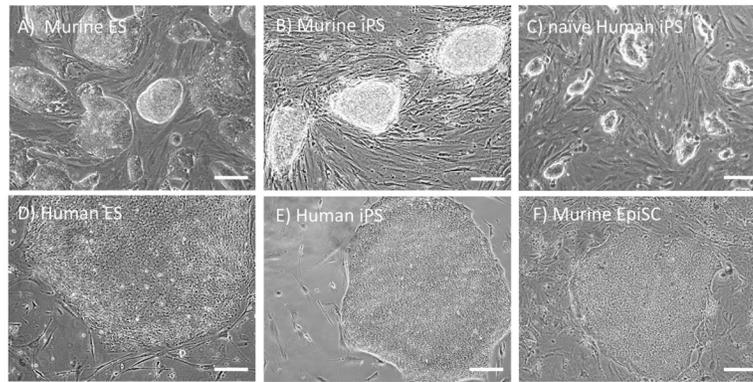
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**Figure 1. Morphology of pluripotent stem cell types**

Mouse ES (a) and iPS (b) cells form dome-shaped, refractile colonies. These colonies are in contrast to the flat morphology of mouse epiblast-derived stem cells (f), which resemble human ES (d) and iPS (e) cells. Human iPS cells induced into a naive pluripotent state by treatment with chemical inhibitors^{82–85} (c) parallel the morphology of mouse ES and iPS cells. Scale bars, 50 μ m.

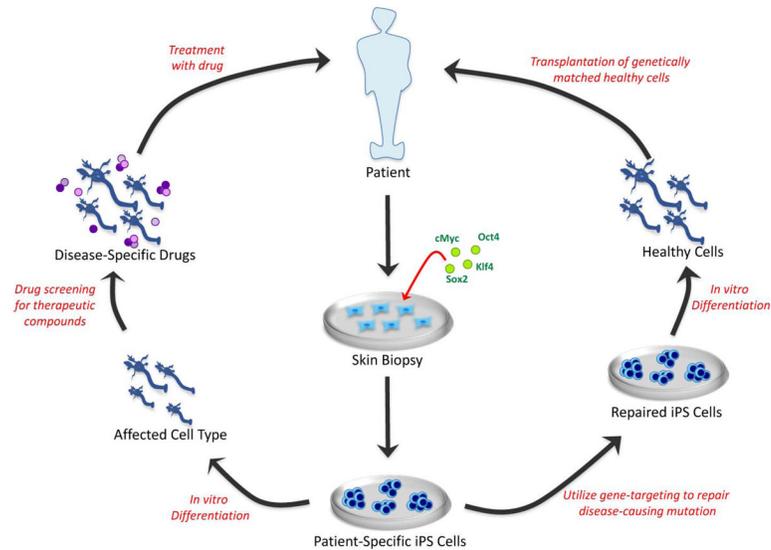


Figure 2. Medical applications of iPS cells

Reprogramming technology and iPS cells have the potential to be used to model and treat human disease. In this example, the patient has a neurodegenerative disorder. Patient-specific iPS cells — in this case derived by ectopic co-expression of transcription factors in cells isolated from a skin biopsy — can be used in one of two pathways. In cases in which the disease-causing mutation is known (for example, familial Parkinson's disease), gene targeting could be used to repair the DNA sequence (right). The gene-corrected patient-specific iPS cells would then undergo directed differentiation into the affected neuronal subtype (for example, midbrain dopaminergic neurons) and be transplanted into the patient's brain (to engraft the nigrostriatal axis). Alternatively, directed differentiation of the patient-specific iPS cells into the affected neuronal subtype (left) will allow the patient's disease to be modelled *in vitro*, and potential drugs can be screened, aiding in the discovery of novel therapeutic compounds.

