HUMAN INDUCED PLURIPOTENT STEM CELLS AS A PLATFORM FOR PERSONALIZED AND PRECISION CARDIOVASCULAR MEDICINE

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Matsa E, Ahrens JH, Wu JC. Human Induced Pluripotent Stem Cells as a Platform for Personalized and Precision Cardiovascular Medicine. Physiol Rev 96: 1093–1126, 2016. Published June 22, 2016; doi:10.1152/physrev.00036.2015.—Human induced pluripotent stem cells (hiPSCs) have revolutionized the field of human disease modeling, with an enormous potential to serve as paradigm shifting platforms for preclinical trials, personalized clinical diagnosis, and drug treatment. In this review, we describe how hiPSCs could transition cardiac healthcare away from simple disease diagnosis to prediction and prevention, bridging the gap between basic and clinical research to bring the best science to every patient.

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I. INTRODUCTION

The ability to reprogram terminally differentiated human cells into induced pluripotent stem cells (hiPSCs) has fundamentally altered the previous belief that cellular differentiation is a unidirectional, nonreversible developmental process (84, 257, 259). The discovery of cellular reprogramming has opened the possibility to create in vitro models of human diseases by isolating and reprogramming somatic cells from patients carrying familial disease-associated mutations. These patient-specific platforms have been used extensively to understand the molecular mechanisms leading to pathophysiological perturbations in humans, and test the pathways via which several drugs may ameliorate a disease phenotype or cause cytotoxicity (181, 182).

The capacity of hiPSCs to retain patient-specific genomic, transcriptomic, proteomic, metabolomic, and other individualized big data information makes it possible to extend their application beyond disease modeling, and into the field of precision medicine which encompasses the adoption of novel prevention and treatment strategies based on individual variability (11, 45). Progress in genetic and genomic research has highlighted some disease-associated genomic DNA (gDNA) polymorphisms [e.g., single nucleotide polymorphisms (SNPs) or insertions/deletions (indels)] that provide causal relationships among genotypes, disease susceptibility, and pharmacokinetics (i.e., drug absorption, distribution, metabolism, and excretion) or pharmacodynamics (i.e., the physiological effects of the drug). Such relationships account for phenotypic variations of clinical importance in drug therapy and disease progression (173). However, large-scale studies to establish causal associations between genetic variations and clinical indications have been difficult to conduct. The lack of robust methods to experimentally test the functional relevance of naturally occurring polymorphisms in human cells has delayed progress, leaving the determination of the molecular and cellular basis of human phenotypic variation an unresolved challenge.

However, revolutionary and rapidly evolving genome editing technologies such as clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), transcription activator-like effector nucleases (TALEN), and zinc finger nucleases (ZFN), can significantly decrease the time and effort it takes to generate human cell lines carrying specific gDNA mutations or polymorphisms (46). Thanks to their abilities to undergo indefinite proliferation and single-cell clonal expansion, hiPSCs serve as a particularly useful platform for genome editing (82, 97, 308). Therefore, hiPSCs can facilitate identification of exactly how disease mutations and polymorphisms result in cellular phenotypes, providing an experimental platform for precision medicine. By advancing a new model of patient-powered research combined with a patient-focused drug development program, precision medicine promises to accelerate biomedical discoveries and offer clinicians novel tools, knowledge, and therapies.
that will enable them to select optimized treatments for each individual patient (36a, 233a).

In this review, we focus on the ability of hiPSCs to facilitate precision medicine in relation to heart disease. Heart disease, whether familial, congenital, or acquired, remains the leading cause of mortality in men and women worldwide (150, 198). Currently, over 17 million deaths per year are attributed to heart disease, and this number is projected to rise to 30 million over the following 15 years (300a). Despite decades of extensive and expensive research, few interventions have significantly improved patients’ survival in heart failure (34). Even the most commonly prescribed treatments (e.g., β-adrenoreceptor blockers) act primarily by delaying disease progression, and can have adverse side effects such as fainting, seizures, or bradycardia (71, 78, 143). These facts point to the need for enhancing our understanding of the molecular mechanisms leading to heart failure, and for developing improved preventive, preventive, and reparative therapies. As detailed later on in this review, small and large animal models ranging from fruit flies to primates have been utilized extensively to model the pathophysiology of human heart disease (192). However, species-specific variation has hindered significant progress, making any therapeutic advancements disproportional to the amount of time and money invested (226). Thus cutting-edge 21st century heart failure research is required to use novel human-based and human-relevant research methodologies to efficiently address the lack of effective therapies against heart failure.

We hereby show examples of how hiPSC-derived cardiomyocytes (hiPSC-CMs) can be used to generate human models of heart diseases in a dish, which faithfully recapitulate the human heart’s pathophysiology and respond appropriately to clinically relevant pharmacological intervention. We also outline how, in the relatively short time since their discovery, hiPSCs have been married with next generation sequencing (NGS) and genome editing technologies to highlight molecular mechanisms leading to heart disease, enabling us to link disease-associated mutations or polymorphisms to disease outcome, severity, and response to therapy. Finally, we highlight the future prospects of hiPSCs to ably address the critical need for incorporating a human-based platform into drug discovery, disease diagnosis, and therapeutic pipelines.

II. TOOLS FOR REPROGRAMING SOMATIC CELLS TO hiPSCs

The first reprogramming methods published by the Yamanaka (257) and Thomson (319) labs utilized retroviral and lentiviral vectors to generate hiPSCs from human skin fibroblasts. They were able to demonstrate that transgenic overexpression of OCT4, SOX2, KLF4, and c-MYC (OSKM) or OCT4, SOX2, NANOG, and LIN28 (OSNL) was necessary and sufficient to induce pluripotency in fibroblasts. Subsequent interrogation of reprogramming mechanisms identified that the OSKM or OSNL factors synergistically activate the endogenous pluripotency machinery following a series of consecutive events (FIGURE 1). The first step to reprogramming is initiation, and it is characterized by loss of somatic cell gene expression, metabolic changes, increased cellular proliferation rate, inhibition of apoptosis and cellular senescence pathways, and initiation of mesenchymal-to-epithelial transition (MET) (48). These changes are followed by maturation, a stage when cells acquire expression of pluripotency-associated genes, but are still dependent on transgene overexpression to become fully reprogrammed. The final stage of reprogramming, known as the

<table>
<thead>
<tr>
<th>Initiation</th>
<th>Maturation</th>
<th>Stabilization</th>
</tr>
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<tbody>
<tr>
<td>↓ somatic cell gene expression</td>
<td>↑ pluripotency gene expression (e.g., OCT4, NANOG)</td>
<td>Epigenetic remodeling</td>
</tr>
<tr>
<td>≠ in metabolism</td>
<td></td>
<td>↑ telomere length</td>
</tr>
<tr>
<td>↑ proliferation rate</td>
<td></td>
<td>X-chromosome reactivation</td>
</tr>
<tr>
<td>→ of apoptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>→ of cellular senescence pathways</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenchymal-to-epithelial transition</td>
<td>Transgene dependence</td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 1.** Stages of hiPSC reprogramming. Mechanistic analyses have demonstrated that reprogramming of somatic cells to hiPSCs consists of three major steps. The first step is "initiation," and it is characterized by loss of somatic cell gene expression, metabolic changes, increase in cellular proliferation rate, inhibition of apoptosis and cellular senescence pathways, and initiation of mesenchymal-to-epithelial transition (MET). These changes are followed by "maturation," a stage when cells acquire expression of pluripotency-associated genes, but are still dependent on transgene overexpression to become fully reprogrammed. The final stage of reprogramming is "stabilization," and it is achieved when cells undergo X-chromosome reactivation and telomere elongation, acquire epigenetic characteristics of pluripotent cells, and are transgene independent. ↑, increased; ↓, decreased; ≠, change; →, inhibition.
stabilization phase, is achieved when cells undergo X-chromosome reactivation and telomere elongation, acquire the epigenetic characteristics of pluripotent cells, and become transgene independent. hiPSCs are thought to continue stabilization and to acquire genetic and epigenetic signatures that more closely resemble those of embryonic stem cells (ESCs) (270) at late passages, typically over passage 16 (14, 221).

Reprogramming efficiency (i.e., the number of starting somatic cells which transition to hiPSCs) has been reported to be significantly enhanced by addition of supplementary pluripotency factors (12) or epigenetic modifiers (160) to the reprogramming mix. Rais et al. (225) and Lujan et al. (168) also demonstrated in both human and mouse cells that almost 100% of cells in a differentiated population are capable of reprogramming following depletion of Mbd3, a core member of the Mbd3/NuRD (nucleosome remodeling and deacetylation) repressor complex. Even though some controversy exists around this topic, likely due to due technical differences (64), these studies potentially demonstrate that while iPSC reprogramming is typically a stochastic process, it can become deterministic upon epigenetic manipulation.

Beyond the investigation of hiPSC reprogramming mechanisms, clinically relevant advances have been achieved by the development of automated, high-throughput derivation and characterization protocols for hiPSCs (215, 245), use of chemically defined media (24), and substitution of retroviral and lentiviral transgene overexpression vectors by integration-free systems that circumvent the risk of spontaneous tumor formation due to insertional mutagenesis. Such methods include the use of excisable viral vectors, nonintegrating viruses (250, 251), and even virus-independent approaches such as plasmid episomal, DNA, protein, or mRNA mediated transgene overexpression (114, 293, 324). The advantages and disadvantages of the most prominently used nonintegrating reprogramming techniques are outlined in Table 1 and are discussed in detail below.

A. Nonintegrating Sendai Virus

One of the most common currently used techniques to induce pluripotency is based on F-deficient Sendai virus (SeV/ΔF), a nontransmissible negative-sense single-stranded RNA virus that replicates in the cytoplasm of infected cells without DNA intermediates or genomic integration into the target cell genome. Despite being one of the most reliably integration-free reprogramming methods currently available (72), the use of Sendai virus is expensive and requires more stringent biosafety containment measures than nonviral reprogramming methods. Moreover, residual viral material persists for an extended period of cell culture (up to 10-20 passages), thus delaying the establishment of virus-free hiPSC lines for downstream analysis and differentiation experiments (80). Recently, temperature-sensitive Sendai virus particles have been developed by engineering specific backbone mutations that affect key viral proteins, and allow faster and more efficient removal of viral material from the cytoplasm of host cells by a temperature shift (10). This technology decreases the time and increases the efficiency of establishing desired hiPSC lines. With the use of this method, various cell types, including fibroblasts and peripheral blood mononuclear cells (PBMCs), have been effectively reprogrammed (41, 60, 72).

B. Reprogramming With Episomal Plasmids

As an alternative to retroviral or lentiviral-mediated induction of pluripotency, several DNA-based reprogramming methods have also been developed using either nonreplicating (56, 114, 209) or replicating episomal vectors (317). These techniques are attractive because they reduce biosafety concerns involved in the production and use of viral particles, as well as the risk of tumorigenesis due to potential reactivation of the KLF4 and c-MYC oncogenes, or due to insertional mutagenesis. Nevertheless, the reprogramming efficiency achieved using nonreplicating vectors is relatively low and requires multiple transfections of the target cells (114, 209). Potential explanations for this phenomenon include the low transfection efficiencies of large polycistronic plasmids and enhanced transgene silencing mechanisms operating on plasmid-based vectors in mammalian cells (167).

To overcome these limitations, the Epstein-Barr virus (EBV)-based reprogramming technology was developed. This potent methodology takes advantage of the EBV-encoded nuclear antigen-1 (EBNA-1) trans-acting element, and EBV-encoded oriP cis-acting element to enable plasmids to persist by dividing within human cells as multicopy episomes that attach to chromosomes during mitosis (104, 209). Therefore, the use of episomal vector systems significantly increases reprogramming efficiency through prolonged transgene expression within transfected cells (209). Following multiple cell divisions, oriP/EBNA-based vectors progressively diminish from the targeted cells, thus eliminating potential tumorigenesis or differentiation deficiencies due to transgene reactivation.

Minicircle vectors were also developed as an improvement to classical episomal vectors (114). Minicircles are episomal vectors devoid of any bacterial plasmid and are therefore smaller in size than standard plasmids. This attractive feature enhances their transfection and expression rates (38). However, the reprogramming efficiency achieved using minicircle vectors is low, and their production and purification methodology is relatively complex and time consuming (202). To address the aforementioned drawbacks of this system, a novel, single plasmid reprogramming system called 4-in-1 codon optimized mini intronic plasmid (CoMiP) was developed, which carries codon-optimized sequences of the canonical OKSM reprogramming factors to achieve more accurate and faster translation of transgenes.
Similar to minicircles, the CoMIP vector system overcomes transgene silencing limitations that are often observed with regular plasmids, and provides at least 2–10 times higher levels of transgene expression, as demonstrated in vitro with HEK293 cells and in vivo with mouse liver cells (166).

Furthermore, the 4-in-1 CoMIP vector is a highly efficient, integration-free, and cost-effective methodology applicable for hiPSC reprogramming in a wide variety of cell types (57).

One area of potential concern when using episomal DNA-based reprogramming methods is that they maintain a small potential to integrate into the host genome. Careful downstream screening methods must be used to confirm the derivation of integration-free pluripotent cells to guarantee the safety of these systems (80).

