Induced pluripotent stem cells: the new patient?

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Abstract | Worldwide increases in life expectancy have been paralleled by a greater prevalence of chronic and age-associated disorders, particularly of the cardiovascular, neural and metabolic systems. This has not been met by commensurate development of new drugs and therapies, which is in part owing to the difficulty in modelling human diseases in laboratory assays or experimental animals. Patient-specific induced pluripotent stem (iPS) cells are an emerging paradigm that may address this. Reprogrammed somatic cells from patients are already applied in disease modelling, drug testing and drug discovery, thus enabling researchers to undertake studies for treating diseases 'in a dish', which was previously inconceivable.

The prevalence of chronic and age-associated disorders is increasing owing to population ageing and better survival of patients with genetic diseases. The development of new treatments has proven to be challenging because experimental tools, including *in vitro* cultures and animal models, recapitulate only some of the specific traits of human disease. Heart failure, neurocognitive disorders, atherosclerosis and diabetes are all cases in point. As a result, and despite huge investments by the pharmaceutical industry, few new therapeutic compounds are presently entering the market[1,2].

However, the recent advent of reprogramming technology that allows somatic cells of the body to be modified into a special type of stem cell, called induced pluripotent stem (iPS) cell[3,4], may soon change this scenery. Human iPS cells can in principle form any cell type of the body. For example, heart, brain or liver cells can be generated from individuals of any ethnic background who are either healthy or have a disease of genetic or unknown origin. Such patient-specific iPS cells provide unprecedented human models to study both disease pathology in different genetic backgrounds and their response to drugs. Not only may new windows of opportunity be discovered for slowing disease progression, but it may also be possible to target specific disease mechanisms and develop cures. If the symptoms in human iPS cells and their derivatives can be alleviated, the same result may be achieved directly in the patient. If adverse drug responses are identified in specific populations, it may be possible to predict these before clinical disaster. Human iPS cells can differentiate into many disease-relevant cell types, like cardiomyocytes (cardiac muscle cells) and neurons, with efficiencies now approaching those of human embryonic stem (ES) cells[5,6]. Therefore, it is now feasible to obtain cardiac and neuronal cells that capture entire genetic profiles, not only of mutated genes (when the gene is known), but also all of the genetic modifiers that have important, yet largely unknown, roles in the pathology of neurological and cardiovascular diseases. Thus, beyond anticipated uses in cell replacement therapy, patient-specific human iPS cells have rapidly emerging applications in disease modelling, drug testing and drug discovery[7].

Heart and brain tissues are particularly inaccessible through patient biopsies. By using reprogrammed cells from patients disease models 'in a dish' are being developed, allowing experiments that, until recently, would have been inconceivable. The concept of human iPS cells as 'patients' has also received support from experimental data. Reprogrammed cells from patients with rare genetic disorders are already experimental paradigms providing new clues on how diseases manifest and how this might be alleviated or reversed. The route to clinical application is thus finding novel shortcuts. New diseases are continuously being added to the list of those that recapitulate not only genetic but also sporadic forms of diseases. Here, we review the current state of the field with respect to cardiac and neural disorders. These disorders illustrate why there is an interest in exploring the potential of human iPS cell technology for disease modelling and therapeutic interventions. Finally, we will attempt to predict how this rapidly changing field might ideally evolve.

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Somatic cell nuclear transfer
Reprogramming technique in which a nucleus from a differentiated somatic cell is transplanted into an enucleated oocyte.

Generating iPS cells

2007 was a landmark year in pluripotent stem cell biology. Building on an earlier report in mice[8], Takahashi and Yamanaka[3] and Yu *et al.*[4] described the reprogramming of human somatic (skin) cells to pluripotency simply through the ectopic expression of four transcription factors (that is, OCT4 (also known as POUF51 and OCT3) and SOX2 in combination with either Krüppel-like factor 4 (KLF4) and MYC or NANOG and LIN28). Human iPS cells derived in this way seemed to have all of the properties of human embryonic stem (ES) cells, such as the ability to grow indefinitely and form many or all somatic cell types of the body, but without the use of embryos or the need for somatic cell nuclear transfer to derive pluripotent cells from an individual of choice.

Several methods are available for generating human iPS cells[9–11] (BOX 1). Since the first experiments using integrating retroviral vectors, various approaches to deliver the reprogramming genes have been described, most notably several free of viral and transgene integration. These new techniques avoid insertional mutagenesis and transgene reactivation and should consequently result in less variability between the cell lines that are generated. Even though the non-integrating methods could benefit both disease modelling and future cell transplantation therapies, most publications (~76%) on disease modelling to date have still used the original Yamanaka retroviral method (BOX 1). This can be explained in part by the low efficiencies of these new methods. Nonetheless, as the technology becomes more refined, we anticipate that non-integrating technologies will be preferred. Among these, plasmids and Sendai viruses are being introduced in many laboratories, and first indications are that these may soon be the methods of preference. Reprogramming can also be achieved by using synthetic mRNAs and small molecules, and these methods could become widely applied if protocol efficiencies and ease of use further improve.

Box 1 | **Methods for reprogramming human somatic cells into iPS cells**

Delivery of reprogramming factors can either be achieved with integrating methods, resulting in human induced pluripotent stem (iPS) cell lines that carry randomly distributed insertion of the transgenes, or with non-integrating approaches, generating human iPS cells without any resultant permanent genetic modification. Integrating reprogramming approaches include the use of retroviruses (generated using the retroviral vectors pMXs, pLib, or pMSCV), lentiviruses (usually derived from HIV) and transposons (piggyBac). The reprogramming factors were originally delivered by using single retrovirus vectors (each carrying one of the reprogramming genes)[8]. Retroviruses allow delivery of genes into the genomes of dividing cells and are usually silenced following reprogramming. Lentiviral vectors[4] allow infection of both dividing and non-dividing cells and are also available as inducible or as recombinase-excisable systems. iPS cell lines generated with these vectors contain randomly distributed viral transgene insertions, and even after excision exogenous sequences from the viral construct might remain. Transposons[86,87] are instead non-viral constructs, but they are also randomly integrated into the cell genome; they can be subsequently excised using a transposase. Although recombinase-excisable transgenes leave a scar upon excision, the piggyBac transposase mediates a precise removal. Non-integrating systems include viral and non-viral delivery. Replication-defective adenoviruses[88] and F-deficient Sendai viruses[84] can generate transgene-free human iPS cells, even if they are difficult to eliminate from host cells. Non-viral delivery alternatives include transient transfection with episomal vectors[89], with synthetic mRNAs[90] or with mature double-stranded microRNAs (miRNAs)[91]. Proteins[53,92] can also be directly transduced when fused with specific peptides. Furthermore, small molecules, such as valproic acid (VPA), the transforming growth factor-β inhibitor E-616452 (REF. 93) or vitamin C[94] can accelerate or replace the action of individual reprogramming factors.

Method of reprogramming	Delivery method	Percentage of publications*	Refs (disease modelling)
Integrating			
Viral‡	Retrovirus	76%	37,49,50,59,62-67, 71-73,76,77,95–134
	Lentivirus	20%	38,51,59,61,68–70,72,74, 99,104,131,135–138
Non-viral	Transposon (excisable)	–	–
Non-integrating			
Viral‡	Adenovirus	–	–
	Sendai virus	–	–
Non-viral	mRNA	–	–
	miRNA	–	–
	Small molecules	–	–
	Episomal vectors	4%	124,139,140
	Protein	–	–

*Approximate calculation was performed by Pubmed advanced searching on disease modelling studies that used iPS cells over the past 5 years. ‡National regulations surrounding the use of retroviruses, lentiviruses and Sendai viruses vary among countries (for example, in the USA retrovirally-derived cell lines are considered virus-free after two passages, whereas in parts of Europe these cell lines can never leave bio-safety level II laboratories).