Table 1

Methods for reprogramming somatic cells to iPS cells

Vector type		Cell type	Factors*	Efficiency (%)	Advantages	Disadvantages
Integrating	Retroviral ^{1,14,86,87}	Fibroblasts, neural stem cells, stomach cells, liver cells, keratinocytes, amniotic cells, blood cells and adipose cells	OSKM, OSK, OSK + VPA, or OS + VPA	~0.001-1	Reasonably efficient	Genomic integration, incomplete proviral silencing and slow kinetics
	Lentiviral ^{15,16,88,89}	Fibroblasts and keratinocytes	OSKM or <i>miR302/367</i> cluster + VPA	~0.1-1.1	Reasonably efficient and transduces dividing and non-dividing cells	Genomic integration and incomplete proviral silencing
	Inducible lentiviral ^{23,28}	Fibroblasts, β cells, keratinocytes, blood cells and melanocytes	OSKM or OSKMIN	~0.1-2	Reasonably efficient and allows controlled expression of factors	Genomic integration and requirement for transactivator expression
Excisable	Transposon ⁹⁰	Fibroblasts	OSKM	~0.1	Reasonably efficient and no genomic integration	Labour-intensive screening of excised lines
	<i>loxP</i> -flanked lentiviral ⁹¹	Fibroblasts	OSK	~0.1-1	Reasonably efficient and no genomic integration	Labour-intensive screening of excised lines, and <i>loxP</i> sites retained in the genome
Non-integrating	Adenoviral ^{92,93}	Fibroblasts and liver cells	OSKM	~0.001	No genomic integration	Low efficiency
	Plasmid ^{94,95}	Fibroblasts	OSNL	~0.001	Only occasional genomic integration	Low efficiency and occasional vector genomic integration
	Sendai virus ⁹⁶	Fibroblasts	OSKM	~1	No genomic integration	Sequence-sensitive RNA replicase, and difficulty in purging cells of replicating virus
DNA free	Protein ^{97,98}	Fibroblasts	OS	~0.001	No genomic integration, direct delivery of transcription factors and no DNA-related complications	Low efficiency, short half-life, and requirement for large quantities of pure proteins and multiple applications of protein
	Modified mRNA ⁹⁹	Fibroblasts	OSKM or OSKML + VPA	~1-4.4	No genomic integration, bypasses innate antiviral response, faster reprogramming kinetics, controllable and high efficiency	Requirement for multiple rounds of transfection
	MicroRNA ¹⁰⁰	Adipose stromal cells and dermal fibroblasts	miR-200c, miR-302s or miR-369s	~0.1	Efficient, faster reprogramming kinetics than commonly used lentiviral or retroviral vectors, no exogenous transcription factors and no risk of integration	Lower efficiency than other commonly used methods

* OSKM and similar factor names represent combinations of reprogramming factors: K, KLF4; L, LIN28; M, c-MYC; N, NANOG; O, OCT4; S, SOX2; and VPA, valproic acid.

Table 2

Diseases modelled with iPS cells

Disease	Molecular defect of donor cell	Cell type differentiated from iPS cells	Disease phenocopied in differentiated cells	Drug or functional tests
Neurological				
Amyotrophic lateral sclerosis (ALS)	Heterozygous Leu144Phe mutation in <i>SOD1</i>	Motor neurons and glial cells	ND	No
Spinal muscular atrophy (SMA)	Mutations in <i>SMN1</i>	Neurons and astrocytes, and mature motor neurons	Yes	Yes
Parkinson's disease	Multifactorial; mutations in <i>LRRK2</i> and/or <i>SNCA</i>	Dopaminergic neurons	No	Yes
Huntington's disease	72 CAG repeats in the huntingtin gene	None	NA	No
Down's syndrome	Trisomy 21	Teratoma with tissue from each of the three germ layers	Yes	No
Fragile X syndrome	CGG triplet repeat expansion resulting in the silencing of <i>FMR1</i>	None	NA	No
Familial dysautonomia	Mutation in <i>IKBKAP</i>	Central nervous-system lineage, peripheral neurons, haematopoietic cells, endothelial cells and endodermal cells	Yes	Yes
Rett's syndrome	Heterozygous mutation in <i>MECP2</i>	Neural progenitor cells	Yes	Yes
Mucopolysacchar idosis type IIIB (MPS IIIB)	Homozygous mutation in <i>NAGLU</i>	Neural stem cells and differentiated neurons	Partially	Yes
Schizophrenia	Complex trait	Neurons	Yes	Yes
X-linked adrenoleukodystrophy (X-ALD), childhood cerebral ALD (CCALD) and adrenomyeloneuropathy (AMN)	Mutation in <i>ABCD1</i>	Oligodendrocytes and neurons	Partially	Yes
Haematological				
ADA SCID	Mutation or deletion in <i>ADA</i>	None	ND	No
Fanconi's anaemia	<i>FAA</i> and <i>FAD2</i> corrected	Haematopoietic cells	No (corrected)	No
Schwachman–Bodian–Diamond Syndrome	Multifactorial	None	NA	No
Sickle-cell anaemia	Homozygous HbS mutation	None	NA	No
β -Thalassaemia	Homozygous deletion in the β -globin gene	Haematopoietic cells	ND	No
Polycythaemia vera	Heterozygous Val617Phe mutation in <i>JAK2</i>	Haematopoietic progenitors (CD34 ⁺ CD35 ⁺)	Partially	No
Primary myelofibrosis	Heterozygous mutation in <i>JAK2</i>	None	NA	No
Metabolic				
Lesch–Nyhan syndrome (carrier)	Heterozygous mutation in <i>HPRT1</i>	None	NA	No
Type 1 diabetes	Multifactorial; unknown	β -Cell-like cells (express somatostatin, glucagon and insulin; glucose-responsive)	ND	No
Gaucher's disease, type III	Mutation in <i>GBA</i>	None	NA	No