### C. Non-DNA Approaches for the Induction of Pluripotency

One of the first non-DNA tools used for the generation of hiPSCs was transient transfection of modified mRNAs. This

<table>
<thead>
<tr>
<th>Tool</th>
<th>Subtype</th>
<th>Vector</th>
<th>Transgenes</th>
<th>Efficiency, %</th>
<th>+</th>
<th>–</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral</td>
<td>Integrating</td>
<td>Retrovirus</td>
<td>OSKC, OSK, OS, OK, O</td>
<td>0.001–0.1</td>
<td>↑ Efficiency, robust</td>
<td>Risk for random integration into somatic genome</td>
<td>58, 213, 257</td>
</tr>
<tr>
<td>Lentivirus</td>
<td>OSKC, OSNL, OSN, OSL</td>
<td>0.0001–0.1</td>
<td>Can infect nondividing cells</td>
<td>Same as above</td>
<td>103, 201</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excisable</td>
<td>Cre-LoxP Lentivirus</td>
<td>OSKC, OSK</td>
<td>0.005–0.01</td>
<td>↓ Risk for IM</td>
<td>Labor intensive, may leave footprint</td>
<td>246</td>
<td></td>
</tr>
<tr>
<td>Nonintegrating</td>
<td>Adenovirus</td>
<td>OSKC</td>
<td>0.001</td>
<td>No risk for IM</td>
<td>↓ Efficiency</td>
<td>325</td>
<td></td>
</tr>
<tr>
<td>Nonintegrating</td>
<td>Sendai virus</td>
<td>OSKC</td>
<td>1</td>
<td>Same as above</td>
<td>↑ Cost</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Nonviral, DNA based</td>
<td>Plasmids</td>
<td>EBV Episome</td>
<td>OSKCNL &amp; SV40LT</td>
<td>0.00003–0.02</td>
<td>↓ Risk for IM</td>
<td>↓ Efficiency, need for additional factors</td>
<td>316, 318</td>
</tr>
<tr>
<td>Minicircle</td>
<td>OSL</td>
<td>0.0005–0.005</td>
<td>Inexpensive, easy to use, ↓ Risk for IM</td>
<td>Same as above</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CoMIP</td>
<td>OSKC</td>
<td>0.02–0.03</td>
<td>Same as above</td>
<td>Same as above</td>
<td>56, 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transposons</td>
<td>PiggyBac</td>
<td>OSKC</td>
<td>0.1</td>
<td>↑ Efficiency</td>
<td>May leave footprint, risk for random insertion</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Sleeping Beauty</td>
<td>HAC</td>
<td>OSKC &amp; p53 shRNA</td>
<td>0.00007–0.00014</td>
<td>Built-in safeguard system based on herpes simplex virus</td>
<td>↓ Efficiency</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>HAC</td>
<td>OSKC</td>
<td>0.02</td>
<td>↓ Risk for IM</td>
<td>Same as above</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanoparticle</td>
<td>CPP</td>
<td>OSKC &amp; SKN &amp; NR5A2</td>
<td>0.001–0.049</td>
<td>Stable transgene source</td>
<td>Labor intensive</td>
<td>29, 151</td>
<td></td>
</tr>
<tr>
<td>Nonviral, non-DNA based</td>
<td>RNA replicon</td>
<td>VEE</td>
<td>OSKC, OSKG</td>
<td>0.25</td>
<td>↑ Efficiency, easy, ↓ risk for IM</td>
<td>Possibility of RT to cDNA, leading to IM</td>
<td>315</td>
</tr>
<tr>
<td>Synthetic mRNAs</td>
<td>Cationic lipid</td>
<td>OSKC &amp; VPA, &amp; hypoxia</td>
<td>1.4–4.4</td>
<td>Same as above</td>
<td>Expensive, labor intensive</td>
<td>293</td>
<td></td>
</tr>
<tr>
<td>miRNAs</td>
<td>Cationic lipid</td>
<td>miR-200c, 302, 369 s</td>
<td>0.1</td>
<td>Same as above</td>
<td>Same as above</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>Small molecules (mouse)</td>
<td>N/A</td>
<td>0 &amp; VC6T</td>
<td>0.00005</td>
<td>Minimal genetic modification, easy to use</td>
<td>Greatly ↓ efficiency, not demonstrated in humans</td>
<td>158</td>
<td></td>
</tr>
<tr>
<td>VC6TFZ</td>
<td>0.2</td>
<td>No genetic modification, no risk for IM, easy to use</td>
<td>Not demonstrated in human cells</td>
<td>98</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A summary of the plethora of methods available for the reprogramming of somatic cells to hiPSCs, with reference to their individual efficiencies, advantages (+), and disadvantages (–). O, OCT4; S, SOX2; K, KLF4; C, c-MYC; N, NANOG; L, LIN28; SV40LT, simian vacuolating virus 4D large T antigen; IM, insertional mutagenesis; VPA, valproic acid; EBV, Epstein Barr virus; CoMIP, codon optimized mini intronic plasmid; RT, reverse transcription; VEE, Venezuelan equine encephalitis self-replicating RNA virus; HAC, human artificial chromosome; CPP, cell penetrating peptides; VC6T; VPA, CHIR99021, 616452, and tranylcypromine; VC6TFZ, VC6T, forskolin and 3-deazaadenosine; O, high; ↓, low.
approach not only guarantees the derivation of integration-
free hiPSCs due to the lack of any DNA intermediates, but it
also obviates further time-consuming screening experi-
ments (177, 293). Initial reprogramming experiments using
mRNA transfection required a laborious series of consecu-
tive mRNA transfections for 14 days, as well as pretreat-
ment of target cells with the expensive interferon-α antag-
onist B18R (177) to prevent cell death from repeated
mRNA transfections (44). Another limitation of initial pro-
tocols for mRNA-mediated reprogramming for clinical ap-
proaches was the requirement of mouse embryonic fibro-
blast (MEF) feeder cells and conditioned media, which po-
tentially increases the risk of transmitting undetected
animal pathogens to human cells (4). Recent optimizations
of the established mRNA reprogramming factors addressed
some of the aforementioned problems, allowing the deriva-
tion of footprint-free hiPSCs from human fibroblasts with-
out the use of feeder cells or other potentially xeno-contam-
inated reagents (294), using only a single mRNA transfec-
tion (315). Still, mRNA-based reprogramming has thus far
only allowed reprogramming of adherent cells, which limits
the types of differentiated cells that can be reprogrammed.

Another successful DNA-free method for hiPSC generation
involves the use of transmembrane-permeable fusion pro-
teins (135, 152). However, due to slow kinetics, low effi-
ciency, and high cost (80), this technique has not been
widely adopted. In addition, an exciting alternative ap-
proach has been the elimination of classical reprogramming
factors by exclusively using small-molecule compounds to
reprogram mouse somatic cells (98). Reproducing this tech-
nique using human somatic cells would greatly broaden its
clinical interest.

D. Selecting the Right Reprogramming Tools
for Clinical Applications

Recent evidence suggests that the number and size of karyo-
type abnormalities, such as copy number variations
(CNVs), are significantly higher in isogenic hiPSC lines gen-
erated using integrating reprogramming methods compared
with nonintegrating methods (126). This confirms the need
for adopting safe reprogramming methods for clinical ap-
lications. It is important to note that not all reprogram-
mapping routes may lead to the same pluripotency state.
Although genetic background and cell type of origin contribu-
to heterogeneity among hiPSC lines (231), the type of
reprogramming strategy used can also affect the stoichiom-
etry and expression levels of individual transgenes, thus
leading to transcriptional diversity amongst reprogrammed
populations (327). Carefully assessing the quality of the
reprogrammed state is vital as new technologies emerge.
Quality control (QC) testing and release assays that ensure
the identity, safety, purity, and viability of new hiPSCs lines
include the expression of pluripotency-associated markers
(e.g., OCT4, SSEA4, TRA-1-81, TRA-1-160) in over 70%
of the population, normal 46 XX or 46 XY karyotype,
identical short tandem repeat (STR) profiles between parent-
tal and hiPSC lines, sterility, absence of mycoplasma or
endotoxin (<0.5 EU/ml) contamination, lack of episomal
or other vector integration into gDNA, viral panel clearance
(e.g., retroviruses, adeno-associated viruses, HIV, EBV,
ETC, and bovine or porcine viruses), and over 50% cell
viability per cryopreserved vial (9). These assays should be
conducted prior to the use of hiPSCs for clinical applica-
tions.

III. GENERATION OF FUNCTIONAL HUMAN
CARDIOMYOCYTES FROM hiPSCs

A. Genetic and Epigenetic Control of Cardiac
Specification

Cardiac specification during development is thought to be a
four-stage process tightly controlled by transcription factor
networks, signaling pathways, and epigenetic modifications
(25). On the basis primarily of mouse studies, it is thought
that the first stage of cardiac specification during develop-
ment consists of cardiac mesoderm formation around the
time of gastrulation (261). This process is facilitated by
upregulation of NODAL and BMP4 signaling (6). Subsequent
induction of WNT signaling by BMP4 activates the
expression of essential mesoderm transcription factors,
such as brachyury (T), which in turn stimulate Mesp1,
a master regulator and marker of cardiogenic mesoderm
formation (15). During the second stage of cardiac specifi-
cation, Mesp1+ cardiac mesoderm progenitors give rise to
two distinct progenitor cell populations known as the first
heart field (FHF) and second heart field (SHF) progenitors
(154). This process is controlled by intrinsic signaling as
well as complex extrinsic signaling by BMP4 from the
underlying anterior ectoderm, and BMP4, FGF, and WNT
signaling from the overlying anterior endoderm (247).

In the FHF, WNT signaling inhibition through the Mesp1-
activated gene Dkk1 leads to upregulation of the cardiac
progenitor marker Nkx2-5 and the FHF-specific marker
T-box transcription factor Tbx5 (70). This incorporates the
third stage of cardiac specification, known as patterning of
the heart fields, which is followed by the fourth and final
stage of terminal cardiac differentiation. The latter is
brought about by Nkx2.5 and Tbx5-mediated activation of
connexin 40 (Gja5), Nppa, and sarcomere-specific genes
(18) which induce the formation of atrial cardiomyocytes
(CMs), left ventricular CMs, and cardiac bundle of His
pacemaker cells from the FHF (95). Nkx2.5 and Tbx5
physically interact to coregulate their downstream targets
during cardiac differentiation and morphogenesis (18),
whereas their independent binding activity also serves to
prevent transcription factors from distributing to ectopic
loci and activate lineage-inappropriate genes (169). Positive
regulation of *Nkx2.5* by polycomb repressive complex 1 (PRC1)-mediated chromatin remodeling (241) and negative regulation by histone 3 trimethylation (205) add an additional level of complexity to these temporally and spatially controlled gene regulatory pathways.

In the SHF, *Isl1* and *Mef2c* act downstream of *Mesp1* (125) to activate cardiac-specific factors such as *Smyd1*, *Hand1*, *Irx4*, and *Fgf10* (217, 295) and ultimately give rise to atrial CMs, right ventricular CMs, and sinoatrial node pacemaker cells. *Mef2c* is known to be regulated by DNA methylation and histone acetylation (33, 260), further supporting the importance of epigenetic modifications during cardiac specification. Even though many details of this process are yet to be untangled, our current understanding of cardiac specification during development has greatly facilitated in vitro cardiac differentiation of hiPSCs, as described below.

**B. Protocols for Cardiac Differentiation**

Cardiac differentiation of pluripotent stem cells has progressed from an original efficiency of 5–10% to over 90% efficiency within the past 15 years (23). Original protocols were based on random differentiation of three-dimensional pluripotent stem cell aggregates, known as embryoid bodies, to CMs. Techniques including the enrichment with Percoll separation gradients (305), flow cytometry sorting using the mitochondrial dye tetramethylrhodamine methyl ester perchlorate (TMRM) (92), and the use of the surface markers EMILIN2 (280), SIRPA (62), or VCAM1 (277) have been shown to selectively enrich CM populations. However, the most widely reproduced method for CM enrichment has been based on the distinct metabolic differences in glucose and lactate metabolism between CMs and other differentiated or undifferentiated cell types, where glucose-deprived culture media containing abundant lactate was found to favor the survival of CMs over other cell types (272).

Advances have also been made in cardiac specification of hiPSCs based on the observation that this process is highly dependent on precise modulation of both endogenous (131, 212) and exogenous BMP and WNT signaling pathways (122, 307). Targeted protocols that use key signaling growth factors such as BMP4 and Activin A enabled conversion of pluripotent stem cells to CMs with an improved efficiency (26, 321). This discovery also emphasized the importance of using hiPSC reprogramming strategies that do not allow for continued overexpression of reprogramming factors, because OCT4, SOX2, and NANOG each have known roles in inhibiting differentiation by modulating BMP4-mediated mesendoderm induction (188, 292).

In protocols developed more recently, growth factors were also replaced by small molecules (e.g., CHIR99021 and IWR1) that modulate BMP and WNT signaling (22, 24, 81, 159). Small molecules are advantageous compared with growth factors in that they are cheaper to produce, simpler to store, more stable, and more amenable to quality control (137). Finally, the labor intensive process of embryoid body formation has been replaced by hiPSC differentiation in monolayer (145) or suspension cultures (37) that generate over 90% pure CM populations, and are amenable to automation and scale-up of 1.5–2 billion cells per liter of culture. These remarkable advancements in cardiac specification of hiPSCs have greatly enhanced their potential for clinical application.

**C. Generation and Characterization of hiPSC-CMs at a High-Throughput Scale**

The clinical application of hiPSC-CMs necessitates their production and functional characterization in large scale (52). In parallel to the evolution of cardiac differentiation protocols in suspension bioreactors as mentioned above (66, 132, 235, 252), automated liquid handling platforms (e.g., Tecan Freedom Evo 100, Sarotous CompactT SelectC, and Beckman Coulter Biomek FXP) for robotic scale-up of mouse and human pluripotent stem cells and their differentiated cardiac derivatives have been evaluated and demonstrated to improve the consistency and quality of cell cultures (105, 139, 215, 267).

Industrial-scale phenotyping of hiPSC-CMs properties is also advancing. High content imaging (HCI) platforms have been used to perform 96, 384, or 1,536-well screening for small molecules to improve reprogramming efficiency, hiPSC culture, and cardiac differentiation and maturation (52). Importantly, HCI has been used to assess drug-induced cardiotoxicity in hiPSC-CMs. For example, Sirekno et al. (242) have used HCI platforms to perform CalceinAM, Hoechst, and MitoTracker imaging in hiPSC-CMs to evaluate the cardiotoxicity of 131 drugs affecting Na+, K+, and Ca2+ channels, as well as adreners, dopamine, and histamine receptors. Mioule et al. (193) also used the Thermo Scientific Arrayscan imaging platform to study cellular stress induced by chelerythrine, a cell-permeable protein kinase C inhibitor, by performing combined TMRM, caspase-3, and BOBO-1 staining to measure mitochondrial content, apoptosis, and cell death, respectively. More detailed high content assessment of hiPSC-CM mitochondrial function has been performed by measuring glycolysis and oxidative phosphorylation (OXPHOS) using the Seahorse Extracellular Flux Analyzer (63, 289).

