Advancing cardiac safety and drug discovery screening using human stem cell-derived cardiomyocytes

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In recent decades, drug development costs have increased by approximately a hundredfold, and yet about 1 in 7 licensed drugs are withdrawn from the market, often due to cardiotoxicity. This review considers whether technologies using human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) could complement existing assays to improve discovery and safety while reducing socioeconomic costs and assisting with regulatory guidelines on cardiac safety assessments. We draw on lessons from our own work to suggest a panel of 12 drugs that will be useful in testing the suitability of hiPSC-CM platforms to evaluate contractility. We review issues, including maturity versus complexity, consistency, quality, and cost, while considering a potential need to incorporate auxiliary approaches to compensate for limitations in hiPSC-CM technology. We give examples on how coupling hiPSC-CM technologies with Cas9/CRISPR genome engineering is starting to be used to personalize diagnosis, stratify risk, provide mechanistic insights, and identify new pathogenic variants for cardiovascular disease.

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Unlocking personalized biomedicine and drug discovery with human induced pluripotent stem cell-derived cardiomyocytes: fit for purpose or forever elusive?

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Introduction and context

In the drug development pipeline, the conventional wisdom is that large drug libraries are whittled down via a series of assays designed to identify and characterize pharmacological activity associated with molecular targets, off-targets, and safety liabilities[1]. These assays involve in silico screens, heterologous cell lines, primary cells, organotypic models, and preclinical assessment in animals before putative drugs are progressed into phase I–III clinical trials. The ultimate aim for the desired target/patient population is to find a highly efficacious and safe drug, which can be launched to market for significant patient health and socioeconomic benefits. This strategy has been productive, as evidenced by the marketing of, on average, 37 drugs per year over the last 20 years. However, three core challenges faced by the pharmaceutical industry are prompting discussion on whether at least some aspects of the development pipeline could be replaced or complemented by newer approaches, including those involving models based on human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs)[2].

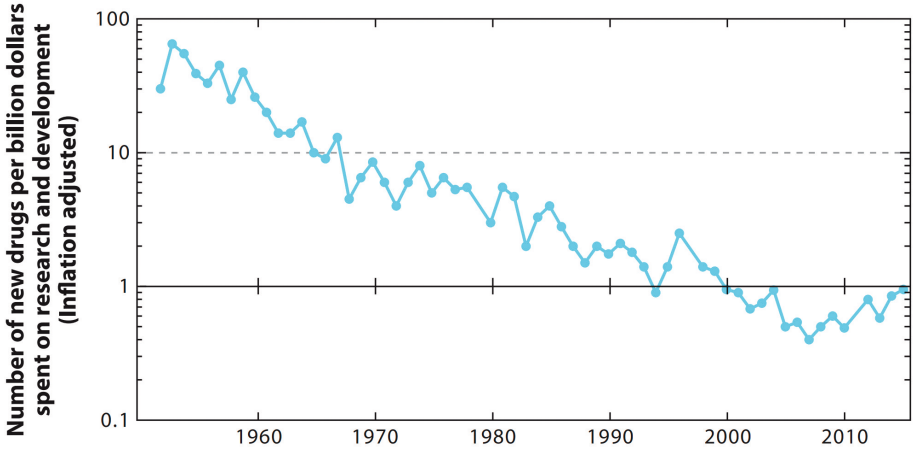

Figure 1. Inflation adjusted figures for drug discovery between 1953 and 2014 shows that cost of identifying new drugs has increased by up to ~100-fold[3].

First, many of the easy-to-find drugs have been discovered, which has increased the costs of identifying new tractable targets and discovering novel therapeutics. Blockbuster drugs such as sildenafil, omeprazole, ramipril, or amlodipine are rare finds, and, in general, the costs associated with successful regulatory approval of a drug have risen substantially. As shown in Figure 1, during the 1950s and 1960s, for each billion dollars (inflation adjusted) spent on research and development (R&D), up to 70 drugs were marketed (as little as $14 million per drug). Over the last six decades, there has been a steady decline in the ease of discovery such that each marketed drug now costs $1–1.8 billion. By this reckoning, the 56 drugs launched in 2015[3] alone cost a total of $56–101 billion, which constitutes the highest R&D expenditure of any industrialized sector and is not sustainable.