Driving human iPS cell differentiation

Human iPS cells and ES cells are biologically very similar but have different origins. iPS cells are pluripotent cells that are derived artificially, typically from adult somatic cells, by 'forced' expression of endogenous pluripotency genes induced by delivery of exogenous 'reprogramming' transcription factors. By contrast, ES cells are pluripotent cells that are derived from pre-implantation stage embryos. These two cell types can be referred to collectively as human 'pluripotent stem cells'. In principle, these cells have the same potential to differentiate into all somatic cell types of the body. In practice, however, there may be cell line-to-cell line differences that depend on the specific reprogramming method that is used to generate iPS cells, the tissue origin of the somatic cell that is being reprogrammed, the level of endogenous growth factor production or exogenous transgene expression after reprogramming and natural stochastic variation that comes with culturing clonal cell lines[12–14]. Several protocols have been described that direct differentiation *in vitro* to neurons, haematopoietic cells, hepatocytes, smooth muscle cells and cardiomyocytes, among various other cell types[15,16]. Many of these methods are based on recapitulating differentiation events in the developing embryo and use signalling pathway agonists and antagonists in specific concentrations and sequences to induce stepwise progression through a developmental programme. Alternatively, some protocols use a cell co-culture system to achieve this purpose, whereas others use concentration gradients of defined factors combined with appropriate proteins[17–21].

The result, for both human iPS cells and human ES cells, is typically a heterogeneous population of relatively immature differentiated cells that need subtype-specific selection for further application. Although this would preferably be achieved by exploiting cell surface markers or vital cell type-specific dyes that allow live-cell selection, these markers do not exist for many lineages, and morphology is often the only way to distinguish the different cell populations. In the case of neurons and cardiomyocytes this is feasible (because of respective neurite formation and spontaneous contraction)[5,6], although some cell surface markers have recently been described for cardiac cells[22,23]. The best markers for these lineages, however, are transcription factors or cytoplasmic proteins, which are not suitable for live-cell selection unless a genetic selectable marker is included as a reporter. This can be done either by insertion of a fluorescent protein, a cell surface protein like the glycoprotein cluster of differentiation 4 (CD4) or an antibiotic resistance gene into the endogenous locus by homologous recombination or by including an ectopic promoter sequence[5,23–28]. However, specification of cardiac or neuronal subtypes (such as atrial, ventricular and pacemaker or conduction system cells of the heart; different subtypes of neurons and glial cells for the central and peripheral nervous system) is still challenging even by using markers, and specification is presently the focus of most protocol development studies both for biotechnology and pharmaceutical applications as well as for cell transplantation therapies[29]. In addition, differentiated derivatives of human pluripotent stem cells are usually immature and often more similar to fetal than adult cells[30,31]. Although this might be a disadvantage in studying late onset diseases, age-associated disorders or in fact any disease in which the phenotype is only revealed postnatally, the similarity to fetal cells might represent an opportunity to study and treat very early stages of diseases before onset of overt symptoms (BOX 2; see Supplementary information S1 (box)).

As human pluripotent stem cell differentiation often recapitulates embryonic development, this provides an opportunity to isolate cell type-specific progenitors. This is especially interesting when studying developmental or congenital diseases. For example, cardiac progenitors have been extensively characterized and several markers are available for their purification[21]. This approach could be helpful in studying certain mutations that interfere with the differentiation of cardiac progenitors to cardiomyocytes[32].

For many diseases, inducing maturity is likely to be crucial for modelling diseases using human iPS cell technology. For example, fetal cardiomyocytes are often resistant to hypertrophy, a pathological condition in which the cells enlarge, and in the case of myopathies, which are diseases of the sarcomere contractile apparatus, it may be difficult to detect differences between control and afflicted cells when the force of contraction is much smaller than in adult cells[33]. As a result, many groups are aiming to develop methods to produce homogenous populations of mature atrial, ventricular and conduction system cells by using approaches on the bases of forced contraction and electrical 'pacing', which mimic the function of a beating heart. Alternatively, tissue-engineering solutions, in which cardiomyocytes are grown in three-dimensional structures, may also be an option[34–36] (BOX 3).

Revealing disease phenotypes

In order to be able to use human iPS cells for disease modelling, but also as a platform for drug discovery and toxicity tests, it is essential to have appropriate and robust assays to detect one or more specific pathological traits.

Detecting cardiomyocyte dysfunctions. Whether using immature or mature cells, sensitive and accurate read-outs of cardiac function *in vitro* are necessary for detecting disease phenotypes in a robust and quantitative way. The differences between cardiomyocyte characteristics from a set of control individuals derived by using different reprogramming methods (which might be referred to as control 'noise') need to be statistically significantly less than the noise between individual sets of diseased cells. Variability among reprogrammed cardiomyocytes would be low if, for example, similar results are obtained using independent human iPS cell clones from the same reprogramming experiment and biological repeats from the same individual but a different reprogramming method. Additionally, the gene mutation is likely to be the sole cause of the phenotype if similar results are obtained using human iPS cells from affected siblings and/or human iPS cells from non-family members with a similar mutation in the same gene or the same disease.

Cell co-culture system
In vitro differentiation technique in which pluripotent stem cells are cultured together with another cell type that is able to induce and support the specification of a particular lineage by mimicking *in vivo* tissue niches.

Neurite
A projection from the cell body of a neuron.

Homologous recombination
A type of genetic recombination in which DNA is exchanged between two DNA molecules that share high sequence similarity. This process is exploited for genetic manipulation of mouse and human embryonic stem cells.

Glial cells
Non-neuronal cells that provide support and protection for neurons in the brain and in other parts of the nervous system.

Sarcomere
The contractile unit of the skeletal and cardiac muscle fibre that consists of several contractile proteins.

Box 2 | **Promises and hurdles in using human iPS cells for disease modeling and drug discovery**

Controls

Human induced pluripotent stem (iPS) cells from the same patient can show biological variability[30,73,124,141]. Identifying disease-relevant phenotypes depends on comparison with 'healthy' controls. Increased targeting efficiencies in human pluripotent stem cells using classical methods[52], zinc-finger nucleases (ZFNs)[53] or transcription activator-like effector nucleases (TALENs)[54] now enable genetic rescue and monogenetic alterations for the generation of relevant controls.

Drug discovery and toxicity

Early risk prediction for new drugs reduces attrition rates and costs. Human iPS cell-based bioassays could reduce animal use, although enhanced sensitivities might result in the premature arrest of valuable drug developments. Disease-specific human iPS cells are nonetheless starting to be used for drug screening. For example, human iPS cell-derived cardiomyocytes with LQT2 syndrome showed arrhythmogenicity after exposure to repolarization-prolonging compounds[61,63,65] and enhanced sensitivity to HERG (human ether-a-go-go; also known as KCNH2) channel blockers and may therefore replace conventional assays[57]. Various drugs showed dose-dependent responses in human embryonic stem (ES) cell-derived cardiomyocytes that overlap with serum concentrations affecting QT intervals in patients[142]. Independent validation is required before human iPS cell-based assays become acceptable for regulatory authorities.

Cell-autonomous versus non-cell-autonomous

Human iPS cells are most suitable for investigating monogenetic diseases with complete penetrance that display cell-autonomous defects on differentiated cells. Examples include ion channel mutations that affect cardiomyocytes or neurons. Non-cell-autonomous diseases, in which environmental, nutritional and hormonal factors may induce symptoms, might need other cell types to become evident *in vitro*. For example, amyotrophic lateral sclerosis (ALS) involves degeneration of motor neurons, which is partially caused by enhanced sensitivity to factors that are secreted by glia cells. Although both cell types can be differentiated from iPS cells[107], most studies have examined the contribution of motor neurons and not that of glial cells[106,124,129,143,144]. For late-onset diseases, human iPS cell derivatives may not recapitulate symptoms that patients develop during life. One solution may be to expose cells to environmental factors, such as oxidative stress[112,113].