Importantly, the development of 96-well noninvasive multi-electrode array (MEA) systems (e.g., Multichannel Systems and Axion Biosystems Maestro) and contractility analysis platforms (e.g., Cellelog Pulse and SONY S18000) enable recording of field potentials and contraction profiles of hiPSC-CMs in an automated fashion. Besides enabling
high-throughput functional analysis of drug-induced effects in hiPSCs, such platforms also enable monitoring of chronic toxicity using long-term recordings over several hours, days, or weeks. For example, Gilchrist et al. (79) performed high-content MEA recordings at 0.5, 1, 2, and 4 h post exposure to compounds with known proarrhythmic properties (e.g., verapamil, ranolazine, flecainide, amiodarone, ouabain, cisapride, and terfenadine) and found that cardiotoxicity could be recapitulated in vitro using hiPSC-CMs. Similar data were presented using video-edge contractility analysis by Maddah et al. (174).

The evolution of hiPSC-CM technologies which enable high-throughput culture and integration of these cells into high-content industrial platforms that assess structure, mitochondrial function, electrophysiology, calcium transients, and contractility are paramount towards their potential applications in precision medicine.

IV. BIOMEDICAL DATA SCIENCE

Our ability to generate pure hiPSC-CM populations in large scale has opened the route towards performing in vitro functional characterization assays (e.g., structural, electrophysiological, contractile, and metabolic profiling) using automated high-throughput screening platforms (52, 128). The combination of these platforms with next generation biomedical data science is transforming hiPSC-based exploration of molecular mechanisms that underlie cellular function and contribute to human health. High-throughout sequencing information is becoming increasingly important in biomedical research (286), and it has gradually become more cost effective and easier to perform thanks to continuous improvement in related technologies (178, 228, 269). The most prominent NGS technologies are compared and contrasted in Table 2 and are outlined in detail below.

A. Prominent High-Throughput Sequencing Technologies

A key vendor for NGS platforms is Illumina (http://www.illumina.com), whose bridge amplification technology allows billions of reads to be sequenced per run. The bridge amplification method relies on segregation of identical amplified sequences into millions of small clusters, which are immobilized on a flow cell surface to facilitate enzymatic access to the DNA. Amplified sequences are then detected using fluorescence-labeled polymerization terminator dNTPs that allow parallel sequencing of millions of clusters by detecting the fluorescence emission following addition of each dNTP. A different detection chemistry is employed by the SOLiD System, which is currently marketed by Thermo Fisher Scientific (http://www.thermofisher.com). In this platform, a set of four fluorescently labeled di-base probes compete for ligation to the sequencing primer, and the specificity of ligation is analyzed by interrogating the first and second bases that are incorporated in each ligation reaction. Multiple cycles of ligation and detection finally enable reading of the full fragment. Another platform marketed by ThermoFisher is the Ion Torrent, which encompasses the Ion PGM, Ion Proton, and Ion S5 systems. This platform utilizes yet another unique detection technology, which relies on identification of proton release when a nucleotide is incorporated into a DNA fragment by the polymerase enzyme, resulting in a detectable local shift in pH for each dNTP introduced. The Ion Torrent also uses a water-in-oil emulsified droplet method for initial PCR-based fragment amplification, which is a methodology shared with Roche’s 454 platforms (http://www.454.com). However, in the 454 platforms, reads are detected using a method known as sequencing-by-synthesis or pyrosequencing, which relies on the recognition of pyrophosphate release on nucleotide incorporation, rather than chain termination with dNTPs or a change in pH. Revolutionary technology is also incorporated into Pacific Biosciences’ RS and Sequel tools (http://www.pacb.com) which utilize a proprietary Single Molecule, Real-Time (SMRT) technology to deliver sequencing of the longest reads on the market without the requirement of a PCR amplification step. Increased read length can improve the alignment process, especially in stretches of highly repetitive elements. SMRT is based on zero-mode waveguides that permit light to specifically illuminate the bottom of a well in which a DNA polymerase/template complex is immobilized. Phospholinked nucleotides then enable detection of the immobilized complex as the DNA polymerase enzyme produces a natural DNA strand based on the immobilized template. Other NGS platforms include SeqLL’s HeliScope (http://seqll.com), the first microscope-based NGS tool that eliminates the need for a PCR amplification step and, therefore, avoids any associated amplification bias. The advantages and disadvantages of each technology are outlined in Table 2.

Despite their differences in sequencing chemistries, most platforms rely on a common experimental workflow, which consists of random fragmentation of gDNA or RNA, and ligation of platform-specific adapters to the flanking ends of the fragments generated to produce a library. Libraries are then amplified through platform-specific PCR (e.g., on glass or beads) and undergo coupled DNA synthesis and detection processes. As multiple sequencing reactions are run simultaneously, this process is also known as Massively Parallel Sequencing (42). Desynchronization of reads during a sequencing and detection cycle is a source of NGS errors, which can range from base substitutions to insertions or deletions, depending on the sequencing platform used (286). The average number of times a given region is sequenced by independent reads in a sequencing experiment (known as read “depth”) can be increased to boost confidence in the accuracy of sequencing information (3).
B. Biological Information Obtained Via High-Throughput Technologies

NGS platforms enable the deciphering of complex yet valuable biological information. For example, whole genome (WG) or exome sequencing can be used to identify mutations that may cause genetic disorders, or genomic polymorphisms that may underlie disease progression, disease severity, and response to pharmacotherapy. A read depth of 30–100× is recommended to enable accurate detection of gDNA variation (146). However, as the read depth increases, so does the sequencing cost (279). Using targeted capture arrays that focus on a subgroup of target genes involved in a specific pathway or disease can provide a balance between performance and cost. Notably, in a recent publication, Wilson et al. (298a) used a padlock probe approach to create a cost-effective and rapid clinical-grade NGS platform that enables detection of thousands of mutations in over 50 genes (e.g., TNNT2, MYH7, and LMNA/C) that are associated with familial cardiomyopathies. Such platforms promise to facilitate the identification of underlying disease mutations, and gather personalized information that will expedite precision medicine endeavors.

RNA-sequencing (RNA-seq) is a widely used method to generate dynamic and quantitative information regarding transcribed mRNA present in a tissue or cell population at the time of sample collection. Its high sensitivity allows detection of low abundance transcripts and splice variants, which can contribute towards unraveling the molecular mechanisms of cellular processes (144). As mentioned

### Table 2. Overview of high-throughput platforms available for biomedical science applications

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<td>Illumina</td>
<td>Reversible termination</td>
<td>MiSeq</td>
<td>15 M</td>
<td>4–55 h</td>
<td>A</td>
<td>↓ Equipment cost</td>
<td>↑ Cost per sample</td>
<td>222</td>
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<td></td>
<td></td>
<td>MiSeq FGx</td>
<td>130–400 M</td>
<td>11–29 h</td>
<td>B</td>
<td>Same as above</td>
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<td>NextSeq 500</td>
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<tr>
<td>HiSeq 2500</td>
<td></td>
<td></td>
<td>300 M-4 B</td>
<td>7 h-6 days</td>
<td>C</td>
<td>↓ Cost per sample, ↑ scale and throughput</td>
<td>↑ Instrument cost, ↑ time, ↓ read length</td>
<td>165</td>
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<tr>
<td>HiSeq 3000</td>
<td></td>
<td></td>
<td>2.5 B</td>
<td>1–3.5 days</td>
<td>Same as above</td>
<td>Same as above</td>
<td>Same as above</td>
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<tr>
<td>HiSeq 4000</td>
<td></td>
<td></td>
<td>2.5–5 B</td>
<td>Same as above</td>
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<td>Same as above</td>
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<tr>
<td>Thermo Fisher</td>
<td>Proton detection</td>
<td>Ion PGM</td>
<td>400 K-6 M</td>
<td>2.3–7.3 h</td>
<td>A</td>
<td>↓ Cost and time</td>
<td>↓ Read no. and sample throughput</td>
<td>222</td>
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<tr>
<td>Ion Proton I</td>
<td></td>
<td></td>
<td>60–80 M</td>
<td>2–4 h</td>
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<td>Ion S5</td>
<td></td>
<td></td>
<td>3–80 M</td>
<td>2.5–4.5 h</td>
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<td>Same as above</td>
<td>Same as above</td>
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<tr>
<td>Ion S5 XL</td>
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<td></td>
<td>Same as above</td>
<td></td>
<td>↑ Sample throughput</td>
<td>↑ Equipment cost</td>
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<tr>
<td>Ligation</td>
<td>SOLID 5500/5500xl</td>
<td>1.4–2.8 B</td>
<td>8 h</td>
<td>C</td>
<td>↓ Error rate, ↓ cost, ↑ throughput</td>
<td>↑ Read length, ↑ run time</td>
<td>165</td>
<td></td>
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<tr>
<td>SOLID 5500W/5500xl</td>
<td>2 h</td>
<td>Same as above</td>
<td></td>
<td>↑ Time</td>
<td>↑ Instrument cost</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PacBio</td>
<td>Real-time sequencing</td>
<td>RS I</td>
<td>23 K</td>
<td>1–12 h</td>
<td>B</td>
<td>Longest read length, no PCR step, simple preparation</td>
<td>↑ Error rate</td>
<td>222, 229</td>
</tr>
<tr>
<td>RS II</td>
<td></td>
<td></td>
<td>62 K</td>
<td>Same as above</td>
<td></td>
<td>Same as above</td>
<td>↑ Cost</td>
<td>229</td>
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<tr>
<td>Sequel</td>
<td></td>
<td></td>
<td>400 K</td>
<td>1–6 h</td>
<td>A</td>
<td>Same as above</td>
<td>Same as above</td>
<td></td>
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<tr>
<td>Roche</td>
<td>Pyro-sequencing</td>
<td>454 GS Junior</td>
<td>20–100 K</td>
<td>3.5 h</td>
<td>A</td>
<td>↑ Read length</td>
<td>↑ Error rate, ↑ cost, ↓ throughput</td>
<td>165</td>
</tr>
<tr>
<td></td>
<td></td>
<td>454 GS FLX+</td>
<td>20 K-1 M</td>
<td>6 h</td>
<td>Same as above</td>
<td>Same as above</td>
<td>Same as above</td>
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<tr>
<td>SeqLL</td>
<td>Single molecule</td>
<td>Heliscope</td>
<td>600–800 M</td>
<td>8 days</td>
<td>C</td>
<td>Nonbiased representation of reads</td>
<td>↑ Run time, cost, and error rate</td>
<td>268</td>
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<tr>
<td>Oxford Nanopore</td>
<td>Exonuclease sequencing</td>
<td>gridIO*</td>
<td>4–10 M</td>
<td>Flexible</td>
<td>C</td>
<td>Greatly ↑ read length, ↓ run time</td>
<td>↑ Error rate</td>
<td>88</td>
</tr>
</tbody>
</table>

A summarized cross-comparison of the sequencing abilities, applications, advantages (+) and disadvantages (−) of next generation sequencing instruments commercialized by leading vendors in the field. WG, whole genome; K, thousand; M, million; B, billion; PacBio; Pacific Biosciences; PGM, Personal Genome Machine; ↑, high; ↓, low; no., number; A, targeted gene panels; B, exome and RNA sequencing and targeted gene panels; C, de novo sequencing; WG, exome, RNA, ChiP, and methyl sequencing, and targeted gene panels; *Instrument is under development.
above for gDNA sequencing, several gene targeted platforms (e.g., Ion AmpliSeq panels for genes involved in developmental, heart, or metabolic disease) are available for RNA-seq, all of which are designed to reduce the cost of experimentation, and increase sample throughput. RNA-seq is also not limited to the quantification of mRNA transcripts; depending on the methods used for RNA isolation and library preparation, it can sequence other RNA populations such as miRNA, tRNA, or ribosome-bound RNA to detect mRNA actively being translated into proteins (108). The ability to measure the abundance of different RNAs has in recent years refined the investigation of a variety of biological problems, including optimizing experimental conditions (e.g., drug dose and timing), time-course experiments (e.g., to monitor developmental processes, or disease progression), population-specific response profiling (e.g., in healthy and diseased individuals), studying response to treatment (e.g., to several different drugs), and performing large-scale RNA interference (RNAi) studies (249).

Because gene expression can also be greatly influenced by epigenetic modifications such as histone methylation or acetylation, as well as gDNA methylation of cytosine guanine dinucleotides (CpGs) (112), chromatin immunoprecipitation sequencing (ChIP-seq) and DNA methylation sequencing (Methyl-Seq) can also provide valuable information regarding biological processes. ChIP-seq involves cross-linking of histone proteins to gDNA (e.g., using formaldehyde), followed by an immunoprecipitation reaction with antibodies against histone modifications associated with actively transcribed genes (e.g., H3K4Me3 and H3K9ac) or silenced genes (e.g., H3K27Me3). The resulting immunoprecipitated DNA is then sequenced in an NGS platform to identify genes associated with each type of histone epigenetic modification (224). On the other hand, Methyl-seq relies on the comparison of native gDNA to bisulfite-treated gDNA, in which non-CpG cytidine nucleotides are converted to uracil nucleotides (141). Regions lacking bisulfite conversion are thought to be methylated and silenced, since DNA methylation is strongly correlated with the suppression of gene expression (278). Epigenetic modifications influence developmental processes (107), and a variety of disorders, including heart disease (1, 47), and their study helps us understand the physiological and pathological mechanisms involved.

Large international projects aim to capture genome-wide relationships using large population samples and a variety of platforms and sequencing approaches. These include the 1000 Genomes Project Consortium and the 100,000 Genome Project (100kGP) (282). De-identified genomic data are freely available and publicly accessible through resources such as the ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/cbi.nlm.nih) or the ClinGen (http://www.clinicalgenome.org) databases. In addition to the collection of samples, large efforts are underway to integrate different classes of omic information. These include the interactive personal omics profiling (iPOP) initiative (155), which is intended to assemble an individual’s exome, transcriptome, proteome, metabolome, microbiome, and other omics to create a dynamic profile of the physiology and health state of the individual. Such collectives can become stepping stones towards precision health by improving risk prediction as well as disease diagnosis, monitoring, and treatment.

C. The Combination of hiPSC and NGS Technologies

In hiPSCs, transcriptional and epigenetic variation originating from genetic background have been shown to dominate over cell type of origin or derivation method in regards to the variation observed among lines from different patients (40). This demonstrates that hiPSCs preserve patient-specific omic information and, therefore, provide a useful experimental platform to study the pathophysiological effects of individualized biomedical data. An interesting study related to hypertrophic cardiomyopathy (HCM) was published by Zhi et al. (323), who performed whole exome sequencing in seven sibling trios to identify missense or nonsense mutations that were associated with increased left ventricular (LV) mass. Functional correlation of these mutations to hypertrophy-related gene signaling changes in hiPSC-CMs led to the conclusion that a predicted conserved and damaging nonsense variant in exon 13 (A2099G) of the THBS1 gene was significantly associated with pathologically increased LV mass. It is anticipated that, in combination with NGS tools, hiPSC technology will serve as a next-generation biomedical interface enabling us to establish strong phenotype-to-genotype correlations and understand complex biological systems (90). Further examples of studies which provide proof-of-principle that hiPSCs can be used to understand how complex gene networks and polygenic modifications may lead to heart disease are discussed in more detail in the following section.