Second, the use of animals is considerable. This leads to high costs but does not necessarily provide accurate predictive data on efficacy or toxicity in humans. In 2015, there were over 6,000 putative medicines in the preclinical development stage, requiring millions of animals at a total cost of $11.3 billion, a financial value only exceeded by phase III clinical trials ($15.3 billion). Of these medicines, about 1,700 (~30%) progressed to phase I clinical trials, but only 56 (<1%) made it to market[3,4]. This highlights the major molecular and functional physiological differences between species (reviewed in 2). For example, endothelin receptor antagonists were shown to be effective in preventing heart failure in rodents[5,6], but the outcome was neutral when tested in clinical trials[7]. Conversely, furosemide could have been rejected because it causes hepatotoxicity in mice, rats, and hamsters, but it does not in humans. There is greater similarity in physiology between humans and large animals[8]. This is seen by recent work from the Institute of Zoology of the Chinese Academy of Sciences in Shanghai[9,10], where nonhuman primate models of autism, Parkinson's disease, and macular degeneration show symptoms more closely related to humans than do models of other species, particularly rodents[11]. Moreover, stem cells from monkeys, especially rhesus or cynomolgus macaques, are similar to their human counterparts[10], and phenotypes of gene knockouts in nonhuman primates more closely replicated those seen in humans than mice. However, the widespread use of large animals, and particularly nonhuman primates, is costly, challenging, ethically sensitive, and contrary to the "3 Rs" principles of refinement, reduction, and replacement of animal use. Therefore, there is a pressing need to develop alternative human-based models that have translational relevance.

Third, continuing issues include high levels of late-stage attrition, post-market withdrawals, and usage restrictions. Analysis of reasons for attrition of drug candidates within AstraZeneca, Eli Lilly & Co, GlaxoSmithKline (GSK), and Pfizer shows that safety and toxicology are the largest sources of failure[12]. Unexpected cardiotoxicity is problematic, accounting for up to 45% of the liability depending on the stage of drug development[13]. Indeed, of the 462 drugs withdrawn from market between 1953 and 2013, cardiotoxicity accounted for 79 (17%) of these withdrawals and was only exceeded by hepatotoxicity (83 drugs, 18%)[14]. From 1980 to 2009, about 1 in 7 licensed drugs deemed efficacious in phase III trials were withdrawn from the market[15]. This is exemplified by the anti-inflammatory drug rofecoxib, which was withdrawn in 2004 because its long-term use may double the risk of heart attack and stroke among users. Soon after, it emerged that rofecoxib could have caused up to 140,000 extra cases of serious coronary heart disease in the United States[16], despite the drug being considered safe in animal tests and human clinical trials. Even for drugs that are approved and marketed, potential risks to patients are highlighted as adverse drug reaction (ADR) notifications in product information/labeling and, for serious or life-threatening ADRs, by black box warnings. While the top 200 drugs account for 66.6% of the 4.3 billion prescriptions administered in the United States each year, 81 of them carry black box warnings. These risk notices can cause financial burden through reduced sales[17].