Disease	Molecular defect of donor cell	Cell type differentiated from iPS cells	Disease phenocopied in differentiated cells	Drug or functional tests
α 1-Antitrypsin deficiency (A1ATD)	Homozygous mutation in the α 1-antitrypsin gene	Hepatocyte-like cells (fetal)	Yes	No
Glycogen storage disease Ia (GSD1a)	Defect in glucose-6-phosphate gene	Hepatocyte-like cells (fetal)	Yes	No
Familial hypercholesterol aemia	Autosomal dominant mutation in <i>LDLR</i>	Hepatocyte-like cells (fetal)	Yes	No
Crigler-Najjar syndrome	Deletion in <i>UGT1A1</i>	Hepatocyte-like cells (fetal)	ND	No
Hereditary tyrosinaemia, type 1	Mutation in <i>FAH1</i>	Hepatocyte-like cells (fetal)	ND	No
Pompe disease	Knockout of <i>Gaa</i>	Skeletal muscle cells	Yes	No
Progressive familial cholestasis	Multifactorial	Hepatocyte-like cells (fetal)	ND	No
Hurler syndrome (MPS IH)	Genetic defect in <i>IDUA</i>	Haematopoietic cells	No	No
Cardiovascular				
LEOPARD syndrome	Heterozygous mutation in <i>PTPN11</i>	Cardiomyocytes	Yes	No
Type 1 long QT syndrome	Dominant mutation in <i>KCNQ1</i>	Cardiomyocytes	Yes	No
Type 2 long QT syndrome	Missense mutation in <i>KCNH2</i>	Cardiomyocytes	Yes	Yes
Primary immunodeficiency				
SCID or leaky SCID	Mutation in <i>RAG1</i>	None	NA	No
Omenn syndrome (OS)	Mutation in <i>RAG1</i>	None	NA	No
Cartilage-hair hypoplasia (CHH)	Mutation in <i>RMRP</i>	None	NA	No
Herpes simplex encephalitis (HSE)	Mutation in <i>STAT1</i> or <i>TLR3</i>	Mature cell types of the central nervous system	No	No
Other category				
Duchenne muscular dystrophy	Deletion in the dystrophin gene	None	NA	No
Becker muscular dystrophy	Unidentified mutation in dystrophin	None	NA	No
Dyskeratosis congenita (DC)	Deletion in <i>DKC1</i>	None	NA	No
Cystic fibrosis	Homozygous deletion in <i>CFTR</i>	None	NA	No
Friedreich's ataxia (FRDA)	Trinucleotide GAA repeat expansion in <i>FXN</i>	Sensory and peripheral neurons, and cardiomyocytes	Partially	No
Retinitis pigmentosa	Heterogeneity in causative genes and mutations: mutations in <i>RP9</i> , <i>RPI</i> , <i>PRPH2</i> or <i>RHO</i>	Retinal progenitors, photoreceptor precursors, retinal-pigment epithelial cells and rod photoreceptor cells	Yes	Yes
Recessive dystrophic epidermolysis bullosa (RDEB)	Mutation in <i>COL7A1</i>	Haematopoietic cells, and epidermis-like keratinocytes that differentiate into cells of all three germ layers <i>in vivo</i>	Partially	Yes
Scleroderma	Unknown	None	NA	No
Osteogenesis imperfecta	Mutation in <i>COL1A2</i>	None	NA	No

An extended version of this table includes references and more information about drug and functional tests (Supplementary Table 1). *ABCD1*, ATP-binding cassette, sub-family D, member 1; *ADA*, adenine deaminase; *CFTR*, cystic fibrosis transmembrane conductance regulator; *COL1A2*,

α 2-chain of type I collagen; *COL7A1*, α 1-chain of type VII collagen; *DKCI*, dyskerin; *FAA*, Fanconi's anaemia, complementation group A; *FAD2*, Fanconi's anaemia, complementation group D2; *FAHDI*, fumarylacetoacetate hydrolase; *FMR1*, fragile X mental retardation 1; *FXN*, frataxin; *Gaa*, acid α -glucosidase; *GBA*, acid β -glucosidase; HbS, sickle haemoglobin; *HPRT1*, hypoxanthine phosphoribosyltransferase 1; *IDUA*, α -L-iduronidase; *JAK2*, Janus kinase 2; *KCNH2*, potassium voltage-gated channel, subfamily H (eag-related), member 2; *KCNQ1*, potassium voltage-gated channel, KQT-like subfamily, member 1; LDLR, low-density lipoprotein receptor; *LRRK2*, leucine-rich repeat kinase 2; *MECP2*, methyl CpG binding protein 2; NA, not applicable; *NAGLU*, α -N-acetylglucosaminidase; ND not determined; *PRPH2*, peripherin 2; *PTPN11*, protein tyrosine phosphatase, non-receptor type 11; *RAG1*, recombination activating gene 1; *RHO*, rhodopsin; *RMRP*, RNA component of mitochondrial-RNA-processing endoribonuclease; *RP*, retinitis pigmentosa; SCID, severe combined immunodeficiency; *SMN1*, survival of motor neuron 1; *SNCA*, α -synuclein; SOD1, superoxide dismutase 1; *STAT1*, signal transducer and activator of transcription 1; *TLR3*, Toll-like receptor 3; *UGT1A1*, UDP glucuronosyltransferase 1 family, polypeptide A1.