V. hiPSC-BASED MODELS OF HUMAN INNATE DISEASE

The most widely studied hiPSC-based models of human cardiovascular disease are those caused by familial or congenital mutations. Following clinical consultation and obtaining informed consent, patients carrying familial mutations associated with cardiovascular disease are recruited for hiPSC generation (FIGURE 2). Subsequent cardiac specification of hiPSCs generates beating cells (24) which, despite their relatively immature structural and electrophysiological state (25, 184), have been shown to recapitulate the clinical phenotypes observed in the donors. Disease model derivation has also been achieved in integration-free chemically defined conditions, making them more clinically relevant (20). The growing body of research regarding innate
models of cardiac diseases is summarized in Table 3 and below.

### A. Cardiac Channelopathies

Among the most extensively researched diseases using hiPSC-based models are arrhythmic disorders caused by mutations in cardiac ion channels, which are known as cardiac channelopathies. This category of disorders includes congenital long QT (LQT) syndrome, catecholaminergic polymorphic ventricular tachycardia (CPVT), Timothy syndrome, and Brugada syndrome, which are clinically characterized by arrhythmias, ventricular fibrillation, seizures, and even sudden death (248). As summarized in Table 3, characterization via MEA and patch-clamp electrophysiological techniques has enabled identification of prolonged field potential duration (FPD) and action potential duration (APD) in hiPSC-CMs from patients with LQT syndrome type 1 and type 2 loss-of-function autosomal dominant mutations in potassium ion channels (184, 197). These abnormalities were found to be due to diminished \( I_{Ks} \) or \( I_{Kr} \) currents resulting from trafficking defects in the proteins responsible for the respective ion channel formation. \( \beta \)-Adrenergic stimulation or pharmacological blockade of cardiac repolarization currents (e.g., with E4031) caused LQT syndrome hiPSC-CMs to develop arrhythmias in the form of early afterdepolarizations (EADs), which could be corrected by treatment with \( \beta \)-blockers (e.g., propranolol and nadolol). Novel therapeutic avenues, such as small molecule-mediated modulation of chaperone proteins with \( \mathcal{N} - (\mathcal{N} - \text{acetyl-L-leucyl}) - \text{L-leucyl}) - \text{L-norleucine} (\text{ALLN}) \) (187) and allele-specific RNA interference (183), were also able to ameliorate the disease phenotypes. These findings were among the first to provide proof-of-principle that hiPSC-CMs can recapitulate the human pathophysiology of LQT syndrome.

Other arrhythmia syndromes have also been studied, including LQT syndrome type 3 (LQT3), which is caused by gain-of-function mutations in the sodium ion channel-encoding gene \( \text{SCN5A} \). These hiPSC-CMs showed markedly prolonged APD (due to decreased voltage-dependent inactivation of \( Na^+ \) channels) and late or persistent \( Na^+ \) currents. These phenomena were reversed by a combination of increased pacing rate and \( NaV1.5 \) sodium channel blockade (e.g., with mexiletine). This treatment regimen closely mirrors clinical management of LQT3 (172, 175, 209, 264). Similarly, hiPSC-CMs generated from patients carrying mutations in the L-type calcium channel Ca(v)1.2 that are associated with LQT syndrome type 8, also known as Timothy syndrome, showed pro-arrhythmia, prolonged APD, excess \( Ca^{2+} \) influx, and abnormal \( Ca^{2+} \) transients. Roscovitine, a Ca(v)1.2 activator, ameliorated the electrophysiological and \( Ca^{2+} \) handling perturbations observed in these hiPSC-CMs (311).

Finally, hiPSC-CMs from patients with CPVT types 1 and 2, carrying mutations in the ryanodine receptor (RYR2) or cardiac calsequestrin (CASQ2) genes, showed pro-arrhythmia in the form of delayed afterdepolarizations (DADs), which were reversed following administration of flecainide, a Nav1.5 sodium channel and RYR2 blocker that is clini-
<table>
<thead>
<tr>
<th>Model</th>
<th>Class</th>
<th>Disorder</th>
<th>Gene</th>
<th>Mutation</th>
<th>Reference Nos.</th>
<th>Key Phenotypes</th>
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<td>Arrhythmia syndromes</td>
<td>LQT1</td>
<td>KCNQ1</td>
<td>R190Q</td>
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<td>Protein trafficking defects</td>
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<td>Exon 7 del</td>
<td>171</td>
<td>↑ FPD and APD in atrial and ventricular CMs</td>
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<td>LQT2</td>
<td>KCNH2</td>
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<td>Serve ↓ or absence of Ik, (LQT1) or Ik (LQT2)</td>
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<td>A561T</td>
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<td>Arrhythmias characterized by EADs</td>
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<td></td>
<td>A614V</td>
<td>110</td>
<td>↑ Sensitivity to proarrhythmic drugs</td>
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<td>A561P</td>
<td>120</td>
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<td>LQT3</td>
<td>SCN5A</td>
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<td>Defective Na(^+) channel caused by deficiency in open-state inactivation of the Na(^+) channel</td>
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<td>F1473C</td>
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<td>V240M</td>
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<td>Enhanced late Na(^+) channel current (I_{Na})</td>
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<td>R535Q</td>
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<td>Delayed repolarization and prolonged APD</td>
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<td>CPVT1</td>
<td>RYR2</td>
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<td>Diastolic [Ca(^{2+})]</td>
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<td>F2483I</td>
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<td>S406L</td>
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<td>Frequency and duration of elementary Ca(^{2+}) release events (sparks)</td>
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<td>M4109R</td>
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<td>Arrhythmias characterized by DADs, broad and double-humped transients</td>
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<td>↑ Sensitivity to proarrhythmic drugs</td>
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<td>R420Q</td>
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<td>CPVT2</td>
<td>CASQ2</td>
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<td>Myofibrillar disarray, ↑ SR cisternae size, ↑ caveolae number</td>
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<td>TS</td>
<td>CADNA1C</td>
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<td>Irregular contractility and electrical activity, ↑ APD</td>
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<td>G406R</td>
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<td>↑ Ca(^{2+}) influx</td>
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<td>TNNT2</td>
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<td>Abnormal Ca(^{2+}) regulation, ↓ contractility</td>
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<td></td>
<td>R636S</td>
<td>304</td>
<td>↑ Nuclear blebbing and micronucleation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LMNA/C</td>
<td>243</td>
<td>↑ Nuclear senescence and apoptosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R225X</td>
<td></td>
<td>Abnormal DES aggregations</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DES</td>
<td>275</td>
<td>↓ Max rate of Ca(^{2+}) re-uptake and ↓ beating rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>↓ Tolerance to inotropic stress</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>HCM</td>
<td>MYH7</td>
<td>147</td>
<td>Cellular size and sarcomere disorganization</td>
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<td>R663H</td>
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<td>Contractile arrhythmias</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R442G</td>
<td>89</td>
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<th>Model</th>
<th>Class</th>
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<th>Gene</th>
<th>Mutation</th>
<th>Reference Nos.</th>
<th>Key Phenotypes</th>
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<tr>
<td></td>
<td></td>
<td>MYBPC3</td>
<td>G999-Q1004del</td>
<td>262</td>
<td>Dysregulation of Ca$^{2+}$ cycling and ↑[Ca$^{2+}$], FRDA-associated HCM</td>
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<tr>
<td></td>
<td></td>
<td>FXN</td>
<td>Silencing</td>
<td>153</td>
<td>↑ Rate of iron accumulation, ↓ Ca$^{2+}$ uptake and release kinetics, Disorganized and fragmented mitochondrial network, ↑ ROS accumulation</td>
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</tr>
<tr>
<td>LS</td>
<td>PTPN11</td>
<td>T468M-</td>
<td>A324fs335X</td>
<td>31</td>
<td>↑ Nuclear NFATC4 localization, ↑ Expression of genes associated with cardiac hypertrophy</td>
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</tr>
<tr>
<td>ARVD</td>
<td>PKP2</td>
<td>c.2484C&gt;T</td>
<td>134</td>
<td>↑ Lipogenesis and apoptosis, ↓ PKP2 and ↓ PPAR-γ gene expression</td>
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<tr>
<td></td>
<td></td>
<td>c.2013delC</td>
<td>170</td>
<td>↓ PKP2, plakoglobin, and Cx43 protein density, ↓ β-Catenin activity</td>
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<td></td>
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<tr>
<td>DMD</td>
<td>DMD</td>
<td>exon 45-52del</td>
<td>164</td>
<td>Dystrophin deficiency, ↑ Levels of resting Ca$^{2+}$, Mitochondrial damage and ↑ apoptosis</td>
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<tr>
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<td>Structural</td>
<td>Not identified</td>
<td>Not identified</td>
<td>↑ Ability for cardiac differentiation, ↑ Myofilament organization, ↑ Fetal gene expression pattern</td>
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<tr>
<td></td>
<td>HLHS</td>
<td>Not identified</td>
<td>Not identified</td>
<td>115</td>
<td>↑ Levels of ROS and toxic aldehydes, ↑ Cell cycle arrest and activation of apoptotic signaling</td>
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<tr>
<td></td>
<td></td>
<td>Metabolic</td>
<td>ALHD deficiency</td>
<td>ALDH2</td>
<td>E487K</td>
<td>63</td>
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<tr>
<td></td>
<td></td>
<td>BTHS</td>
<td>TAZ</td>
<td>c.517delG c.517ins</td>
<td>289</td>
<td>↑ Levels of ROS, ↓ mitochondrial function, ↑ Sparse and irregular sarcomeres, weak contractility</td>
</tr>
<tr>
<td></td>
<td>Pompe disease</td>
<td>AAA</td>
<td>D645E</td>
<td>101</td>
<td>↑ Levels of glycogen, defective respiration, Deficit of Golgi-based protein glycosylation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>exons 18del 1441delT</td>
<td>227</td>
<td>Ultrastructural aberrations, lysosomal enlargement</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>c.796C&gt;T c.1316T&gt;A</td>
<td>233</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Engineered arrhythmias</td>
<td>LQT1</td>
<td>KCNQ1</td>
<td>R190Q</td>
<td>290</td>
<td>Protein trafficking defects, ↑ APD in atrial and ventricular CMs, Severe ↓ or absence of $k_o$ (LQT1) or $k_a$ (LQT2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>G269S</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>G345E</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>A614V</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>N986I</td>
<td>13</td>
<td>Arrhythmias characterized by EADs</td>
<td></td>
</tr>
</tbody>
</table>
cally used against CPVT (109). Thapsigargin, a sarcoplasmic reticulum calcium ATPase pump inhibitor, also ameliorated the CPVT phenotype in hiPSC-CMs by depleting internal Ca\(^{2+}\)/H\(^{+}\) stores. This supported the mechanistic explanation that increased diastolic Ca\(^{2+}\)/H\(^{+}\) can lead to the development of arrhythmias. In a separate study, ultrastructural characterization demonstrated that hiPSC-CMs from CPVT1 patients also have enlarged mitochondrial, glycogen, and lysosome clusters, as well as thinner Z-bands, reduced number of sarcomeres per cell, and increased sarcomere disarray (206). These phenotypes were more pronounced in CPVT2 hiPSC-CMs, demonstrating that hiPSCs provide a setting for the investigation of the similarities and differences in the pathophysiological consequences of different mutations.

**B. Cardiomyopathies**

A second class of cardiac disorders that have been widely studied using the hiPSC in vitro platform are cardiomyopathies. These include familial HCM, dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), LV noncompaction (LVNC), and restrictive cardiomyopathy (RCM) (91). Most cardiomyopathies are characterized by disrupted sarcomeric alignment, a phenomenon known as myocardial disarray that is linked to deterioration in myocardial function, heart failure, and cardiac sudden death (111). However, each cardiomyopathy has its own distinct pathophysiological features: DCM is identified by ventricular dilation and systolic dysfunction; HCM manifests as thickening primarily of the left ventricular wall; ARVC is distinguished by cardiac enlargement, aneurysm formation, and fatty infiltration of the ventricles; and RCM manifests as atrial arrhythmias, abnormal right- and left-sided filling pressures, and other clinical signs and symptoms characteristic of right ventricular failure (210).