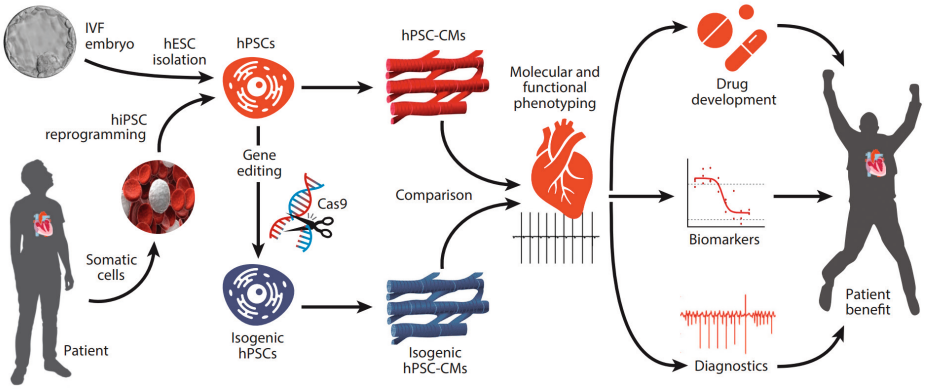

Figure 2. Derivation, engineering, and utility of hPSC-CMs. *While hESCs are derived from preimplantation embryos, hiPSCs are produced by epigenetic reprogramming of somatic cells such as skin fibroblasts or mononuclear blood cells. These hPSCs have similar properties,including long-term proliferation and expansion, and the capacity for differentiation to hPSC-CMs. Cross comparison of molecular and functional phenotypes in isogenic pairs of hPSC-CMs that differ only in specific engineered differences, e.g., by Cas9/CRISPR technologies, can provide mechanistic insight into genetic disease. This information has the potential to enable the development of drugs, biomarkers, and diagnostics, all with the potential for patient benefit. Abbreviations: CM, cardiomyocyte; hESC, human embryonic stem cell; hiPSC, human induced pluripotent stem cell; hPSC, human pluripotent stem cell; IVF, in vitro fertilization.*

Biopharma is adapting by recognizing that collaboration between industry and academia can be fruitful. This has encouraged large pharma toward developing open innovation platforms, which vary from direct funding schemes to accessing well-annotated drug libraries. There is an increasing appetite for humanized screening platforms on the premise that these will improve the prediction of efficacy and/or toxicity in patients if incorporated into preclinical data packages. If this notion is accurate, it would facilitate a so-called fail early, fail cheap approach to avoid late-stage attrition and post-market withdrawal. Conversely, drugs that are harmful to animals but potentially beneficial to humans would not be discontinued during development. Accordingly, it has been calculated that reducing drug attrition in phase I clinical trials by 5% could reduce development costs by 5.5–7.1%[18].

For these reasons, the potential utility of new models such as those based on hiPSC-CMs is an active area of interest *(Figure 2)*. Whereas hiPSCs are derived by epigenetic reprogramming of human somatic cells, human embryonic stem cells (hESCs) are derived from the inner cell mass of the preimplantation embryo. However, while both cell types have similar properties, industry prefers hiPSCs since there are fewer ethical issues relative to the embryo-derived origin of hESCs. Also, hiPSCs can be created with relative ease from a wide variety of somatic cell sources, including skin fibroblasts, mononuclear blood cells, and urinary cells[19,20]. Therefore, unless otherwise specified, this article focuses on hiPSCs.

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)

A key property of hiPSCs is that they can be propagated in long-term culture and yet be induced to differentiate into many cell types, including cardiomyocytes (CMs) *(Figure 2)*. Reviews have documented the evolution of the technology, which now allows for efficient production of hiPSC- CMs of >85% purity at scale[21,22] in both academic and commercial laboratories. More recently, approaches to specify atrial- and ventricular-like hiPSC-CM subtypes have been described by several groups using modulation of retinoic acid signaling[23–25].

There are many attractive reasons for using hiPSC-CMs to complement or replace existing model systems. Notably, they overcome the issues of primary CMs for which large-scale use is hindered by near-nonexistent proliferation and/or almost immediate dedifferentiation in culture, while limited availability and poor consistency are additional burdens for human sources[2,26]. In contrast, hiPSC-CMs can be maintained for months, and 1–2 years in extreme cases, in culture and can be shipped between labs in either live or cryopreserved formats.