Immaturity of differentiated cells

Differentiated cells from human pluripotent stem cells are immature. Cells may need to mature for disease phenotypes to become evident[30,31], although maturation might not be required if the (mutated) gene is expressed in immature cells. For example, LQTS iPS cell-derived cardiomyocytes express appropriate ion channels to recapitulate the salient traits of the disease and responses to chronotropic stimuli and arrhythmogenic molecules[61–63,69]. Maturation protocols are under development for many cell types[35,39] (BOX 3).

Effects of genetic and ethnic backgrounds

Many diseases manifest with incomplete penetrance so that patients harbouring the same mutation have different phenotypes. Human iPS cells 'capture' genetic backgrounds, presenting opportunities to examine their effects. For example, LQT2 human iPS cell-derived cardiomyocytes from an asymptomatic patient showed a milder phenotype than those from a severely affected patient[61]. Coupling genome-wide association studies and medical histories with functional analysis in patient-specific human iPS cells may help to identify new disease susceptibility markers.

Novel biological insights

A key challenge in human iPS cell biology is to generate new (patho)physiological insights. A salient example is familial dysautonomia, for which mice cannot be used as a model system. The pathological traits for this disease, which is known to be caused by IκB kinase-associated protein *(IKBKAP)* mRNA mis-plicing[70], were recapitulated in human iPS cell-derived neurons. Moreover, the use of iPS cells unravelled that the pathogenesis is also caused by reduced *ASCL1* (achaete-scute complex homologue 1) expression in neural crest precursors, indicating defective primary autonomic neurogenesis. Kinetin restored the normal phenotype in human familial dysautonomia iPS cell-derived peripheral neurons and contributed to its use in an early-stage clinical trial[145,146]. Another example is insulin-like growth factor 1 (IGF1)-induced recovery in Rett syndrome (RTT) human iPS cell-derived neurons, and IGF1 is being considered for clinical trials[71,147].

Without knowing the extent of this noise, it is difficult to determine whether comparing one control with one patient-specific human iPS cell line is sufficient to draw conclusions on disease phenotypes. For electrical diseases of the heart, such as those caused by mutations in ion channel genes (see TABLE 1 for a summary of ion channel gene mutations and the corresponding human iPS cell lines), conventional patch clamp electrophysiology or microelectrode arrays have been widely used to demonstrate disease characteristics. However, establishing the disease phenotype for cardiac disease caused by mutations in genes other than ion channels genes has proven more challenging and has relied on less robust assays such as cardiomyocyte size, redistribution of cardiac proteins from the cytoplasm to the nucleus and altered force of contraction[37,38]. This is clearly an area that would benefit from multidisciplinary research in the future with inputs from biochemistry, biophysics, molecular biology and proteomics. Assays that are being considered to detect abnormal phenotypes include measuring mitochondrial activity, analysing glucose and fatty acid metabolism and examining the expression of stress-associated proteins, apoptosis and disease markers.

Revealing neuronal defects. Neuronal differentiation of human iPS cells also shows variability that is independent of transgene integration[30]. Even though the most recent neuronal differentiation protocols have resulted in more homogeneous populations of neuron subtypes relevant to particular diseases[6] and in different classes of

Box 3 | **New tools and technologies**

In the future it will be necessary to develop new tools and technologies to reveal relevant phenotypes when modelling diseases using human induced pluripotent stem (iPS) cells. Improving cell differentiation and maturation protocols and finding new ways to measure cell function will be essential parts of this process.

Bioengineering techniques

Three-dimensional bioscaffolds can be obtained by seeding differentiated cells in three-dimensional structures that consist of either natural or synthetic polymers. This helps cells to become organized into functional tissues, for example cardiomyocytes align as in the native heart tissue and neurons become multipolar and also align[148]. When cells are conductive and/or contractile, incorporation into scaffolds with tensile strengths that match native tissue can reveal disease phenotypes independent of stress created by culture on plastic. For example, this allows the study of cardiac cells that are able to contract synchronously[35].

***In vivo* grafting**

Another approach to stimulate maturation of human iPS cell derivatives is to transplant them into adult rodent tissues, for example into the heart or the brain, and analyse them as a function of time when they have engrafted into the host tissue and possibly acquired specific subtype phenotypes[149]. Functional assays can also be carried out on transplanted cells after reisolation in culture.

cortical neurons that can form functional synapses and networks in culture[39], much work remains to be done before these protocols are widely tested and validated[40]. In addition, better methods are needed to evaluate neuronal connectivity, synaptic plasticity and functional electrophysiological outcomes *in vitro* to characterize disease-related phenotypes[41]. Alternatively, functional analysis of mature, circuit-integrated neurons can be carried out after *in vivo* grafting of human iPS cell-derived cells into rodent brains[42,43] (BOX 3). Studying the anatomy and function of transplanted neurons over time could help to understand neurodevelopmental aspects of the disease and the cell-autonomous versus non-cell-autonomous characteristics. In addition, bioengineering techniques that simulate tissue architecture, much like those techniques mentioned earlier for cardiomyocytes, could be useful to accelerate and improve functional neuron maturation. Neuronal maturation in culture is presently notoriously slow, and it may take many months before reaching a stage in which a disease phenotype becomes evident[39] (BOX 2; see Supplementary information S1 (box)).

Human iPS and ES cells are complementary

The concept of diseased human iPS cells as patients that await cures still has some important caveats, largely related to the question of what the right controls are, as mentioned above. It is essential that variability between individual human iPS cell lines is not confused with disease phenotype. The common features of all pluripotent stem cells, such as their characteristic colony morphology, expression of pluripotency markers, typical epigenetic state and the ability to form teratomas *in vivo* and to differentiate *in vitro,* have not yet explained why all cell lines are not the same. Even the use of common reagents for their derivation, characterization and maintenance in dedicated core facilities, banks, repositories and registries and the growing use of standardized operating procedures (see Further information for a list of links to organizations that establish or fund iPS cell banking projects) have not eliminated cell line-to-cell line differences. The genetic background of human cell lines also has an impact on experimental results[44]. Good manufacturing practice (GMP) protocols, input from the European Medicines Agency, the Food and Drugs Administration and the pharmaceutical industry will be required to create accessible resources of well-validated disease and control cell lines with different ethnic and genetic backgrounds. Genomic instability is also a feature that is shared by human ES cells and human iPS cells, and translocations have been observed on the same specific chromosomes in both[45], although human iPS cells may be more susceptible to genetic and epigenetic instability[46,47]. In particular, human iPS cells might bear more mutations that are acquired not only from the reprogramming process and during culture but also from the somatic cell type of origin[48] (for example, keratinocytes that are exposed to sunlight are more prone to DNA damage).