<table>
<thead>
<tr>
<th>Model Class</th>
<th>Disorder</th>
<th>Gene</th>
<th>Mutation</th>
<th>Reference Nos.</th>
<th>Key Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiomyopathies</td>
<td>DCM</td>
<td>PLN</td>
<td>R14del corrected to WT</td>
<td>130</td>
<td>Correction of: Ca(^{2+})/H(^{+}) handling abnormalities, Electrical instability, Abnormal cytoplasmic distribution of PLN protein, ↑ Expression cardiac hypertrophy molecular markers, ↓ Contractile performance due to A-band truncations, Sarcomere insufficiency, Impaired responses to mechanical and β-adrenergic stress, Attenuated growth factor and cell signaling activation</td>
</tr>
<tr>
<td>Metabolic</td>
<td>BTHS</td>
<td>TAZ</td>
<td>c.517delG, c.517insG</td>
<td>289</td>
<td>↑ Levels of ROS, ↓ mitochondrial function, Sparse and irregular sarcomeres, weak contractility</td>
</tr>
</tbody>
</table>

Together, Tables 3 and 4 provide a detailed outline of hiPSC-based models of cardiac disease published to date, with correlation to the gDNA mutations studies, and the key phenotypes observed for each disorder. APD, action potential duration; FPD; field potential duration; ↑, increased; ↓, decreased; EAD, early afterdepolarization; DAD, delayed afterdepolarization; hiPSC, human induced pluripotent stem cell; CMs, cardiomyocytes; SM, small molecule; GF, growth factor; SR, sarcoplasmic reticulum; VPA, valproic acid; ET-1, ETA-b, endothelin receptor type A blocker; ROS, reactive oxygen species; CDI, Cellular Dynamics International; LQT1, 2, 3, long-QT syndrome type 1, 2, 3; JLNS, Jarvel and Lange-Nielsen syndrome; CPVT1, 2, catecholaminergic polymorphic ventricular tachycardia type 1, 2; TS, Timothy syndrome; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; FRDA, Friedreich's ataxia; DFP, deferiprone; LS, LEOPARD syndrome; ARVD, arrhythmogenic right ventricular dysplasia; DMD, Duchenne muscular dystrophy; HLHS, hypoplastic left heart syndrome; BTHS, Barth syndrome.
tile force, altered regulation of Ca$^{2+}$, and decreased tolerance to β1-adrenergic challenge (254, 301). These phenotypes were attenuated by clinically relevant β1-blockade (e.g., with metoprolol), or more experimental pharmacological inhibition of PDE2A and PDE3A (e.g., with Bay-60-7550 and milrinone), or transgenic overexpression of sarcoplasmic reticulum Ca$^{2+}$ adenosine triphosphatase (SERCA2a). Microarray analysis in hiPSC-CMs that had undergone SERCA2a gene therapy showed that Ca$^{2+}$ signaling, protein kinase A (PKA) signaling, and G protein-coupled receptor (GPCR) signaling were mainly responsible for the phenotypic rescue. It should be noted that the R173W mutation identified in these DCM patients had not been previously reported, and was discovered in this family cohort following exome sequencing of the skin fibroblasts used to generate hiPSCs. In separate studies, hiPSC-CMs carrying DCM-associated mutations in the spliceosome genes (243, 275, 304) also demonstrated abnormalities that closely recapitulated the morphological and functional phenotypes of the affected human heart, as outlined in Table 3.

hiPSC models of HCM associated with thick myofilament myosin heavy chain 7 (MYH7) (89, 148) or myosin binding protein C3 (MYBPC3) (262) mutations showed that in vitro-derived hiPSC-CMs are significantly enlarged, as seen in the diseased human myocardium. hiPSC-CMs also demonstrated increased myofilament content, contractile arrhythmias (DADs), Ca$^{2+}$ cycling perturbations, and intracellular Ca$^{2+}$ elevation, which were worsened by adrenergic stimulation (e.g., with isoproterenol) or environmental exposure to the potent vasoconstrictor endothelin-1 (ET-1) (310). These disease phenotypes were reversed either by β-blockade (e.g., with propranolol or metoprolol), treatment with endothelin receptor type A blockers (e.g., BQ-123), or treatment with L-type Ca$^{2+}$ channel blockers (e.g., verapamil). The latter observation provided mechanistic validation that Ca$^{2+}$ handling defects and elevated intracellular Ca$^{2+}$ levels are underlying disease mechanisms for HCM. Whole transcriptome sequencing and pathway enrichment analysis in HCM hiPSC-CMs also showed a widespread enrichment of genes associated with cellular proliferation (e.g., WNT1), Notch signaling (e.g., DLL1/4), and FGF signaling (e.g., FGFR4) which could serve as potential therapeutic targets (89). HCM manifestations associated with the multi-organ condition known as LEOPARD syndrome were likewise studied in a hiPSC-CM platform, showing that irregularities in RAS-MAPK signal transduction are implicated in development of disease pathology (31). In addition, hiPSC-CMs from patients with HCM associated with the recessive neurodegenerative disorder Friedreich’s ataxia (FRDA) showed an increase in reactive oxygen species (ROS) levels caused by silencing of the mitochondrial gene frataxin (FXN) (153). Additional disease phenotypes, including disorganization of the mitochondrial network, intracellular iron accumulation, and abnormal Ca$^{2+}$ handling kinetics were evident following iron loading-induced stress. Treatment with molecules that are currently under clinical trial for FRDA, such as the iron chelator deferiprone (DFP), significantly suppressed ROS synthesis, modulated mitochondrial stress, and improved electrical coupling during hiPSC-CM contraction.

Finally, hiPSC-CMs carrying ARVC-associated mutations in desmosome proteins have also recapitulated disease pathologies (e.g., increased lipogenesis and apoptosis, and desmosome disorganization) following induction of adult-like metabolic energetics from glycolysis to fatty acid oxidation, therefore demonstrating the ability of hiPSC-CMs to model adult-onset cardiac disorders (134, 170).

Collectively, these studies not only demonstrate that hiPSC-CMs can serve as disease modeling and drug discovery platforms, but can also be used to assess the unique interactions between individual patients’ genetic backgrounds and environmental factors, and serve as biomedical data interfaces to unravel complex molecular pathways implicated in cellular dysfunction and human cardiac or syndromic disease.

C. Structural Defects

Developmental heart defects such as hypoplastic left heart syndrome (HLHS), characterized by severe underdevelopment of the left ventricle, are another class of cardiac disorders that have been modeled in hiPSC-CMs. hiPSCs from HLHS patients were found to have a reduced ability to form beating CMs in vitro, whereas hiPSC-CMs also demonstrated myofilament disorganization, induction of fetal gene expression profiles (e.g., HIF1α), Ca$^{2+}$ handling abnormalities, and increased susceptibility to β-adrenergic stimulation and caffeine treatment (73, 116). Further studies in hiPSC-CMs revealed that disease pathologies could be associated with alternative Ca$^{2+}$ release mechanisms from the sarcoplasmic reticulum via the inositol trisphosphate receptor rather than the ryanodine receptor, thus providing evidence that hiPSC-CMs can be used for mechanistic investigations of developmental heart defects. Although no known mutations linked to HLHS were identified in these studies, hiPSC-CMs offer an opportunity for further research regarding causative gDNA alterations.

D. Metabolic Disorders

Familial metabolic disorders, also known as inborn errors of metabolism (IEMs), represent more than 15% of monogenic disorders (8). Initiating during early infancy or childhood, over 40% of IEMs patients manifest a cardiovascular phenotype that includes limited exercise capacity, HCM, DCM, LVNC, RCM, and fatal arrhythmias (162). Due to the high dependence of cardiac muscle on aerobic metabolism (up to 70% of energy in the heart muscle stems from oxidative me-
IEM caused by mutations of the lysosomal glycogen-hydrolyzing enzymes. Infantile-onset Pompe disease, an autosomal recessive disorder, accounts for a minority of cardiomyopathy cases, identifying an IEM as the underlying cause of disease has significant prognostic and therapeutic implications (297).

Regardless of the clinical avenues used for therapy, the mechanisms by which IEMs lead to cardiomyopathy remain poorly understood. To bridge this gap, hiPSCs have been used as platforms to study the mechanisms and physiological perturbations underlying cardiomyopathy related to metabolic diseases. Infantile-onset Pompe disease, an autosomal recessive IEM caused by mutations of the lysosomal glycogen-hydrolyzing enzyme acid α-glucosidase (GAA), is one of the disorders studied in a hiPSC-CM setting. Pompe and control hiPSC-CMs demonstrated no significant differences in contractile strength, kinetics, or clearance rates of autophagosomes. However, similar to the patients’ myocardium, Pompe hiPSC-CMs had diminished GAA activity, lysosomal glycogen accumulation, and lysosome enlargement (227). MALDI-TOF-MS analysis of N-linked glycans revealed that Pompe cardiomyopathy could be caused by a glycan-processing abnormality, as often seen in congenital glycosylation disorders with an HCM phenotype, therefore providing mechanistic insight into the disease pathological mechanisms. Gene therapy by lentiviral GAA transfer was able to reverse the disease-specific hiPSC-CM phenotypes (233).

Another metabolic disorder to be studied in a hiPSC setting is Barth syndrome (BTHS), a mitochondrial disorder caused by mutations in the tafazzin (TAZ) gene. Engineered muscular thin film (MTF) chips (276) constructed from BTHS hiPSC-CMs had defined metabolic (e.g., excess accumulation of ROS), structural (e.g., sparse and irregular sarcomeres), and functional (e.g., weak contractility of engineered heart muscle) abnormalities (289). These were reversed either by gene replacement therapy or genome editing, demonstrating that the TAZ mutation under investigation was necessary and sufficient for these phenotypes. Therefore, the combination of tissue engineering, genome editing, and hiPSC technologies in this study provided new insights into the pathogenesis of BTHS, delivering a biomedical platform to confirm the phenotype-to-genotype correlation of a gDNA mutation.

Taking a step further to demonstrate the association of gDNA alterations to disease manifestation, Ebert et al. (63) showed that the aldehyde dehydrogenase 2 (ALDH2) genetic polymorphism E487K, which is found in nearly 8% of the human population and is linked to increased susceptibility towards ischemic heart damage and coronary artery disease (CAD), was associated with elevated accumulation of ROS and toxic aldehydes (e.g., 4-hydroxynonenal; 4HNE) in heterozygous hiPSC-CMs. These pathological phenotypes were connected with the induction of cell cycle arrest and activation of apoptotic signaling genes (e.g., JUN) and were aggravated during exposure to an ischemia-mimetic solution and hypoxic conditions. Alda-1-mediated activation of ALDH2, treatment with the independent ROS scavenger catalase, or inhibition of the Jun regulator Jun-NH2-terminal kinase (JNK) were all able to decrease pathological ROS levels. Therefore, the authors were able to shed light onto the underlying molecular bases of a genetic polymorphism often found in East Asians that is associated with adverse cardiac function in response to ischemia.

VI. ENGINEERED DISEASE MODELS THROUGH TARGETED GENOME EDITING

Targeted gene editing has been historically achieved by homologous recombination (HR) (157); however, the low efficiency of HR events in human cells impeded extended application of this approach. Recent advances in endonuclease-based gene editing systems have transformed the field of genome engineering (46). Following discovery that induction of DNA double-strand breaks (DSBs) increases the frequency of HR events by several orders of magnitude, targeted nucleases such as ZFN, TALEN, and CRISPR/Cas9 have emerged as the preferred methods for improving the efficiency of HR-mediated gene modification (74). Once endonuclease enzymes induce a DSB in the DNA, the cell’s natural repair mechanism initiates one of two different processes: 1) the more likely but also more error prone nonhomologous end joining (NHEJ) pathway, whereby the two broken strands are modified by either adding or removing sheared nucleotides and then physically ligating the two strands back together; or 2) the more precise homology directed repair (HDR) pathway, kinetically favored over NHEJ only if a DNA strand with homology to the cut gDNA is present (199). This process can utilize an exogenously provided homologous sequence or plasmid as a template from which a complementary strand is synthesized. The newly synthesized strand then ligates with the original, resulting in a repaired double strand. Endonuclease-based techniques take advantage of this repair system by providing a template of semi-homologous sequence with specific and intended mutations that will be copied into the cell’s DNA.

The next section of the review provides more details regarding novel genome editing technologies, by comparing the ZFN, TALEN, and CRISPR/Cas9 systems, and highlighting the future research applications of these technologies.

A. DNA Binding Designs

In addition to the induction of DSBs, current nuclease-based approaches distinguish themselves from previous gene editing
systems by the ease with which their DNA recognition domains are customized, a feature that ultimately improves genome editing efficiency (214). ZFN and TALEN technologies share a mechanism where repeating domains in their protein-based DNA binding modules recognize individual nucleotides. The zinc finger domain, first discovered in 1997, is the core component of a zinc finger protein (ZFP), which recognizes a three nucleotide sequence. ZFNs typically comprise 3–6 zinc finger proteins and bind 9–18 nucleotides of the target DNA. ZFNs may be combined through a modular assembly to recognize nearly any genetic sequence; however, the codon-based gene editing limits flexibility and the ability to potentially target any gDNA sequence. Additionally, even though ZFNs rely on a very modular assembly, their production remains labor intensive and time consuming (136).

Similar to the role of the ZFPs, the core TALEN domain binds to DNA based on a protein-DNA interaction. The TALEN binding domain is a sequence of 33–35 repeating amino acids that forms a repeat-variable di residue (RVD). The 12th and 13th amino acids in each RVD, known as hypervariable positions, are responsible for binding different nucleotides. Based on the affinity of A, T, G, and C to the respective RVDs NI, HD, NN, and NG, TALENs may be utilized to recognize any sequence of DNA. This one-to-one interaction is a large advantage over ZFN in that it provides increased specificity and ease of customization (83). However, like ZFNs, to target a new DNA sequence one must reengineer the primary binding domains. Thanks to intensive research in recent years, customized TALENS can be constructed in <1 mo, following minimally labor intensive procedures that also reduce or eliminate the number of cuts and ligations required, thus reducing the possibility of orientation error (59, 234).

The most prominent technology for modern genome editing is the CRISPR/Cas9 system, first reported in 1987. A form of the CRISPR/Cas9 system can be found throughout bacteria and prokaryotes. However, the most thoroughly researched and most applicable to DNA modification is the modular type 2 CRISPR/Cas9 system found only in bacteria. Cas9 is an endonuclease specific to the bacteria *Streptococcus pyogenes* and is the only nuclease involved in silencing of foreign DNA (117). Within bacteria, different CRISPR systems exhibit slight modifications in length and DNA recognition, but all can be engineered through modular assembly of three main components: 1) the nuclease that cuts DNA; 2) a specificity determining RNA strand, known as the CRISPR RNA (crRNA); and 3) the trans-activating crRNA (tracrRNA) (118). Unlike the ZFN and TALEN recognition of DNA via protein interactions involving complex α-helix recognition of nucleotide triplets or repeating amino acids with hypervariable regions, the CRISPR-Cas9 system binds to DNA in the same fashion as an RNA oligo (302). Twenty nucleotides on the crRNA bind with the 20 nucleotides of the target DNA, recruiting the Cas9 endonuclease, which on its own has no sequence-specific bind-

While it is tempting to further stratify ZFN, TALEN, and CRISPR/Cas9 by ranking them based on binding efficiencies, it would be potentially misleading to do so. A clear and fixed ranking system is impossible to construct because each system binds differently to different targets. Currently no molecular basis explains this discrepancy. However, it has been suggested that DNA methylation, chromatin state, genomic position, and DNA sequence may affect DNA binding and thus explain why different editing efficiencies are reported for different targets (100).

### B. Off-Target Binding

Recent analysis of whole genome sequencing applied to hiPSCs reveals minimal differences in off-target activity among ZFN, TALEN, and CRISPR/Cas9 technologies (244, 255, 281). In addition, the frequency of off-target activity is typically lower than the rate of naturally accruing mutations that result from culture maintenance (51). Studies specific to TALEN and CRISPR/Cas9 identified significantly more mutations as a result of cell maintenance than those causally linked to the application of gene editing (106). Although some reports indicate high rates of off-target mutations, these numbers stem from analysis of transgenic mammalian cell lines such as HEK293, and thus cannot be put into context relevant to hiPSC culture and the development of disease models (274). Nevertheless, off-target binding remains a significant hurdle to clinical adoption of gene editing techniques. More research is required to develop improved computational algorithms able to identify sequences with low risk of off-target binding, and to predict off-target binding even when DSBs do not occur (291).