A notable advantage of undifferentiated hiPSCs is that they can be cryo-banked in large numbers, which enables genetically identical seed stocks to be differentiated into CMs. This overcomes issues of genetic variability between individuals and also makes it possible to create prescribed, large-scale banks of hiPSCs with desired genotypes such as disease classes, genetic groups, and so on[27]. The utility of hiPSC-CMs from patients was realized early on, as they provided new *in vitro* models for genetic diseases of the heart, including those caused by defects in ion channels [e.g., long QT syndrome (LQTS)] and structural proteins [e.g., hypertrophic cardiomyopathy (HCM)], as explored elsewhere[2,28].

Additional possibilities have been created by improvements in genetic engineering of hiPSCs *(Figure 2)*, particularly the advent of efficient gene-editing approaches using Cas9/clustered regularly interspaced short palindromic repeat (CRISPR) systems[29]. With various configurations of Cas9/CRISPR, it is now possible to create hiPSC-CMs that carry targeted gene knockouts, knock-ins, and polymorphic substitutions, while gene activation, repression, and epigenetic regulation are also feasible (for a review, see 30). This precision enables the effect of particular polymorphisms on cell function to be assessed in an otherwise constant genome (known as isogenics), which is important because the impact of genetic background on phenotype can exceed that of the mutation[31]. Using this approach, we recently described suites of isogenic sets, wherein mono- and/or bi-allelic substitutions were made in two structural proteins associated with HCM. Thus, R453C and E99K changes were introduced to the beta myosin heavy chain protein and cardiac actin, respectively[21,22]. Diverse phenotyping using 12 different approaches (e.g., high-content imaging, metabolism, Ca2+ handling, contractility) were used to show a high degree of recapitulation of the disease characteristics seen in patients while also clarifying hypotheses and providing approaches for evaluating new therapeutics[21,22].

We have also used high-throughput efficacy screening to discover compounds that may be of use in treating HCM *(Figure 3)*.