There are two types of control now being proposed to validate human iPS cells as reliable disease paradigms. One control consists of human iPS cell lines in which the candidate gene mutation has been corrected either by removing the mutant sequence and replacing it by the correct wild-type version[49,50] or, in the case of dominant-active mutations, by knockdown of the mutant allele. The second control is obtained by introducing the single mutant allele into an isogenic pluripotent stem cell background[51]. Although the genetic basis of many diseases is incompletely understood, the advent of new techniques for efficient genetic manipulation through homologous recombination (either by using the classical approach[52] or by using zinc-finger nucleases (ZFNs)[53] or transcription activator-like effector nucleases (TALENs)[54]) now allows targeted mutations to be introduced into human ES cells to study monogenetic diseases. These same techniques can also be used in human iPS cells to correct the mutation[55] to generate genotyped matched cell lines, which then allow proper comparisons of disease-specific genotypes. However, both approaches are labour intensive, inherent to genetic manipulation and depend on knowing the mutation that is related to the disease. It is therefore unlikely that every disease modelling study will include such a control. Another option is to generate a database with a limited number of well-characterized human pluripotent stem cells, ideally from different ages, gender, ethnic and genetic backgrounds, as common references for the scientific community, and such a database could be compared with both disease models and controls. It is now becoming clear that there are large differences in the allelic frequencies for many single nucleotide polymorphisms among different ethnicities[56]. The availability of human pluripotent stem cells from various ethnic backgrounds representative of the three major ethnic groups (that is, Asian, Caucasian and African) would ensure the validity of generally-applied results. This is particularly important for the use of human pluripotent stem cell-derived cells for drug screening. Several idiosyncratic effects of drugs have been attributed to specific genetic variations[57], and the efficacy of numerous drugs is presumed to be influenced by genetic factors.

Cortical neurons
Nerve cells that make up the cerebral cortex, which is the outer part of the brain.

Synapses
Structures that, in the nervous system, allow a neuron to communicate with another cell. The transmitted signal can be electrical or chemical (neurotransmitter).

Zinc-finger nucleases
(ZNFs). Artificial proteins that contain a sequence-specific DNA-binding domain (composed of engineered zinc-finger arrays) fused to a nuclease domain (from the FokI restriction enzyme). ZNFs cleave DNA in a non-sequence-specific manner.

Transcription activator-like effector nucleases
(TALENs). Like ZFNs, they consist of a non-specific FokI nuclease domain fused to a DNA-binding domain derived from bacterial transcription activator-like effectors (TALEs).

Table 1 | **Diseases with phenotype including cardiac and/or neurological traits for which human iPS cells have been derived**

Disease	Genetic defect	Cardiac and neurological traits in patients	Phenotype in human iPS cell-derived cells	Rescue and drug testing	Refs
Cardiovascular diseases					
LQT1	*KCNQ1*	QT-prolongation	Increased APD, impaired rate adaptation, decreased slow activating potassium current (I_{Ks}), arrhythmia under β-adrenergic stress	Propanolol	62, 126
LQT2	*KCNH2*	QT-prolongation	Increased APD, reduced rapidly activating potassium current (I_{Kr}), arrhythmia	Nifedipine, pinacidil, ranolazine, propanolol, nadolol, nicorandil, PD-118057, sotalol, erythromycin, cisapride	61,63, 65
LQT3, Brugada syndrome	*SCN5A*	QT-prolongation, slower conduction	Increased APD, reduced sodium current (I_{Na})	N/A	69
CPVT1, CPVT2	*RYR2*, *CASQ2*	Stress-induced ventricular arrhythmia	Increased diastolic Ca^{2+}, arrhythmia under β-adrenergic stress	Dantrolene	66–68
DCM	*TNNT2*	Ventricular dilatation, systolic dysfunction	Altered Ca^{2+} regulation, reduced contractility, altered distribution of sarcomeric protein	SERCA2 overexpression, metoprolol	38
ARVC	*PKP2*	Myocardial fibro-fatty infiltration, ventricular arrhythmia	Large cardiomyocytes, reduced Z-bands organization, reduced desmosome density, increased lipid droplets upon adipogenic stimulus	N/A	125
Neurological diseases					
Adrenoleukodystrophy	*ABCD1*	Myelin loss, central and peripheral nervous system function loss	Increased very long-chain fatty acids in oligodendrocytes	Lovastatin, 4-phenylbutyrate	98
Alzheimer's disease	Multifactorial, *PS1*, *PS2*, *APP* duplication	Progressive memory disorientation, impaired cognition	Increased amyloid-β secretion, increased phosphorylated TAU (on Thr 231), increased active GSK3B	γ-secretase inhibitor, β-secretase inhibitors	77, 130
ALS	*SOD1*, *VAPB*, *TDP43*	Neuromuscular degeneration, upper and lower motor neuron progressive loss, weakness, paralysis	*VAPB*: reduced VAPB expression in motor neurons *TDP43*: increased soluble and detergent-resistant TDP43 protein expression in neurons, increased vulnerability to PI3K pathway antagonism, shorter neurites	Anacardic acid, trichostatin A, spliceostatin A, garcinol	106, 107, 124, 129
Huntington's disease	*HTT* (CAG repeat expansion)	Progressive chorea and dementia, medium spiny neuron loss in the striatum, neuron loss in the cortex	Increased neural stem cell caspase activity upon growth factor deprivation, increased lysosomal activity, huntingtin aggregate formation upon proteasome inhibition in GABAergic striatal neurons	N/A	59,121, 122,150
Familial dysautonomia	*IKBKAP*	Sensory and autonomic neuron degeneration	*IKBKAP* mis-splicing, reduced expression of neurogenesis genes, defects in neural crest migration	Kinetin	70
Parkinson's disease	*LRRK2*, *PINK1*, *SNCA*, idiopathic	Age-related degeneration of central and peripheral nervous systems	*PINK1*: impaired mitochondrial function in dopaminergic neurons *LRRK2* and *SNCA*: oxidative stress sensitivity in neurons	N/A	51,55, 59,73, 112,113, 137
RTT	*MECP2*, *CDKL5*	Spectrum of autistic characteristics, impaired motor function, regression of developmental skills, hypotonia, seizures	*MECP2*: maturation defects in neurons, reduced synapses, decreased spines, smaller cell soma size, increased LINE1 retrotransposition *CDKL5*: reduced number of synaptic contacts, altered dendritic spine structure	*MECP2*: IGF1, gentamicin *CDKL5*: N/A	71,116, 131,132, 134
Schizophrenia	Multifactorial, *DISC1*	Hallucinations, delusions, disorganized speech, aberrant neurotransmitter signalling, dendritic arborization, impaired myelination	Decreased connectivity in neurons, reduced neurites, decreased PSD95 protein level, reduced glutamate receptor expression, increased extra-mitochondrial oxygen consumption, increased ROS	Loxapine, valproic acid	74,119, 120,140

Table 1 (cont.) | **Diseases with phenotype including cardiac and/or neurological traits for which human iPS cells have been derived**

Disease	Genetic defect	Cardiac and neurological traits in patients	Phenotype in human iPS cell-derived cells	Rescue and drug testing	Refs
SMA	*SMN1*	Selective lower motor neuron loss, muscle weakness, paralysis	Reduced size and number of motor neurons	Valproic acid, tobramycin	138
FXTAS	*FMR1* (CGG-repeat expansion)	Tremor, ataxia, parkinsonism, cognitive decline, peripheral neuropathy, inclusions in neurons and astrocytes	Shorter neurite extension, decreased synaptic PSD95, aberrant Ca^{2+} activity	N/A	97,123
Cockayne's syndrome	*ERCC6*	Neurodegeneration, hypersensitivity to sunlight, premature ageing	Undifferentiated human iPS cells: increased cell death, increased ROS Neurons or other differentiated cells: N/A	N/A	127
MJD	*ATXN3*	Progressive cerebellar ataxia	Insoluble ATXN3 aggregates upon L-glutamate excitation in neurons	Calpain	76
Diseases including cardiovascular and neurological traits					
Timothy syndrome	*CACNA1C*	LQTS, neurological defects	Cardiomyocytes: irregular contraction, increased Ca^{2+} influx, prolonged APD, irregular electrical activity, abnormal Ca^{2+} transients Neurons: reduced expression of genes specific of the lower cortical layers and of the callosal projection neurons, abnormal Tyr hydroxylase expression, increased norepinephrine, increased dopamine	Roscovitine	64, 75
LEOPARD syndrome	*PTPN11*	Electrocardiographic conduction abnormalities, hypertrophic cardiomyopathy, sensorineural deafness	Cardiomyocytes: increased cell size, increased sarcomeric organization, preferential localization of NFATC4 in the nucleus Neurons: N/A	N/A	37
Pompe's disease	*GAA*	Cardiomyopathy, neurological dysfunction	Cardiomyocytes: increased glycogen, deteriorating mitochondria, autophagosome-like structures, short survival Neurons: N/A	*GAA* transgene, rhGAA, 3-MA, L-carnitine	133
Down's syndrome	Trisomy 21	Heart defects (atrial and ventricular septal defects), mental delay	N/A	N/A	59
Gaucher's disease type III	*GBA*	Nervous system and (sometimes) cardiovascular dysfunction	N/A	N/A	59
Duchenne's muscular dystrophy and Becker's muscular dystrophy	*DMD*	Cardiomyopathy, nervous system dysfunction	N/A	N/A	59,96