Recently, research has expanded beyond the optimization of binding domains to explore other creative approaches for reducing off target mutations. For example, engineered nuclelease mutations that induce DNA “nicks” or single-stranded breaks (SSBs) are advantageous because SSB cellular repair circumvents NHEJ repair mechanisms, which would normally increase the probability of mutagenesis. Multiple reports identify that both ZFNNickases and TALEN Nickases induce fewer off-target mutations while demonstrating equal if not better on-target mutation rates (303). Likewise, nickase-based CRISPR systems (CRISPR/Cas9n) result in negligible off-target effects without a sacrifice of on-target mutation frequency (240).

### C. Genome Editing in hiPSC-Based Cardiac Disease Modeling

In hiPSCs, genome editing has thus far been utilized for the correction of genetic disorders as mentioned earlier for
BTHS (289). Karakikes et al. (130) also used genome editing to correct the PLN gene mutation R14del which has been associated with ventricular dilation, ventricular arrhythmias, and heart failure. In this study, hiPSC-CMs heterozygous for the R14del mutation exhibited Ca$^{2+}$ handling abnormalities, electrical instability, abnormal cytoplasmic distribution of PLN protein, and increased expression of molecular markers of cardiac hypertrophy, which were ameliorated following TALEN-based mutation correction.

Similarly, genome editing has been utilized for the generation of hiPSC-based disease models, such as LQT syndrome. In two studies by Wang et al. (290) and Bellin et al. (13), hiPSCs from healthy donors were engineered to introduce dominant negative mutations in the ion channel genes KCNQ1 (p.R190Q, p.G269S, and p.G345E) and KCNH2 (p.A614V and p.N996I) into the safe harbor AAVS1 locus. Compared with isogenic controls, genome-edited lines displayed characteristic LQT syndrome phenotypes, which included significant APD prolongation, as outlined in Table 3. These disease phenotypes were corrected by drug treatment with the L-type calcium channel blocker nifedipine, or the K$_{ATP}$ channel opener pinacidil, therefore providing validation that engineered isogenic hiPSC lines can be used for disease modeling and drug testing.

In combination with genome editing, hiPSC technology has also been utilized in functional genomic screening for identification of the roles essential genes play in specific cellular process. For example, Hinson et al. (93) were able to generate cardiac microtissues using hiPSC-CMs to evaluate the pathogenic mechanism of truncating titin gene variants (TTNtvs), which are commonly associated with DCM. TTNtvs engineered into healthy hiPSCs resulted in microtissues with diminished contractile abilities (e.g., reduced contractile force) and impaired responses to mechanical and β-adrenergic stress. The investigators also found reduced expression of growth factors (e.g., TGFβ1, VEGF, HGF, EGF, and FGF2), hypoxia regulators (e.g., HIF1A and EPAS1 or HIF2A), and MAP kinases (e.g., MEK and ERK). VEGF supplementation of hiPSC-CM culture media ameliorated these phenotypes. The pathogenic mechanisms of TTN-induced DCM were identified as the disruption of critical linkages between sarcomerogenesis and adaptive remodeling when TTNtvs are present in the A-band domain of this protein that is responsible for thick filament-binding. Another interesting finding of this study was that hiPSC-CMs carrying engineered A-band TTNtvs showed less severe phenotypes compared with patient-specific hiPSCs carrying the same mutations, thereby raising the possibility that genetic background can influence the functional severity of TTNtvs. This observation has important functional implications for precision medicine.

### D. Future Directions of Genome Editing Technology

The last decade of research has seen rapid progress in the field of genome editing. It is clear that off-target effects exist, and resolving them will be important for future clinical applications of this technology. As research continues to optimize current protocols, more creative approaches such as the use of recombinant fusion proteins provide new opportunities (75, 76, 189). Fusing nuclease DNA-binding domains to alternate protein complexes can create genome-editing systems with reporter and therapeutic applications. Depending on which protein complex is used to replace the normal endonuclease domain, ZFN and TALEN systems can transcriptionally upregulate or downregulate genes to alter entire genetic pathways, remodel chromatin networks, or even act as reporters for gene activity.

A wide range of successful experiments have validated these supplementary additions to the genetic toolbox and protocols that are readily available through publicly available repositories (e.g., Addgene). In the case of both ZFNs and TALENs, the Folk1 endonuclease domain is replaced by transcriptional activators or repressor domains. Similar lists of protocols and gene regulatory activities are possible for CRISPR applications, where the Cas9 must be rendered catalytically inactive (CRISPR/Cas9d). This allows other protein complexes to be fused with the CRISPR/Cas9d, which is guided to the target sequence via the guide RNA, and can consequently provide targeted gene inhibition (CRISPRi) (149), activation (CRISPRa) (77), as well as spatiotemporal (204) or conditional (306) gene regulation with a higher specificity and reproducibility compared with other technologies such as RNA interference (RNAi) or Cre-LoxP systems. Modified CRISPR/Cas9 systems can also inflict alterations of single or multiple genes at a time. Notably, some groups have synthesized CRISPR/Cas9 libraries consisting of 70,290 guides targeting all human RefSeq coding isoforms (138), demonstrating that this technology constitutes a powerful genetic perturbation tool, with wide applications in precision medicine.

### VII. INDUCED MODELS OF CARDIAC DISEASE

Aside from genetic composition, several environmental factors (e.g., diet, stress, drugs, or pathogens) as well as secondary factors that occur due to failure in other organs can lead to cardiac disease, or affect disease progression and response to pharmacotherapy in heart failure patients (179) (see Table 4). Factoring in the effects of all these parameters is an important part of precision medicine. Very few studies to date, however, have established direct mechanistic links regarding the complex effects of environmental and secondary factors on heart failure. hiPSC-CMs that lack known familial or engineered
gDNA mutations can be exposed in vitro to a variety of environmental conditions (e.g., ischemic injury, hypoxic conditions, dietary supplements, electromechanical stress, and infectious agents) to enable determination of their effects on CM function. Existing studies of induced hiPSC-models are discussed below.

### A. Pathogen Induced

hiPSC-CMs can provide a suitable platform for the investigation of pathological mechanisms and therapeutic targets for life-threatening infectious diseases affecting the heart, such as Chagas cardiomyopathy caused by the *T. cruzi* trypanosome (176), or viral myocarditis caused by the B3 strain of coxsackievirus (CVB3) (5). In a recent study, Sharma et al. (239) demonstrated that hiPSC-CMs lacking any known mutations associated with cardiac disease can be infected by CVB3 particles and undergo pathological phenotypic changes such as increasingly irregular beating patterns, increase in Ca\(^{2+}\) transient duration and time to transient peak, and eventual beating arrest (239). The authors also showed that these phenotypes were ameliorated by treatment with antiviral drugs (e.g., interferon-β1, ribavirin, pyrroolidine dithiocarbamate, and fluoxetine), which acted by triggering viral RNA and protein clearance pathways and reducing the proliferation capacity of the CVB3 virus. This study demonstrated that hiPSC-CMs can be used to predict antiviral drug efficacy and serve as a sensitive platform to screen novel antiviral therapeutics for their effectiveness in a high-throughput fashion.

### B. Drug Induced

Pluripotent stem cell-derived CMs have been shown to respond appropriately to a wide variety of drug classes, including β-adrenergic receptor agonists and class I, II, III, and IV anti-arrhythmic agents (186, 314). As discussed in this review, clinically relevant and experimental drugs have also been used in vitro for the recovery of diseased phenotypes in models of cardiovascular disease. However, only a few studies have investigated the effects of known cardiotoxic drugs in a pluripotent stem cell-based platform that lacks known mutations. One example was given by Holmgren et al. (96), who found severe in vitro abnormalities following a 2-day treatment with varying doses of doxorubicin, a chemotherapeutic agent indicated for the treatment of a variety of cancer types (e.g., leukemia, lymphomas, and solid tumors) that is also associated with severe cardiotoxicity, especially in children and adolescent patients (36). CMs treated with the drug exhibited concentration-dependent alterations in cellular morphology, reduced contractile ability, lactate dehydrogenase leakage, cardiac troponin T release, and increased apoptosis. Transcriptome profiling performed by RNA-seq revealed that differentially expressed genes due to doxorubicin exposure were associated with cellular defense mechanisms such as p53 signaling (e.g., *TP53I3*), DNA damage response (e.g., *GADD45A*), and cellular senescence and response to stress (e.g., *HIST1H4H*). These genes could, therefore, serve as sensitive novel biomarkers for doxorubicin-induced toxicity in human CMs. In a separate study, Burridge et al. (21) demonstrated that doxorubicin treatment caused higher susceptibility to cell death, metabolic dysfunction, and calcium

### Table 4. Induced models of cardiac disease

<table>
<thead>
<tr>
<th>Model</th>
<th>Class</th>
<th>Disorder</th>
<th>Stimulus</th>
<th>Reference Nos.</th>
<th>Key Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induced</td>
<td>Drug</td>
<td>Myopathy</td>
<td>Doxorubicin</td>
<td>21, 96</td>
<td>↑ Contractile ability Cardiac troponin T and lactate dehydrogenase leakage</td>
</tr>
<tr>
<td>Pathogen</td>
<td>Myocarditis</td>
<td>CSV-B3 virus</td>
<td>239</td>
<td>Irregular or ceased beating Calcium handling abnormalities ↑ Cell death</td>
<td></td>
</tr>
<tr>
<td>Secondary</td>
<td>Hypertension</td>
<td>ET-1</td>
<td>2</td>
<td>Investigation of differentially expressed mRNAs and mRNAs upon induction of hypertrophy</td>
<td></td>
</tr>
<tr>
<td>T2D</td>
<td>Glucose, ET-1, cortisol</td>
<td>61</td>
<td>Myofibrillar disarray Lipid accumulation ↑ Oxidative stress Hypertrophy ↑ Cytosolic [Ca(^{2+})]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Together, Tables 3 and 4 provide a detailed outline of hiPSC-based models of cardiac disease published to date, with correlation to the gDNA mutations studies, and the key phenotypes observed for each disorder. ET-1, endothelin-1; CSV-B3, Coxsackievirus B3; T2D, type 2 diabetes.
handling abnormalities in hiPSC-CMs from breast cancer patients who experienced doxorubicin-induced cardiotoxicity (DIC) in the clinic compared with hiPSC-CMs from breast cancer patients who had not experienced DIC. These data support that hiPSC-CMs are a suitable platform for identifying patient-specific predilection to drug-induced cardiotoxicity.

C. Secondary Cardiomyopathy

Cardiomyopathy arising as a secondary feature to medical conditions initiated at a different organ has also been the subject of hiPSC-based studies. For example, induced cardiac hypertrophy was developed in hiPSC-CMs following 5-day exposure to endothelin 1 (ET-1), a potent vasoconstrictor peptide secreted by endothelial cells which leads to pulmonary hypertension (39) and also has known implications in biochemical and structural remodeling of the heart (30). Mechanistic investigation performed by high-throughput transcriptome analysis identified a pathological increase in the expression of fetal genes (e.g., ACTA1, NPPA, and NPPB). Pathological upregulation of NPPB was also confirmed at the protein level, and served as the basis for development of a 384-well HCl phenotypic screening tool for the assessment of compounds suspected to target the NPPB pathway, and therefore modulate the hypertrophic response. Several compounds, including the Ca\(^{2+}\) channel blocker verapamil, were shown to reverse the effects of cellular hypertrophy induced by ET-1. Other beneficial compounds included BEZ-235 (a dual PI3K-mTOR inhibitor), fenofibrate (a PPAR\(\alpha\) activator), and SAHA (a broad-spectrum histone deacetylase inhibitor).

In a separate study, microRNA (miRNA) sequencing showed that ET-1 treatment in hiPSC-CMs leads to differential expression of 250 known (e.g., miR-23a-3p and miR-208a-3p) and 34 novel miRNAs, the majority of which were associated with corresponding mRNA expression changes (2). Some of the complex RNA regulatory mechanisms associated with cardiac hypertrophy were involved in cardiovascular development (e.g., MEF2C), cell proliferation and transformation (e.g., MYC, FOS, TGF\(\beta\)2, and TGF\(\beta\)R3), and mitogen-activated protein kinase signal transduction (e.g., MAP3K9 and MAP2K6). Differentially expressed miRNA-mRNA pairs were consistent with several animal models of cardiac hypertrophy (28, 102). In addition, bioinformatics principal component analysis (PCA) comparing in vitro hiPSC-based findings to human myocardial biopsies from unrelated patients with left ventricular hypertrophy detected overlapping expression changes between the two groups.

Induced type II diabetic cardiomyopathy was also modeled in hiPSC-CMs following prolonged exposure to “diabetic milieu” consisting of a combination of glucose, ET-1, and cortisol (61). Gene set enrichment analysis (GSEA) using RNA-sequencing gene expression data showed significant upregulation in metabolic genes involved in the tricarboxylic acid (TCA) cycle (e.g., PDK1), mitochondrial electron transport chain (e.g., CYCS), glucose metabolism (e.g., ENO2), cell adhesion (e.g., integrins), and extracellular matrix deposition (e.g., collagens), whereas downregulation was observed in genes related to protein synthesis and the cellular response to dysfunctional protein production (e.g., ATF4 and CHOP10). This secondary cardiomyopathy model was consistent with one generated using hiPSC-CMs from patients with type II diabetes (T2D), therefore lending support to the hypothesis that clinical chemistry can induce a phenotypic surrogate of diabetic cardiomyopathy. The use of NPPB-based high-throughput phenotypic assays enabled the investigation of therapeutic targets by screening a library of 480 compounds. Beneficial molecules included regulators of Ca\(^{2+}\) homeostasis, such as inhibitors of voltage-gated Ca\(^{2+}\) channels (e.g., fluspirilene), molecules that deplete intracellular Ca\(^{2+}\) stores (e.g., thapsigargin), and inhibitors of Ca\(^{2+}\)-regulated proteins such as calmodulin (e.g., W7). The use of Na\(^{+}\) and K\(^{+}\) channel blockers (e.g., phenamil and penitrem A), which reduced the frequency of CM Ca\(^{2+}\) transients, also showed a therapeutic benefit.