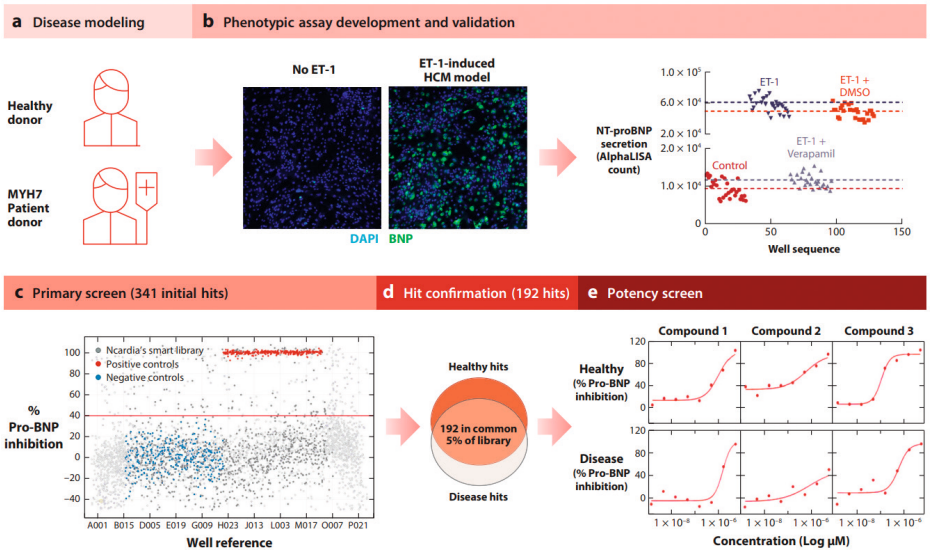

Figure 3. High-throughput efficacy screening of two endothelin-1 (ET-1)-induced hypertrophy models. *(a) Human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) were produced from a healthy and diseased (MYH7-mutated) line. (b) ET-1-induced secretion of NT-proBNP, a clinical marker of cardiac injury/hypertrophy, was rescued by verapamil treatment in hiPSC-CMs using an AlphaLISA assay. (c) 3,600 compounds from Ncardia's Smart Library were screened using calcium transient assays in a 384-well format. Compounds with percent inhibition greater than 40% were identified as hits (n = 341). (d) Hits were confirmed via three distinct assays: NT-proBNP AlphaLISA assay (plus retest), AlphaLISATruHits assay (false positives deselected), and intracellular expression assessed by a high-content-imaging secondary assay (n = 192). (e) To determine compound efficacy, potency analyses with 8-point dose response curves were generated, and hits with various potencies were identified (i.e., low potency, EC50 > 10 µM; moderate potency, EC50 > 1 µM; and high potency, nanomolar to subnanomolar range).*

A role for hiPSC-CMs in re-writing regulatory guidelines on cardiac safety assessment

Soon after the first derivation of hiPSC-CMs, their utility in drug evaluation was identified, particularly for ion channel modifiers. Provisional safety screens often involve simplified cell models (e.g., using CHO or HEK cell lines) genetically engineered to overexpress an ion channel of choice. Although high throughput, these screens do not replicate the complexity of the working CM and can lead to false negatives or positives. As an example, the multichannel-blocking drug verapamil was initially marked as potentially harmful based on single ion channel assays that revealed the relatively high potency of verapamil to block rapid delayed rectifier potassium current (IKr). However, verapamil is considered to be safe on account of its dual

blocking of potassium IKr and calcium ICa,L channels. This result was observed in hiPSC-CMs, emphasizing the importance of using integrated human models to detect drug effects on multiple cardiac ion channels[32].

Of the many studies that have now investigated the impact of drugs on the electrophysiology of hiPSC-CMs, the most prominent is the multinational Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative. A complementary approach has been taken via an initiative from the Japan iPS Cardiac Safety Assessment (JiCSA). JiCSA established a multielectrode array (MEA) protocol to evaluate the applicability of hiPSC-CMs for assessing the torsadogenic potential of drugs and completed a large-scale validation study using 60 drugs, which largely overlapped with the 28 drugs used by CiPA. A detailed comparison of the findings of CiPA and JiCSA has been published[33].

CiPA began after a Food and Drug Administration (FDA) workshop held in July 2013, which was aimed at providing a framework for the proarrhythmic risk assessment of new drugs *in vitro*[34,35] with improved specificity compared to the assays currently recommended by the International Conference on Harmonisation, (ICH)-S7B[36]. Most of the emphasis in the current ICH-S7B guidelines is on inhibition of the IKr potassium channel (hERG), since this is the most predominant mode of drug-induced proarrhythmia. Typically, a primary screen in a heterologous hERG expression system is followed by telemetry in instrumented dogs, with possible confirmatory experiments in rabbit Purkinje fibers[37]. CiPA was ambitious because it proposed collaboration between the pharmaceutical industry, service providers, biotech companies, academia, and global regulatory authorities to identify proarrhythmic risk based on several key assessments[38]. These included (a) assessment of several major ion channels in transfected cell lines, (b) in silico modelling of the ion channel effects, (c) proarrhythmic assessment in hiPSC-CMs, and (d) clinical assessment of electrocardiograms from phase I human studies.