3-MA, 3-methyladenine; *ABCD1*, ATP-binding cassette, sub-family D; ALS, amyotrophic lateral sclerosis; APD, action potential duration; *APP*, amyloid beta (A4) precursor protein; ARVC, arrhythmogenic right ventricular cardiomyopathy; *ATXN3*, ataxin 3; *CACNA1C*, L-type Ca^{2+} channel; *CASQ2*, calsequestrin 2; *CDKL5*, cyclin-dependent kinase-like 5; CPVT1, catecholaminergic polymorphic ventricular tachycardia 1; DCM, dilated cardiomyopathy; *DISC1*, disrupted in schizophrenia 1; *DMD*, dystrophin; *ERCC6*, excision repair cross-complementing rodent repair deficiency, complementation group 6; *FMR1*, fragile X mental retardation 1; FXTAS, fragile X associated tremor/ataxia syndrome; *GAA*, glucosidase alpha acid; *GBA*, glucosidase beta acid; GSK3B, glycogen synthase kinase 3 beta; *HTT*, huntingtin; IGF1, insulin-like growth factor 1; *IKBKAP*, inhibitor of kappa light polypeptide gene enhancer in B cells, kinase associated protein; iPS, induced pluripotent stem; *KCNH2*, potassium voltage-gated channel subfamily H (eag-related) member 2; KCNQ1, potassium voltage-gated channel KQT-like subfamily member 1; LINE1, long interspersed nuclear elements 1; LQT1, long QT syndrome type 1; *LRRK2*, Leu-rich repeat kinase 2; *MECP2*, methyl CpG binding protein 2; N/A, not assessed; MJD, Machado–Joseph disease; NFATC4, nuclear factor of activated T cells cytoplasmic 4; PI3K, phosphoinositide 3-kinase; *PINK1*, phosphatase and tensin homologue-induced putative kinase 1; *PKP2*, plakophilin 2; PS1, presenilin 1; PS2, presenilin 2; PSD95, postsynaptic density protein 95; *PTPN11*, protein Tyr phosphatase, non-receptor type 11; ROS, reactive oxygen species; RTT, Rett syndrome; *RYR2*, ryanodine receptor 2; *SCN5A*, sodium channel voltage-gated, type V alpha subunit; SERCA2, sarcoendoplasmic reticulum Ca^{2+} ATPase 2; SMA, spinal muscular atrophy; *SMN1*, survival of motor neuron 1; *SNCA*, synuclein alpha; *SOD1*, superoxide dismutase 1; *TDP43*, TAR DNA binding protein 43; *TNNT2*, troponin T type 2, cardiac; *VAPB*, vesicle-associated membrane protein-associated protein B and C.

An alternative option is the use of human embryos that have been rejected following pre-implantation genetic diagnosis (PGD). This approach has the benefit of being human ES cell-based but also has the disadvantage of providing a limited range of disease models, as PGD is only used for the most severe genetic diseases for which there is no treatment or cure, such as the neurodegenerative Huntington's disease.

Together, these disease and control cell lines will create opportunities to capture heterogeneity that arises from variables such as gender, ethnicity and gene modifiers that are specific to patients and absent or unknown in human ES cells.

In summary, human ES cells and iPS cells exhibit many equivalent biological features, but due to their subtle differences, they have distinct but complementary roles in research.

For monogenetic diseases, genetically rescued cell lines can represent the ideal isogenic control. In diseases with somatically acquired mutations, human iPS cell lines generated from unaffected cell types could be used instead as control. A second choice, especially suitable for multifactorial disorders, could be the use of control cell lines derived from healthy siblings, which may contribute to minimize background-specific confounding factors. Finally, when these options are not available, a panel of cell lines derived from the same patients and additional unrelated patients suffering from the same disease should be analysed and compared with a panel of unrelated controls, in order to be sure that any phenotypical observation is not specific to a cell line.

Disease modelling using human iPS cells

As human iPS cells can be derived from any patient, can self renew and differentiate into many cell types, they offer a renewable tissue resource for disease modelling, for studying gene–drug interactions and for developing novel strategies for drug discovery or drug 'rescue' (that is, the reassessment of drugs that either have been withdrawn from use or aborted at a late stage of development for safety reasons) (FIG. 1).

iPS cell technology is especially useful for generating cell lines with genomes that are predisposed to disease, in particular when genetically inherited diseases[58,59] affect tissues that cannot be easily accessed. Although adult progenitor cells may some day contribute to solving this issue[60], the extent to which adult human progenitors can be isolated and expanded from all tissues is not yet clear. Here, we focus on disorders of the cardiovascular and nervous systems to illustrate how human iPS cells might be used. For a detailed overview of all diseases that have been modelled with human iPS cells, including metabolic diseases, we refer readers to another review[10].

Several studies have demonstrated a proof of concept that human iPS cells can be used to model genetic diseases by showing that derivative cells affected by the disease in patients recapitulate disease traits *in vitro*. In some situations, patient-specific human iPS cells even seem to be able to reflect the severity of the disease as observed in the patient[61].

Modelling cardiovascular diseases. Heart diseases were among the first diseases to be studied with human iPS cells. Long QT syndromes (LQTSs) that are caused by mutations in ion channel genes, such as the potassium channel genes *KCNQ1* and *KCNH2* (which are responsible for LQT1 and LQT2 syndromes, respectively), the sodium channel gene *SCN5A* (which is responsible for both LQT3 and Brugada syndromes) and the L-type Ca^{2+} channel gene *CACNA1C* (which is responsible for LQT8; also known as Timothy syndrome), constitute an example of such heart diseases. These syndromes can be detected on the electrocardiograms of affected individuals as they show long intervals between the Q and T peaks, which correspond to the opening and closing of the left ventricle. Human iPS cells from patients affected by several types of LQTS showed characteristic electrophysiological features of the disease, such as prolonged action potential duration (APD) and arrhythmia (irregular beating rate), under both basal conditions and β-adrenergic stimulation[62–65].

Cardiomyocytes have also been derived from human iPS cells from patients with LEOPARD syndrome that harbour mutations in the Tyr phosphatase gene *PTPN11*. This gene encodes one protein of the RAS–MAPK (mitogen-activated protein-kinase) signalling pathway that regulates multiple aspects of cellular function, including normal development. These cardiomyocytes showed features of hypertrophy, which is a characteristic trait of this multi-systemic disease. Abnormal phenotypes seen in other cell types affected in patients with LEOPARD syndrome were not investigated[37], possibly because of difficulties in deriving the relevant cell types from the human iPS cells. It might be worth investigating other affected cell types as protocols improve. Similarly, human iPS cells harbouring a mutation in cardiac muscle troponin T, which is a sarcomeric protein, recapitulated some morphological and functional characteristics of dilated cardiomyopathy, such as altered Ca^{2+} regulation, decreased contractility and abnormal sarcomeric protein distribution[38].