Overall, by combining the hiPSC technology with high-throughput screening assays, these studies demonstrate a close correlation between hiPSC-CMs and human heart or animal models, provide mechanistic insight into the pathological gene regulatory pathways of HCM and T2D development, and demonstrate nicely that hiPSCs are valuable platforms in predictive toxicology and safety pharmacology applications that can benefit the early phases of drug discovery and development.

VIII. PATIENT RECRUITMENT VERSUS GENE EDITING

Recent improvements in gene editing may supplement the traditional system of patient recruitment that identifies and compares a group of diseased individuals to a control group that lacks disease phenotypes (FIGURE 2). This cause-and-effect based discovery is hampered by multiple issues, such as the possibility that phenotypic differences between patient and control groups are not due to disease-causing mutations, but arise due to other differences in genetic background (TABLE 5). To reduce the genetic background variability between patient and control cells, hiPSC lines generated from family members of the patient unaffected by the disease have been used (148, 184, 254). However, this approach can limit, but not abolish, genetic background variability, given that even siblings or parent-child pairs only have 50% genetic overlap. Gene editing techniques circumvent this limitation, but present other challenges such as off-target effects and clonal heterogeneity (180), which need to be sufficiently addressed to allow confirmation of casual relationships between the underlying genetics.
how individuals’ genomic variation can affect responses to drugs. Human genetic variations can result from SNPs, indels, or duplication of gDNA sequences. SNPs are by far the most common type of variation, with more than 14 million SNPs identified in the human genome (173). Over 60,000 of these SNPs are located within gene coding regions, and it is estimated that more than 90% of human genes contain at least one SNP. Genetic polymorphisms of proteins involved in pharmacodynamics and pharmacokinetics are known to be important determinants of individual variability in drug efficacy. Over 30 drugs are currently prescribed only under fulfillment of a genetic testing prerequisite due to their association with deleterious gDNA polymorphisms. Such information is shared through large publicly available (e.g., PharmGKB) or private (e.g., PGMD) databases, with the aim of contributing towards more effective, and safer medications and doses that will be tailored to a person’s genetic make-up.

A. Examples of Known Polymorphisms Implicated in Drug-Induced Toxicity

One example of a pharmacogenomic study focused on the therapeutic efficacy of simvastatin, a HMG CoA reductase inhibitor with cholesterol-lowering abilities that is used to lower the risk of stroke, heart attack, and other heart complications in people with diabetes or coronary heart disease. This study involved a cohort of 156 human subjects with low-density lipoprotein (LDL) cholesterol levels of 160 mg/dl, who were treated with simvastatin at varying doses (40, 80, and 160 mg/day) over the period of 6 weeks. Simvastatin treatment led to reduction of LDL cholesterol by 41, 47, and 53%, respectively, therefore providing strong evidence that this drug has a high therapeutic value in reducing LDL cholesterol (49). However, a small subset of the cohort (~5%) showed little to no reduction in LDL cholesterol levels, even at 160 mg/day. Furthermore, 0.7 and 2.1% of patients developed liver toxicity, as measured by elevated activity of alanine aminotransferase in the plasma, at the 80 and 160 mg/day doses. Importantly, one subject developed myopathy (0.7%) at the 160 mg/day dose. This variation in clinical outcome was later linked with genetic polymorphisms in the HMG CoA reductase gene HMGCR (e.g., p.A12T and p.T29G), as well as other polymorphisms in the cytochrome P450 family (e.g., p.F384L and p.F386L) that are involved in drug metabolism.

Table 5. Comparison of hiPSC-based disease modeling approaches

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<tr>
<th>Innate</th>
<th>Engineered</th>
<th>Induced</th>
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<tbody>
<tr>
<td>+</td>
<td>↓ Need for patient recruitment</td>
<td>Enables modeling of noncongenital conditions</td>
</tr>
<tr>
<td>-</td>
<td>Time consuming</td>
<td>Induced conditions may not accurately recapitulate disease</td>
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<tr>
<td></td>
<td>Costly</td>
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<tr>
<td></td>
<td>Potential risk for genomic instability</td>
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The advantages (+) and disadvantages (−) of innate, induced, and engineered hiPSC-based disease models. ↓, Reduced.

and disease phenotypes. To limit the effects of clonal variation, some studies have attempted to generate iPSCs in bulk culture (55, 298). More recently, it has also been shown that underlying genetic background variation is responsible for most heterogeneity between hiPSC lines, while cell type of origin and clonality have minor effects on hiPSC gene expression and methylation profiles (27, 231, 232a).

Another problem that restricts the paradigm of patient recruitment involves patient and family consent. Not only must a patient fit a correct disease profile, but the individual and their family must consent to donate human tissue. As a way to circumvent these problems, ZFNs, TALENs, and CRISPR/Cas9 genome editing tools may be applied to create control groups from diseased cell lines, and vice versa. This reduces the effort needed to recruit additional patients. Nevertheless, even this approach does not account for the possibility that multiple polymorphisms may contribute towards a disease phenotype, or address the fact that some disorders and clinical abnormalities are not yet correlated to any known mutations.

It is clear that there are roles for both patient recruitment and genome editing, and it is becoming the general consensus that both approaches should complement each other to establish large biorepository banks that can serve as immediate resources for the academic and pharmaceutical communities (299).

IX. PHARMACOGENOMICS

When a patient is administered a new drug, three outcomes are possible: 1) the patient experiences a beneficial effect in relation to the condition they are being treated for, 2) the patient’s condition does not improve, or 3) the patient experiences adverse cytotoxic effects or even death. In many cases, the same medication can elicit any of these three responses in different patients, and our understanding of the parameters that influence this outcome is greatly lacking. The field of pharmacogenomics aims to bridge this gap, by combining pharmacology and genomics to determine how individuals’ genomic variation can affect responses to

| +       | Disease modeled against patients’ genetic background | ↓ Need for patient recruitment |
| -       | Patient recruitment difficult | Time consuming |
|        | Nonisogenic disease and control lines | Costly |

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as in the solute carrier drug transporter SLCO1B1 (e.g., p.V174A), and the ATP-binding cassette transporter ABCG2 (e.g., c.421C>A) genes that have varying frequencies among different ethnic groups. The latter genes are known to regulate efflux of statins and statin metabolites and, therefore, contribute to the variability in efficacy and side effects of cholesterol-lowering drugs such as simvastatin, pravastatin, or rosuvastatin (35, 200, 273).

Another example is concerned with drug-induced LQT syndrome. In a cohort of 98 patients with drug-induced LQTS, the KCNE2 variant c.T8A, which is found in \(~1.6\%\) of the general population, was linked to adverse QT interval prolongation following administration of trimethoprim/sulfamethoxazole for the treatment of urinary tract infections (237). Screening for KCNE2-blocking activity is now routinely performed during the development process of any new drug to predict any risk for drug-induced QT prolongation that could lead to sudden death in humans. However, testing is typically performed in transgenic cell lines overexpressing KCNE2, which lack the functional complexity of human CMs such as the interaction between opposing ion currents, as well as the ability to reflect deleterious effects associated with SNPs and other types of individualized gDNA variation.

Patient-specific gDNA variation has also been shown to affect cardiotoxicity induced by the drug doxorubicin, an anthracycline antibiotic with broad antineoplastic activity which is currently one of the most common and most effective chemotherapy agents (265). Despite its anti-cancer efficacy, doxorubicin treatment is associated with fatal heart failure in 5–50% of patients (depending on administered dose) that was not predicted in preclinical drug-testing stages (113). Increased susceptibility to cardiotoxicity has been linked to polymorphisms in genes related to mitochondrial function (e.g., NADPH oxidase subunits) (283, 300). Specific examples include the 212A>G variant of the gene encoding the NCF4 subunit of NADPH oxidase (230), and the rs17863783 variant in the UDP glucuronosyltransferase UGT1A6 (284). Interestingly, some SNPs found in efflux transporters (e.g., ABCB1/MDR1, ABCC2, ABCC3, ABCG2, and RALBP1) improve doxorubicin pharmacokinetics/clearance and have been shown to be cardioprotective against this drug (271). Further study regarding the cardiotoxic or cardioprotective effects of such variants in patient-specific or engineered hiPSC models could lead to a deeper understanding of the genetic determinants of chemotherapeutic cardiotoxicity.

**B. Use of hiPSCs in Pharmacogenomic Investigations**

Preclinical drug testing in hiPSC-CMs can circumvent the limitations of drug testing in transgenic lines (196). Already, hiPSC platforms have been utilized to highlight differences in drug response underlined by genomic variation. In a study that generated a library of hiPSC-CMs from healthy patients as well as patients carrying mutations related to LQT syndrome, DCM, or HCM, it was found that LQT and HCM hiPSC-CMs were more susceptible to development of cardiotoxicity (e.g., EADs and DADs) compared with DCM and control hiPSC-CMs when treated with cисapride (161), a gastroprotective agent that was voluntarily withdrawn from the United States market in 2000 due to arrhythmic and sudden cardiac death episodes in patients with preexisting heart conditions such as LQT syndrome and heart failure (223). In a separate study, Braam et al. (17) observed that two potent blockers of the \(k_{\text{c}}\) current (HMR1556 and JNJ303) were cardiotoxic in LQT hiPSC-CMs, but not in controls. These studies have important clinical implications showing that genetic predisposition to toxicity is reflected in disease-specific hiPSC-CMs and may predict adverse drug responses more accurately than tests in transgenic lines overexpressing a single ion channel or even tests using a single control hiPSC line.

**X. POTENTIAL APPLICATIONS OF hiPSCs IN PRECISION MEDICINE**

**A. Safety and Efficacy Assessment of Novel Compounds in Preclinical Trials**

Despite strict guidelines by regulatory agencies, several drugs continue to present adverse side effects subsequent to their release to the clinic, with deleterious cardiovascular events (e.g., torsades de pointes, ventricular tachycardia, ventricular fibrillation, and sudden cardiac arrest) being implicated in 28% of drug withdrawals (16, 86). For example, milrinone, a phosphodiesterase-4 (PDE4) inhibitor, is an inotropic drug thought to have great clinical promise against heart failure, but was found during clinical trials to be associated with increase in mortality and lack of efficacy due to calcium overload and DADs in some patients (69). Cardiotoxicity has been observed not only with drugs targeted to act on the heart, but also with drugs aimed at other organs. For example, anthracycline chemotherapy agents (e.g., doxorubicin, daunorubicin, idarubicin, and epirubicin) have been frequently linked to cardiac complications such as arrhythmias, or dilated cardiomyopathy which develop either acutely or chronically (216, 285).

It is possible that unanticipated drug-induced cardiotoxicity may be in part attributed to the lack of appropriate preclinical screening platforms. Current drug testing is reliant on preclinical evaluation using human or animal transgenic cell lines (e.g., Chinese hamster ovary (CHO) cells and human embryonic kidney (HEK) cells overexpressing the human ether-a`-go-go-related gene (hERG)), followed by in vivo evaluation in small and large animal models (99, 232).
Despite having several advantages, including ease of maintenance and simplicity in developing assay for transgenic lines and the ability to study systemic effects for animal models, these platforms also have several drawbacks. Transgenic lines are only engineered to overexpress certain human proteins (e.g., the $I_{Kr}$ protein hERG) and lack the structural and functional complexity of relevant human cell types (218). For example, transgenic lines over-expressing a single ion channel failed to predict the false-positive outcome related to the drug verapamil, because while it blocks outward ion flow through the rapid delayed rectifier current, $I_{Kr}$, it also blocks inward ion flow through the L-type calcium channel $I_{Ca-L}$, therefore producing a neutral outcome in functional CMs (191). Notably, Navarette et al. (203) showed no cardiotoxicity in response to verapamil in hiPSC-CMs. In addition, these transgenic immortalized lines accumulate several karyotype aneuploidies that may alter their response to drugs (253, 256). As even SNP changes are known to contribute to variability in drug response (253), aneuploidies causing whole gene duplications or deletions as seen in transgenic lines may significantly alter recorded phenotypes.

In vivo testing in small and large animal models is also limited by significant interspecies variation between animals. For example, mice have a heart beat rate that is eight times faster than that of humans (500 vs. 60 beats/min), and cardiac repolarization in murine CMs relies primarily on $I_{to}$, $I_{K,slow1}$, $I_{K,slow2}$, and $I_{SS}$ ion currents, whereas in humans, repolarization is mostly dependent on the potassium channels $I_{Kr}$ and $I_{Ks}$ (226). Calcium handling (e.g., phospholamban; PLN), myofilament (e.g., MYH6/MYH7), and surface (e.g., SIRPA) proteins also have different expression patterns and pathophysiological roles in mouse versus human hearts. As an example, while truncation mutations have been shown to result in considerably reduced myocardial PLN protein content and loss of PLN inhibition of SERCA2a, leading to development of heart failure and early mortality in homozygous humans, in mice PLN deficiency enhances myocardial inotropy and lusitropy without adverse effects (87). Large animals such as rats, pigs, sheep, or dogs have hearts with closer cardiac electromechanical properties to those of humans, but are more difficult to handle, and more expensive to maintain in animal facilities. In addition, they are known to have different drug dose sensitivity compared with humans. For example, relative to humans, canines can tolerate up to 100-fold higher concentrations of chemotherapy agents such as thioTEPA, myleran, actinomycin D, mitomycin C, mithramycin, and fludarabine (220). It is estimated that animal mod-
els have a combined predictability of human behavior of only 10% (185, 238).

In contrast to transgenic and animal platforms, in vitro derived hiPSC-CMs display many of the complex characteristics associated with in vivo development of the heart, including gene and protein expression, ion channel formation, electrophysiological responsiveness, calcium handling, and excitation-contraction coupling (320). Although their current relatively early maturation state warrants additional attempts to drive further maturation, hiPSC-CMs provide an excellent platform to further improve preclinical drug testing (FIGURE 3). Beyond their properties as a functionally relevant human platform for preclinical drug testing, the introduction of hiPSCs to the current drug development model may allow testing of novel compounds on human cells which will be derived from individuals with diverse ethnicities, gender, and disease history (e.g., cardiovascular disease). This will take into account the expanding role of pharmacogenomics in the management of cardiovascular disorders (313). Preclinical drug testing against a diverse human genetic background has the potential to refine drug response-to-genotype correlations, prevent the release of cytotoxic chemicals to the clinic, and enable informed decisions about drug prescriptions based on gender, ethnic group, disease status, etc. A growing body of evidence indicates that hiPSC-CMs can offer the pharmaceutical industry a powerful, human, and physiologically relevant tool for validating new drug candidates in early drug development stages (65).