For validation and implementation of hiPSC-CMs in cardiac safety pharmacology, CiPA has played a significant role. Data presented by Blinova et al.[39] describe the utility of hiPSC-CMs in evaluating the electrophysiological responses to 28 drugs linked to low, intermediate, and high proarrhythmic (Torsades de Pointes) risk categories. Testing was blinded across 10 sites, with some sites using two commercial hiPSC-CM lines. Five different platforms were used, including MEAs and voltage-sensing optical methods. Despite variations in experimental protocols, cell batch, and cell culture medium composition, the results for all 28 drugs were broadly consistent, including similarity between the two commercial lines for low- versus intermediate- or high-risk prediction. Collectively, data were used to develop regression models to predict risk at a reasonable level of accuracy. Meanwhile, other investigators also started to develop scoring systems for preclinical cardiac safety evaluation. For example, Kopljar et al.[40] used hiPSC-CM calcium transient screening to assign risk points, which were calculated from the level of drug-induced change on transient duration, beat rate, amplitude, and quiescence/

arrhythmia incidence, with the final parameter being most heavily weighted. Summing the risk points allowed the outcomes to be easily interpreted on a color-coded scorecard.

It is important to recognize that CiPA was the first major international effort to use hiPSC-CMs in drug evaluation for proarrhythmia. The use of these cells as testing tools is still undergoing rapid development, hence there were limitations to the study. Most of the selected proarrhythmic drugs had a hERG-related mode of action for which there are well-established evaluation models, and going beyond hERG seems like a missed opportunity. Indeed, there are now very few, if any, drugs withdrawn from market that perturb electrophysiology due to hERG blockade. A closer look at the data in the Blinova study[39] showed that there was high variability between test sites for individual drugs. This may relate to differences in analysis platform and interoperator/interlab variability or to serum content in the cell culture medium, which is known to affect drug solubility and availability. Although capable of predicting low-risk versus combined intermediate- and high- risk drugs with 87% predictivity, the assays did not always correctly distinguish the low-risk from intermediate-risk drugs. Finally, the CiPA study relied solely on the assessment of acute effects at 30 min post drug addition, thereby missing any potential delayed effects on ion channels (e.g., hERG trafficking or PI-3-kinase inhibition by tyrosine kinase inhibitors)[41]. Some of these issues were improved in the JiCSA initiative where, for example, interoperator/interlab variability was reduced by collective training of users across the study[42,43].

Nevertheless, CiPA provided the necessary validation for the ICH assembly to endorse the formation of an implementation working group in November 2018 to begin the process of developing a questions and answers document for ICH-S7B[44–46]. The aim was to set out best practices for the design, conduct, analysis, interpretation, and reporting of these novel nonclinical assays, and it is expected that the first stage of this process will be completed in June 2020.

Assessment of changes in contractility using hiPSC-CMs

While there have been large-scale efforts to evaluate drug-induced changes to electrophysiology using hiPSC-CMs, similar initiatives on other aspects of cardiotoxicity have lagged behind. This is surprising because injury to the myocardium accounts for 30% of drug attrition associated with cardiovascular liability. Xenobiotic-induced CM injury (which can be direct or indirect *in vivo*) can occur through a variety of biochemical mechanisms and may be associated with alteration of CM function (contractility) and/or morphology[47,48]. The latter can manifest as an increase in cell size; disorganization or abnormal cytoplasmic constituents; loss of structural integrity; and, if irreversible, cell death. Assessing changes in contractility is recommended within the ICH-S7B guidelines.

Changes in cardiac contractility can be assessed clinically in altered left ventricular ejection fraction (LVEF)[49]. To assess this preclinically, assays typically involve *in vivo* studies, isolated whole-heart models[50], or ex vivo heart tissue and are limited in throughput. As such, there is an increased focus on the development of novel *in vitro* models with higher throughput to test for contractile liability of drugs early in the drug discovery program. However, translation of hiPSC-CM contractility to clinically relevant data is not always straightforward. Heart function is typically measured by LVEF or maximum rate of change of ventricular pressure (dP/dtmax), and drugs that increase either are categorized as positive inotropes[51]. However, the underlying cardiac myocyte function is described by changes in contraction force (inotropy), contraction time (clinotropy, time to peak contraction), relaxation time (lusitropy, time to relaxation), and chronotropy (beat rate).