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is another congenital disease that is being investigated with the use of human iPS cells. CPVT is a life-threatening disease in young patients who have a structurally normal heart but have an increased susceptibility to arrhythmia under catecholaminergic stress. This feature was clearly evident in human iPS cell-derived cardiomyocytes from patients affected by both the dominant and recessive forms of CPVT compared with healthy controls[66–68]. More recently, cardiomyocytes from patient-specific human iPS cells with mutations in *SCN5A* were shown to exhibit the complex features of a cardiac 'overlap syndrome' in which LQTS coexists with Brugada syndrome[69]. In this case, the derivative cardiomyocytes showed a reduced inward Na^+ current density, reduced upstroke velocity and a prolonged APD, demonstrating that human iPS cells could model the pathognomonic features of the mutation.

Modelling disorders of the nervous system. Many more examples of disease human iPS cell lines exist in the field of neurobiology, perhaps in part due to the availability of more established protocols that allow the generation of a greater variety of neuronal cell types.

For example, peripheral neurons from human iPS cells of patients suffering from familial dysautonomia revealed defects in neurogenic differentiation and migration of

Adult progenitor cells
Self-renewing precursor cells that are present in some adult tissues and that are able to produce one or more specialized cell types.

Long QT syndromes
(LQTSs). Channelopathies characterized by a prolongation of the QT interval in the electrocardiogram and a propensity to ventricular tachycardia and sudden death. These syndromes are not associated with concomitant structural cardiac abnormalities.

Brugada syndromes
Channelopathies characterized by a specific electrocardiographic pattern and susceptibility to ventricular arrhythmia and sudden death. These syndromes are not associated with concomitant structural cardiac abnormalities.

Action potential duration
(APD). Duration of the event in which the membrane potential of an electrically excitable cell rises and falls.

Inward Na^+ current
The specific Na^+ current that passes through the Na^+ channel SCN5A. This current is present at the surface of cardiac cells and is essential for the beginning of an action potential.

Familial dysautonomia
A rare but fatal peripheral neuropathy that is characterized by the degeneration and depletion of autonomic and sensory neurons.

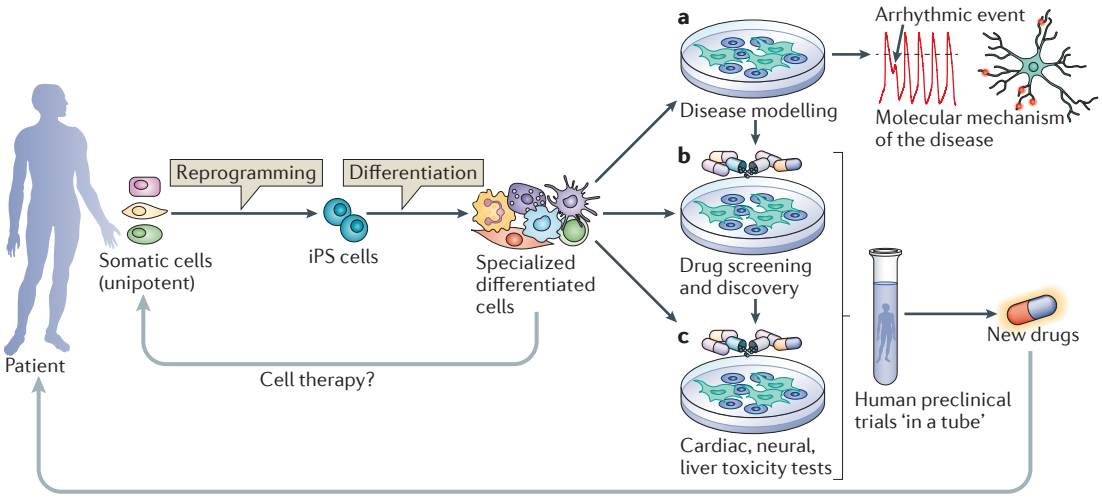

Figure 1 | **Human iPS cell derivation, differentiation and applications.** Adult somatic cells (unipotent) from any patient can be reprogrammed into induced pluripotent stem (iPS) cells. After inducing differentiation *in vitro*, human iPS cells form specialized cells that have several applications. **a** | Human iPS cells can be used in disease modelling to understand the molecular mechanisms underlying disease phenotypes, for example the molecular causes for arrhythmia in cardiomyocytes or for defects in neurogenic differentiation. **b** | Another application of human iPS cells is in drug screening and discovery, to determine the effects of candidate drugs and new compounds and identify target pathways. **c** | Human iPS cells are also valuable in cardiac, neural and liver toxicity tests to assess cellular toxic responses. Drug screening and toxicity tests together represent human preclinical 'trials in a tube' that allow the introduction of 'the patient' in early stages of the drug discovery process.

neuronal precursors due to low expression of *IKBKAP* (IκB kinase complex associated protein gene), which is involved in transcriptional elongation[70]. Similarly, alterations in developing neurons from Rett syndrome (RTT) human iPS cells highlighted synaptic deficiency, altered Ca^{2+} signalling and electrophysiological defects[71].

Patient-specific human iPS cells that are suitable for modelling Parkinson's disease have also been obtained. Derivative dopaminergic neurons carrying mutations in Leu-rich repeat kinase 2 (*LRRK2*), a gene that is clearly associated with this disease but with unclear function, showed increased susceptibility to oxidative stress[72]. On the other hand iPS cell-derived neurons affected by Parkinson's disease and in which the *SNCA* locus was triplicated displayed increased α-synuclein protein levels[73]. Both of these are examples of a disease with genetic or familial origin, the first mutation causing a form of Parkinson's disease with incomplete penetrance and the second mutation causing a very severe form of the disease with full penetrance. Nevertheless, they may serve as models for the study of sporadic forms of the disease.

New human iPS cell models of neural diseases are constantly being added to the list of those that recapitulate not only genetic but also complex and sporadic forms of neuropathies (TABLE 1). Schizophrenia is one example in which neuronal pathology was demonstrated for a complex genetic psychiatric disorder, and new deregulated pathways that have previously not been associated with schizophrenia were identified[74]. The pharmaceutical industry has a significant interest in elucidating whether other psychiatric conditions can be modelled by using human iPS cells, including depression and behavioural disorders such as attention deficit hyperactivity disorder (ADHD) and idiopathic autism.

For more complex syndromes it is also of great importance to uncover whether the pathology is limited to neurons or whether other cell types are also affected. In these cases, human iPS cells could be a useful platform to obtain these other cell types.

Drug–gene interaction

Not only has our understanding of disease pathology in humans been limited by a paucity of appropriate models but also by insufficient research on drug–gene interaction. Human iPS cell technology and the increasing availability of patient-specific iPS cell lines could rectify this, but the development of appropriate assays is still in its infancy. For the ion channel diseases, the assays are relatively well developed, and as a result it has been possible to carry out significant proof-of-concept experiments with respect to drug responses on patient-specific human iPS cells. For example, the addition of propanolol and nadolol, two β-adrenergic blocking agents, attenuated catecholamine-induced tachyarrhythmia in single cardiomyocytes derived from human iPS cells from patients with LQTS. These results confirmed the efficacy of the β-blockade therapy that is already in clinical use for the management of cardiac arrhythmia[61,62] in corresponding human iPS cell-derived cardiomyocytes (FIG. 2a). Furthermore, in LQT2-derived cardiomyocytes other compounds were tested that may possess novel therapeutic benefits for LQTS, through either shortening of the pathological action potential duration or by direct suppression of arrhythmic events. In particular, two channel blockers (nifedipine for the Ca^{2+} channel and ranolazine for the late Na^{+} channel), two ATP-dependent potassium channel (K_{ATP} channel) openers (pinacidil and nicorandil) and one rapidly activating potassium current (I_{Kr}) channel

Rett syndrome
(RTT). A progressive neurological disorder in which patients show a large spectrum of autistic characteristics.