B. Clinical Management

Another goal of precision medicine is to enable physicians to prescribe appropriate medications at the right dose and schedule for individual patients to achieve maximal therapeutic benefit with minimal, tolerable adverse effects (133). In current clinical protocols, clinicians typically rely on the patient’s history and test indications to estimate the best pharmacotherapy, which can be conditional upon careful subsequent monitoring of patients to assess the effectiveness and toxicity of the drug. Often, the clinician may prescribe several different drugs to identify the optimum treatment for each patient (FIGURE 4). During this lengthy process, patients may experience unwanted side effects, and their condition may deteriorate. Furthermore, side effects may be induced by polypharmacy (i.e., the use of multiple medications by a patient). Avoidable adverse drug events are among the most serious consequences of inappropriate drug prescribing (219). However, effective protocols for predicting such events are lacking. A hiPSC-based model for

![Predicted Paradigm Shift in Drug Treatment](image-url)

**FIGURE 4.** hiPSC-based personalized medicine as a novel model for clinical pharmacotherapy. The hiPSC field is growing at an unparalleled pace, with the promise that these cells will become major tools in the advancement of personalized medicine. When treating a patient, clinicians nowadays typically rely on the patient’s history and clinical test indications to choose the presumed best pharmacotherapy. Since there is limited predictive confidence in these indications, the clinician may need to prescribe several different drugs before they can identify one that best suits each patient. During this sometimes lengthy process, the patient may experience unwanted and potentially serious side effects, and their condition may deteriorate. In contrast, a hiPSC-based model for personalized medicine may allow safe and patient-specific testing of several drugs on hiPSC-differentiated derivatives (e.g., cardiomyocytes), and a pharmacogenomics analysis using hiPSC-derived cells may enable precise prediction of the patient’s response to each drug. This paradigm-shifting model of clinical treatment could vastly increase our confidence in the prescription of appropriate medication, saving patients from dangerous side effects or toxicities, and optimally addressing their disease symptoms.
Precision medicine has the potential to provide patient-specific information about drug response by taking into account individualized information such as genomic, transcriptomic, metabolomic, pharmacokinetic, and pharmacodynamic profiles that are reflected in hiPSCs. This may allow safe and patient-specific laboratory-based testing of several drugs on hiPSC differentiated derivatives (e.g., CMs) to enable precise prediction of the patient’s response to a drug or to polypharmacy, as well as to guide the dosing and frequency of drug administration. Overall, this model of clinical treatment could provide increased confidence in the prescription of appropriate medication in the future, which could protect patients from side effects or toxicities, and optimally address their disease symptoms.

In a hiPSC-based study in which the proband had a de novo SCN5A LQT3-associated mutation (F1473C) and a polymorphism (K897T) in KCNH2, the gene associated with LQT2 syndrome, biophysical and molecular analysis of ion channels expressed in hiPSC-CMs demonstrated that phenotypic abnormalities (e.g., arrhythmias) observed in vitro were attributed to the SCN5A mutation, whereas the KCNH2 polymorphism was not a contributing factor to disease severity (264). The abnormally augmented I_{Na} current was corrected either by faster pacing of hiPSC-CMs or treatment with the Na^+/H^+ channel blocker mexiletine. Therefore, in vitro testing in hiPSCs was able to provide functional information as to the mechanisms of disease, indicating that Na^+ channel blockers, rather than K^+ channel activators, would serve as a better pharmacological treatment for this specific patient.

Similar principles can be applied to disease prognosis by utilizing patient-specific hiPSC derivatives to enable extensive phenotypic screening and early prediction of disease outcome and severity (FIGURE 5). Whereas current clinical protocols for prognosis rely on family history and long-term monitoring, hiPSCs may provide a fast track to diagnosis in certain clinical scenarios, thus enabling implementation of prevention or early treatment plans that lead to the best outcome.

One limitation towards clinical applications of hiPSCs is the time required (~3 months) to reprogram somatic cells to hiPSCs and subsequently differentiate them to functional cell types, such as CMs. Optimization of differentiation protocols has already shortened the time needed for hiPSC differentiation to CMs from ~18–25 to ~9–11 days (24). As further improvements in this field are under development, large-scale bio-repositories of both hiPSCs and their differentiated derivatives provide an optimal way to reduce the time required to obtain patient-specific cells for functional assays. It is also possible that hiPSC derivation and bio-banking could become routine, to enable population-wide individualized medicine in the future.

**XI. CONCLUSIONS AND FUTURE PERSPECTIVES**

Precision medicine approaches in which the combination of any given patient’s unique clinical, genomic, and in vitro cellular characteristics can fine-tune decisions regarding the

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**FIGURE 5.** hiPSC-based personalized diagnostics for disease prediction. Familial disease often has a large social impact on family life. In the absence of a known disease-associated mutation, clinicians adopt a disease prediction strategy for young offspring that involves long-term monitoring of biomarkers and heart function. However, this strategy is time-consuming and delays implementation of prevention or early treatment plans. Generation of hiPSC-CMs from family members may enable extensive phenotypic screening and early prediction of disease severity and outcome, to improve patient care.
diagnosis, treatment, and prevention of human disease are starting to take shape thanks to in vitro hiPSC-CM models. To facilitate this field and to promote more efficient development of novel therapies, biobanking of hiPSCs has bloomed over the past 5 years, with participation of several academic groups (e.g., Stanford Cardiovascular Institute) and commercial institutions (e.g., Coriell Institute, Cellular Dynamics International, Stem Cell Theranostics, Pfizer, and Roslin Cells) which are typically funded in partnership with government entities (e.g., NIH, CIRM, etc.). The International Stem Cell Banking Initiative (ISCB) alongside the International Stem Cell Initiative (ISCI) have also coordinated teams of stem cell biologists, bio-banking centers, stem cell society representatives (e.g., ISSCR, ISCT), ethics committees (e.g., ISCF Ethics Working Party), and regulators from over 20 countries to standardize the derivation, culture, and characterization methods of hiPSCs (7). These large multinational efforts have one goal in common: to expedite the clinical uses promised by the hiPSC technology.

While hiPSCs avoid the main controversies associated with hESCs (i.e., the destruction of human embryos during hESC derivation), new ethical concerns have not only clouded the use of hiPSCs, but also the generation and privatization of patient-specific “omic” data. For example, when the company 23andMe initiated sales of a saliva-based Personal Genome Service (PGS) that provides genotyping reports on 254 diseases and conditions without prior marketing clearance or approval from the Food and Drug Administration, this was considered a risk. The concerns were not only that this kit should have been regulated and marketed as a medical device, but also that patients were receiving complex information about their possible predisposition to diseases without appropriate genetic counseling, and without consideration for false-positive or false-negative outcomes (11). This and other examples illustrate the need to set ethical boundaries and rules for generation and distribution of patient-specific NGS data related to hiPSC generation and analysis (43). Careful regulation of genome editing technologies will also be necessary to prevent eugenic misuse (140).

In addition to ethical issues, technical challenges also need to be addressed for each technology. For the hiPSC technology, one major concern is the immature state of differentiated derivatives. This topic has been extensively discussed elsewhere (128, 309), and several physical, chemical, and electrical signals are under investigation to enable these cells to mature to a state more closely resembling the adult human heart (124, 208, 266, 276, 309). Subtype specification of CMs (i.e., into pacemaker, atrial, or ventricular cells of either the right or left side of the heart) is also under investigation. Although a few protocols claim to enable enhancement of the specific generation of ventricular (129, 296), atrial (53, 119, 263), and pacemaker (127, 326) CM sub-types, thus far none has become standard practice. Improving our ability to generate specific CM subtypes from hiPSCs will also enable more precise modeling of diseases that preferentially affect either the ventricles (e.g., HCM, DCM, ARVD), or atria (e.g., atrial arrhythmias), in addition to opening new prospects for creating patient-specific bioengineered pacemakers.

Significant limitations for the genome editing technology include not only the length and cost of the process, but more importantly, its efficiency, and off-target effects, as discussed extensively in previous sections. In addition, the NGS field needs to address the issue of storage and management of the vast datasets generated, as well as to develop more effective methods of data analysis (288). Sequencing throughputs have rapidly outpaced even Moore’s Law for computing power, which states that the number of transistors in a dense integrated circuit doubles approximately every two years (195). Improvement of data compression algorithms would help to address the growing data-to-central processing unit (CPU) ratio (287). To date, thousands of WGs and hundreds of thousands of exomes have been sequenced. However, meaningful biomedical contribution of these data has been greatly outpaced by the rate at which new samples are sequenced (156). Therefore, an additional challenge for the NGS technology is to accelerate the rate of data analysis. Efficient data analysis would lead to the identification of new disease-associated mutations, epigenetic modifications, or gene expression pathways which could in turn generate innovative therapeutic targets, improve the predictive abilities of genetic testing, and ultimately translate into public health benefits. In the future, clinical and hiPSC-based sequencing of patients suffering from disease is expected to guide diagnosis and treatment decisions. The precision medicine initiative is here to ensure that outstanding issues associated with the technologies involved are sufficiently addressed so that these tools can serve to benefit patients. Precision health represents a revolution in patient care, by moving beyond incremental improvements in diagnosis and treatments for acute or chronic disease to achieve fundamental understanding of underlying disease causes and treating individuals based on their unique genetic composition (45). By combining the strengths of basic scientific research, biomedical data science, genome editing, and transformative biomedical platforms such as hiPSCs (299), we can truly begin to understand individual variability in disease development, progression, and response to therapy. Although precision health requires big thinking and bold action, its promise of a revolution in healthcare away from after-the-fact diagnosis, towards prediction and prevention, is approaching fast.

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DISCLOSURES

J. Wu is a co-founder and Scientific Advisory Board member for Stem Cell Theranostics.

REFERENCES

HUMAN INDUCED PLURIPOTENT STEM CELLS


104. Hung SC, Kang MS, Kieff E. Maintenance of Epstein-Barr virus (EBV) oriP-based episomal persistence in cells requires the EBV-encoded nuclear antigen-1 chromosome-binding domains, which can be replaced by high-mobility group-I or histone H1. *Proc Natl Acad Sci USA* 98: 1865–1870, 2001.


232. Sallam K, Li Y, Sager PT, Houser SR, Wu JC. Finding the rhythm of sudden cardiac
dysrhythmia. 


220. Price PS, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data

228. Reuter Jason A, Spacek DV, Snyder MP. High-throughput sequencing technologies.


223. Quigley EM. Cisapride: what can we learn from the rise and fall of a prokinetic?

222. Quail MA, Smith M, Coupland P, Otto TD, Harris SR, Connor TR, Bertoni A, Swerd-

221. Puri MC, Nagy A. Concise review: embryonic stem cells versus induced pluripotent


225. Rais Y, Zviran A, Geula S, Gafni O, Chomsky E, Viukov S, Mansour AA, Caspi I,


217. Phan D, Rasmussen TL, Nakagawa O, McAnally J, Gottlieb PD, Tucker PW, Richard-


212. Pouton CW, Hayes JM. Embryonic stem cells as a source of models for drug discov-
ery. 


209. Pretorius RW, Gataric G, Swedlund SK, Miller JR. Reducing the risk of adverse drug

208. Price PS, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data


203. Qiugley EM. Cisapride: what can we learn from the rise and fall of a prokinetic? J Dig

202. Puri MC, Nagy A. Concise review: embryonic stem cells versus induced pluripotent


200. Price PK, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data

from studies of anti-neoplastic agents in animals and humans. Toxicol Appl Pharmacol
233: 64 –70, 2008.

209. Pretorius RW, Gataric G, Swedlund SK, Miller JR. Reducing the risk of adverse drug

208. Price PS, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data


203. Qiugley EM. Cisapride: what can we learn from the rise and fall of a prokinetic? J Dig

202. Puri MC, Nagy A. Concise review: embryonic stem cells versus induced pluripotent


200. Price PK, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data

from studies of anti-neoplastic agents in animals and humans. Toxicol Appl Pharmacol
233: 64 –70, 2008.

209. Pretorius RW, Gataric G, Swedlund SK, Miller JR. Reducing the risk of adverse drug

208. Price PS, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data


203. Qiugley EM. Cisapride: what can we learn from the rise and fall of a prokinetic? J Dig

202. Puri MC, Nagy A. Concise review: embryonic stem cells versus induced pluripotent


200. Price PK, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data

from studies of anti-neoplastic agents in animals and humans. Toxicol Appl Pharmacol
233: 64 –70, 2008.

209. Pretorius RW, Gataric G, Swedlund SK, Miller JR. Reducing the risk of adverse drug

208. Price PS, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data


203. Qiugley EM. Cisapride: what can we learn from the rise and fall of a prokinetic? J Dig

202. Puri MC, Nagy A. Concise review: embryonic stem cells versus induced pluripotent


200. Price PK, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data

from studies of anti-neoplastic agents in animals and humans. Toxicol Appl Pharmacol
233: 64 –70, 2008.

209. Pretorius RW, Gataric G, Swedlund SK, Miller JR. Reducing the risk of adverse drug

208. Price PS, Keenan RE, Swartout JC. Characterizing interspecies uncertainty using data


203. Qiugley EM. Cisapride: what can we learn from the rise and fall of a prokinetic? J Dig
HUMAN INDUCED PLURIPOTENT STEM CELLS

Mj, Ashley EA, Longaker MT, Robbins RC, Wu JC. Patient-specific induced pluripo-

imally impacts whole-genome mutational load in human-disease-specific induced pluripo-


259. Takaya T, Kawamura T, Morimoto T, Ono K, Kita T, Shimatsu A, Hasegawa K. Identification of p30-targeted acetylated residues in gata4 during hypertrophic re-


261. Takaya T, Watanabe T, Morimoto T, Ono K, Kita T, Shimatsu A, Hasegawa K. Identification of p30-targeted acetylated residues in gata4 during hypertrophic re-

tile vector variability in hypertrophic cardiomyopathy-induced pluripotent stem cell-


266. Thavandiran N, Dubois N, Mlyukov Y, Masse S, Beba B, Simmons CA, Deshpande VS, McGarry JP, Chen CS, Nanthakumar K, Keller GM, Radisic M, Zandstra PW. Design and formulation of functional pluripotent stem cell-derived cardiac microtis-