Another consideration is the method used to define and measure contractility. While some hiPSC-CM-based approaches can calculate absolute force of contraction, most provide indirect measures by, for example, measurement of impedance, Ca2+ flux, sarcomere shortening, image-based contractile motion, and video-based cell geometry technologies. This is illustrated by Pointon et al.[52], who used a live-cell, fast kinetic fluorescent assay with a Ca2+-sensitive dye as a surrogate end point for cardiac contraction. A total of 31 inotropic and 20 noninotropic compounds were tested, wherein hiPSC-CMs provided a high-throughput system with sensitivity and specificity of 87% and 70%, respectively. However, the effect of the positive inotrope isoprenaline was not predicted correctly, and this was attributed to the negative force-frequency relation reported in the relatively immature monolayer of hiPSC-CMs.

Pointon and colleagues[51,53] performed two subsequent studies, which appeared to show that predictivity could be enhanced by using 3-D cardiac microtissues containing hiPSC-CMs, cardiac endothelial cells, and cardiac fibroblasts. Mechanistically, improvements in contractility were attributed to the higher expression of genes for calcium handling, myofilaments, and beta-adrenergic signaling but were not associated with morphological maturation and anisotropy. A bespoke image acquisition workflow and optical flow analysis method was used to test 29 inotropic and 13 noninotropic compounds. The microtissues provided a sensitivity and specificity of 80% and 91%, respectively, which included insight into the direction of the inotropic response. The second report assessed mitochondrial membrane potential, endoplasmic reticulum integrity, and cellular viability after microtissues had been treated with 29 FDA-approved structural or nonstructural cardiotoxins. They concluded that the approach was able to detect changes in cardiac structure at clinically relevant concentrations and provide insights into the phenotypic mechanisms of this liability. This tri-lineage system is now being used by AstraZeneca within their safety assessment pipeline.

Based on these reports, it is likely that the interaction and influence of endothelial cells and cardiac fibroblasts are required to detect certain forms of structural cardiotoxicity, such as a decrease in cardiac functionality caused by fibrosis-induced alterations in myocardial

compliance and contractility. In addition, cardiovascular effects might also originate from the autonomic nervous system or be driven by exposures to metabolites, suggesting that even cell types from different organs (e.g., hepatocytes) might be required to be incorporated in 3-D microtissues or organ-on- a-chip models for accurate cardiac safety assessment[54].

The InPulse CRACK-IT initiative

The need for improved methods for predicting changes in cardiac contractility was also recognized by a public-private partnership between the authors of this manuscript, a UK funding agency (National Centre for the Replacement, Refinement & Reduction of Animals in Research), and a pharmaceutical company (GSK). In 2014, this InPulse CRACK IT initiative aimed to develop medium-throughput technology platforms to measure contractility of hiPSC-CMs as a physiologically relevant functional output and to evaluate their utility in preclinical safety evaluation. Parallel or simultaneous measures for Ca2+ handling and/or voltage, with or without physiological loading where possible, were included as supplementary parameters to multiplex mechanistically relevant end points and thereby inform integrated decision-making.

Within CRACK IT, a training set of eight drugs with known cardiac effects was used to establish the protocols to be used on three platforms, all based on optical techniques: first, a bespoke triple transient measurement (TTM) system that produced an interlaced 1,000-frame/sec movie to measure contractility, Ca2+ handling, and electrophysiology in hiPSC-CMs cultured in 2-D monolayers in 96-well plates loaded with appropriate dyes[55]; second, a commercial CellOPTIQ® system to measure contractility, via bright field images, and electrophysiology, via voltage-sensitive dyes, in hiPSC-CMs cultured in 2-D monolayers in 96-well plates[56]; and third, an engineered heart tissue (EHT) platform, which uses 3-D constructs fabricated from hiPSC-CMs cast within a fibrin hydrogel and multiplexed in 24-well plates[57]. Two polydimethylsiloxane posts at each end of the EHT provide the loading that causes anisotropy in the construct, but the posts also allow absolute force of contraction to be calculated by video-optical recording of the extent of their deflection upon each beat. Ca2+ handling was assessed by transgenic incorporation of a fast variant of the genetically encoded calcium indicator (GECI-GCAMP6f) during EHT fabrication. Finally, as a comparator, CMs isolated from adult rabbit hearts were assessed for contractility and electrophysiology on the CellOPTIQ® platform. To simplify quantification of contractility, we developed and produced an algorithm called MUSCLEMOTION[55].