Penetrance
In genetics, the proportion of individuals within a population carrying a mutation that causes a particular trait and that exhibits the specific associated clinical symptoms.

Roscovitine
A compound that selectively inhibits cyclin-dependent kinases and increases the (voltage-dependent) inactivation of the L-type Ca^{2+} channel CACNA1C.

Calpain
A family of Ca^{2+}-dependent proteases that has an important role in the signal transduction pathway by catalysing the controlled proteolysis of target proteins.

Ataxin 3
(ATXN3). A deubiquitylating enzyme that is involved in protein homeostasis maintenance, transcription, cytoskeleton regulation, myogenesis and degradation of misfolded chaperone substrates.

enhancer (PD-118057) could prevent arrhythmia[61,63]. Of note though, although human iPS cells were able to indicate a novel way to treat cardiac arrhythmias, this form of treatment would never be applicable in the clinic due to severe side effects: cessation of beating (by nifedipine) and excessive APD shortening (by ranolazine). Furthermore, dantrolene, a drug that is effective in malignant hyperthermia, restored normal Ca^{2+} properties and rescued the arrhythmic phenotype in CPVT cardiac cells. These findings indicate dantrolene as a potential novel drug for treatment of patients with mutations in the amino-terminal domain of the cardiac ryanodine receptor 2 (*RYR2*) gene[66], which is essential for Ca^{2+} release from the sarcoendoplasmic reticulum during systole. Finally, roscovitine was effective in altering the electrophysiological properties of both cardiomyocytes and neurons from Timothy syndrome human iPS cells. The electrical and Ca^{2+} signalling properties were restored in both cell types, but because roscovitine has multiple targets, only related molecules could be of value to treat this or other cardiac arrhythmias[64,75]. This example also highlights the potential effect of any of these novel drugs on other cell types, and demonstrates that robust protocols and assays for multiple derivatives of compounds need to be developed to realize the full potential of iPS cells in this field.

Neurons derived from a number of patient-specific iPS cells have also been used to validate the potency of candidate drugs in reversing aberrant disease-specific molecular mechanisms and ameliorating pathological traits. For example, kinetin was able to act at the level of mRNA splicing, by reducing the mutant *IKBKAP* splice form in neuron precursors from patients affected by the familial dysautonomia neuropathy[70]. In the model for Machado–Joseph disease (MJD), which is a late onset neurodegenerative disorder, calpain inhibition in human iPS cell-derived neurons was effective in preventing ataxin 3 (ATXN3) aggregate formation[76]. In the model for RTT, which is part of the autism spectrum disorders, mutant neurons showed increased synapse numbers upon treatment with insulin-like growth factor 1 (IGF1)[71], which is a hormone that mediates growth and development (FIG. 2b). Importantly, human iPS cells from non-monogenetic disorders also provide new clues on possible treatments such as novel antipsychotic drugs for schizophrenia[74] and β-secretase inhibitors for familial and sporadic Alzheimer's disease[77].

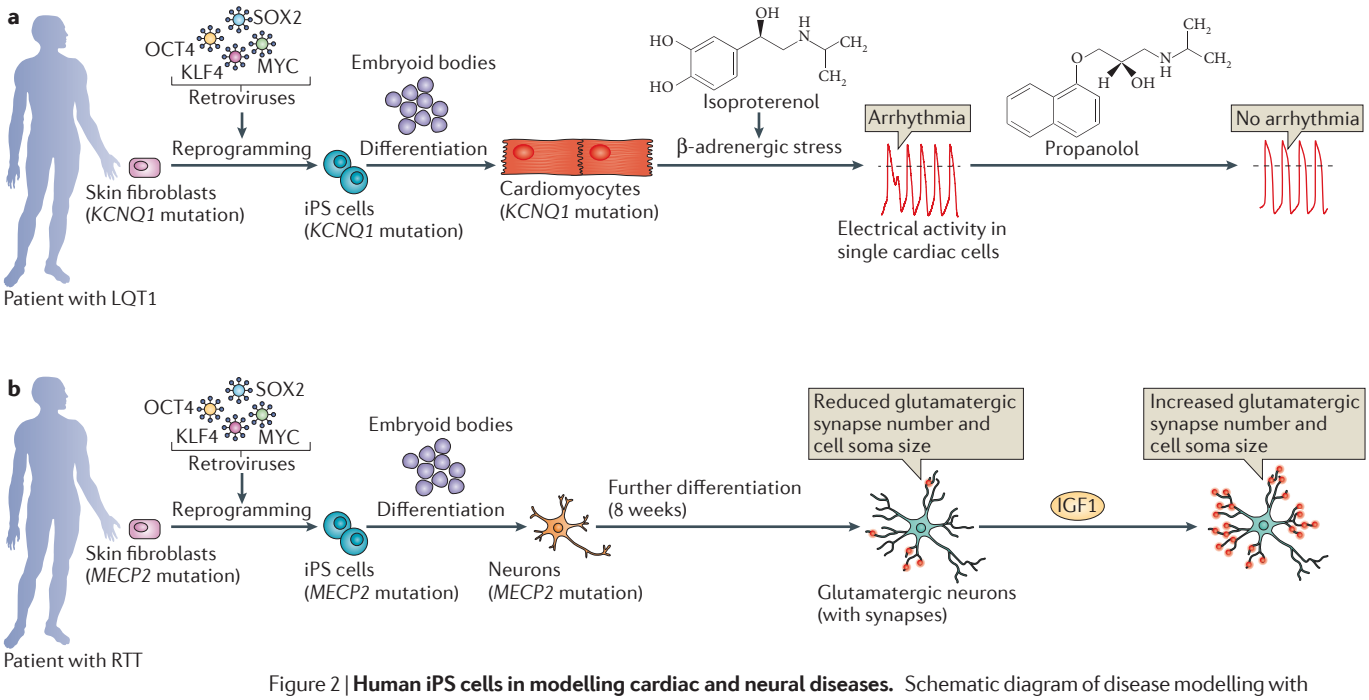

Figure 2 | **Human iPS cells in modelling cardiac and neural diseases.** Schematic diagram of disease modelling with human induced pluripotent stem (iPS) cells, showing amelioration of the disease phenotype. **a** | Skin fibroblasts from a patient affected by type 1 long QT syndrome (LQT1), carrying a mutation in the *KCNQ1* potassium channel gene, were reprogrammed into iPS cells by retroviral transduction of the genes encoding the four reprogramming factors OCT4, SOX2, Krüppel-like factor 4 (KLF4) and MYC[3]. iPS cells were then differentiated as embryoid bodies. Spontaneous contraction indicated the presence of cardiomyocytes that were micro-dissected and plated separately. β-adrenergic stress was mimicked by isoprenaline application, which induced arrhythmic events in these cells, which is the phenotype seen in the heart of patients with LQT1. Treatment with the β-blocker propranolol suppressed arrhythmia[62]. **b** | Skin fibroblasts from a patient affected by Rett syndrome (RTT) that carry a mutation in the epigenetic regulator methyl CpG binding-protein 2 (*MECP2*) gene were reprogrammed into human iPS cells by retroviral transduction of OCT4, SOX2, KLF4 and MYC. iPS cells were then differentiated as embryoid bodies. Appearance of rosettes structures (not shown) indicated the presence of neural precursors that were further differentiated into glutamatergic neurons. These cells showed reduced glutamatergic synapse number (red dots) and reduced the size of the soma (that is, the cell body of the neuron). Treatment with insulin-like growth factor 1 (IGF1) led to increased glutamatergic synapse number and increased cell soma size[71].

Most of these studies as such are of necessity preliminary, and the number of compounds tested has always been less than four. However, these studies demonstrate the feasibility of using human iPS cell-derived cells for predictive drug screening, and they are a potential starting point to identify effective drug dosages without side effects and to determine how molecules could be modified so that they maintain their therapeutic properties but lose their toxicity. In addition, human iPS cells allow many compounds to be tested simultaneously[57], thus enabling to reflect a scenario of patients taking a multiplicity of prescription and non-prescription drugs at the same time.

Novel strategies

The number of new drugs approved per billion US dollars invested in research and development has roughly halved every 9 years since 1950 (REF. 1), and the unmet drug need has as a result increased. The examples given above for cardiac and neural disorders demonstrate the potential of human iPS cell technology to break through this impasse. The use of human iPS cells could contribute to a more efficient development of new drug candidates, rescue those that may have unnecessarily been withdrawn because of negative effects in non-optimal assays and meet the significant need for predictive toxicology bioassay systems that more closely approximate effects observed in humans, particularly identifying susceptible groups.

Some strategies are by definition cell type-specific. For example, only cardiomyocytes are affected by β-blockade therapy[61,62], which impedes the action of endogenous catecholamines on the sympathetic nervous system by administration of antagonists that bind to the β-adrenergic receptors of the heart. Therefore, this therapeutic strategy might be useful in preventing arrhythmias in cardiac diseases other than those for which β-blockers are presently prescribed. However, strategies to rescue disease phenotypes through molecules that act widely on general molecular and cellular processes could potentially be used for multiple pathologies. For example, several diseases are caused by aberrant mRNA splicing, resulting in the expression of unnatural mRNAs or in the inappropriate expression of natural mRNAs. Diverse mechanisms can be responsible for this[78], including disruption of a component of the splicing machinery or of a splicing-regulatory complex. Drugs that are able to reverse aberrant mRNA splicing have therefore the potential to ameliorate abnormal phenotypes, as shown in the case of mutations in *IKBKAP*, which is involved in transcriptional elongation[70].

Some mutations that disrupt splicing result in the introduction of premature stop codons into mRNA, and this typically leads to degradation by nonsense-mediated mRNA decay, as it occurs with nonsense DNA mutations. Pharmacological treatment to suppress nonsense mutations by stop codon read-through could be a common approach to treat diseases caused by such mutations. For example, the aminoglycoside antibiotic gentamicin (which can impair ribosomal proofreading activity leading to the incorporation of a random amino acid at the position of the stop codon) increased the expression levels of the epigenetic regulator methyl CpG binding-protein 2 (MECP2) in affected neurons from a patient that had a stop codon mutation in the *MECP2* gene[71].

For maladies in which protein aggregation has a role in pathology initiation and progression, protease inhibition could be beneficial (for example, in MJD, in which proteolytic processing of ATXN3 triggers the formation of aggregates)[70]. In addition, finding ways to reactivate the normal gene on the inactive X chromosome in female cells by application of specific compounds could be applicable to several X-linked diseases. Of note though, this mechanism can lead to unwanted 'repair' of the disease phenotype if it occurs spontaneously during culture of iPS cells carrying an X chromosome-linked disease. This has recently been described for X-linked Lesch–Nyan syndrome, which is caused by mutations in the *HPRT* (hypoxanthine guanine phosphoribosyl transferase) gene[79].

By providing systems that more closely approximate human cell biology and pathophysiology, human iPS cells could allow patients to enter the drug screening process through preclinical trials much earlier[80] (FIG. 1).

Finally, an important step will be to study a large cohort of patients that represents different ethnic and genetic backgrounds in order to generalize therapies and to determine whether the same drug is equally effective in different patient groups or whether adverse drug events are more likely in a particular population[44]. This would ideally be accomplished by coupling (anonymized) patient medical records that include adverse and positive drug responses in large databases that are linked to corresponding human iPS cells.

Summary and prospects: the crystal ball

One major barrier for research on cardiac and neurological disorders that we have emphasized here is the inaccessibility of diseased tissue for study. Increasingly, and where possible, humanized animal models are being developed. These include transgenic mice that carry human mutations or that are repopulated with cells that constitute a human immune system. However, not all gene sequences or variants are known or sufficiently conserved between species to make this approach feasible. Also, mutations in animals may not recapitulate the human disease, and interspecies differences in cell and organ physiology or cellular and molecular composition may preclude the phenotype being revealed. For diseases of the nervous system, human neuronal network complexity may not be captured; for diseases of the heart, physiological differences (most evident in heart rates of 60 beats per minute (bpm) in humans and 500 bpm in mice) may mean that disorders like arrhythmias are masked. As described above, human iPS cells offer perspectives to address these basic biological questions. However, we are clearly just beginning to explore the value of human iPS cells in combination with proper controls and quantitative assays to understand disease and developing therapies. Nevertheless, because so few human iPS cell lines with the same mutation have been compared directly by using the same read-out or assay,

it is not yet clear whether genetic or ethnic background effects on disease manifestation or drug responses will be captured as detectable modifications of the phenotype (BOX 2). This may actually best be addressed in mice, in which breeding mutations on isogenic backgrounds for many generations and determining the effect of genotype on phenotype before isolation of mouse iPS cell lines is entirely feasible.

Increasing the numbers of human iPS cell lines will eventually demonstrate the feasibility of introducing the patient into the process of drug discovery earlier through the use of a selected repertoire of cell lines that detect drug sensitivities and predict risk and efficacy in high-throughput formats. In the future, these cells may reduce or even replace some animal experiments that are costly, raise ethical issues and are often unreliable and difficult to translate to humans.

As a cautionary note, there is undoubtedly still variability between the human iPS cell lines that emerge from reprogramming. This variability between human iPS cell lines and individual clones is evident in their gene expression profiles, genetic and epigenetic stability and differentiation potential[10,30,81–83]. There may be no other 'golden standard' for human pluripotent stem cells than human ES cells, but the power of knowing how the disease will manifest during life is an enormous incentive to perfect reprogramming technology. The variability between human iPS cell lines needs to be understood better, and as the vast majority of papers (~96%) published on disease modelling to date (BOX 1) have used integrating retrovirus or lentivirus to deliver the reprogramming genes, we will need more experimental studies that are both time consuming and tedious. In addition, human iPS cell expansion requires repeated cell cloning that can also contribute to the amplification of abnormal colonies with growth advantage during passage in culture. New methods of reprogramming that do not require viral integration (BOX 1) are clearly important to determine the best way to decrease the variation between clones from the same individual[84]. In addition, more research to elucidate the actual mechanisms underlying reprogramming would benefit the field enormously. Understanding the processes and steps of reprogramming would shed light onto the causes of the variability among the of generated cell lines and likely increase the efficiency of the process.

Another potential source of variability is the different genetic backgrounds in different individuals (BOX 2). This may be better understood in the near future, as more fully sequenced human genomes become available for comparison[85]. It will be of value to derive human iPS cell lines from these genomes. Naturally occurring genetic variation between individuals is also now being dissected by using new high-throughput genomic tools, such as genomic deep sequencing. Including cohorts of patients who present common clinical histories and/or respond to drugs in a similar manner could also potentially reduce the variability between iPS cell lines. Finally, reprogramming cells from genetically identical individuals (that is, monogenetic twins) who are concordant or discordant for various disorders would also help to understand variability and to generate relevant disease hypotheses.

Large consortia, often based on private–public partnerships, are now receiving financial support to create resources of banked patient-specific iPS cell lines with accompanying medical records and genome sequence data (for more details, see Further information for a list of links to organizations that establish or fund iPS cell banking projects). Common informed consent procedures are also being developed that will make human iPS cell lines more widely available to research and commercial communities. Through this united effort, human iPS cells may emerge as 'the new patient' of the 21st century, ready for treatment and cure.

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Competing interests statement

The authors declare no competing financial interests.

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