This prework enabled a second phase of CRACK IT, which involved blinded testing of up to 28 drugs known to be positive or negative inotropes or to have no effect on heart function. Analysis of the inotropic, clinotropic, and lusitropic responses for each platform-drug combination allowed the consortium to assign an overall prediction for each drug as a positive or negative inotrope or as having no effect. The first nine drugs were tested in adult rabbit CMs as a comparator, achieving a predictivity score of 67%, which was similar to that seen for hiPSC-

CMs on 2-D (44–78%) or 3-D (67%) platforms. High predictivity in the 2-D TTM system may be due to simultaneously quantifying multiple dynamic parameters in hiPSC-CMs (contractility, voltage, and Ca2+ handling). Across the full drug panel, the highest predictivity was from the 3-D platform (81%), which also appeared to have a high level of sensitivity; thus, detection of cardioactive drugs occurred close to the known effective therapeutic concentration. This was likely facilitated by the ability to measure contraction force on the 3-D EHT system instead of cell movement (a surrogate of contractility) on the 2-D platforms.

Refinement to test conditions was addressed after blinded testing. Inclusion of B27 supplement as a protein source improved the signal-to-noise ratio in 2-D systems relative to when serum-free conditions were used. In both 2-D and 3-D, assessing function at 30 min and 24 h enabled detection of immediate and longer-term effects, such as for the anticancer drug doxorubicin.

A particular challenge was the correct detection of positive inotropes, which requires a higher degree of maturation via, for example, enhanced myofilament organization and calcium handling. Two adaptations were made that were beneficial. First, spontaneous beat rates exceeding 1.5 Hz were reduced by the addition of ivabradine, an If-current inhibitor. Lower beating rate revealed not only a positive force-frequency relationship (up to ~2 Hz, but also stronger inotropic effects of most positive inotropes, as shown previously in native heart muscle preparations paced at different rates[58]. Second, the respective drug was applied as a high-concentration bolus. Under these conditions, correct assignment for the drug sets led to an accuracy of up to 93% in the 3-D platform, which is also consistent with the higher level of developmental maturity previously reported for EHTs[57,59,60].

Currently, there are no guidelines or suggested standardized drug sets that specifically and individually challenge each of the most important mechanisms of action affecting contractility and can thus serve as a reference for hiPSC-CM-based contraction assays. In general, contraction can be influenced by a change in cAMP or calcium concentration or by increasing or decreasing the binding affinity of the contractile mechanism. During the CRACK IT project, different drug sets were investigated, and the mechanism of action often turned out to be more complex than expected. For instance, the positive inotrope digoxin also caused negative chronotropic effects and acted on the electrical activity of CMs. This finally led us to a set of reference drugs, of which each has a specific mechanism of action affecting contractility (Table 1). Other investigators who wish to test the predictivity of their hiPSC-CM-based platforms should find this useful. This includes standard drugs, which are usually detected in hiPSC-CM-based assays, such as nifedipine and BayK 8644, and also more challenging drugs such as milrinone, which preferentially acts on the PDE3 pathway[51,61–63] to produce its inotropic effects by modulating cAMP levels. Potential misidentification of milrinone by hiPSC-CM models might be due to low basal receptor tone, resulting in low cAMP levels in hiPSC-CMs[62] and a predominance of PDE4 over PDE3 in hiPSC-CMs[61]. Similarly, hiPSC-CM models may have relatively immature sarcoplasmic reticulum calcium-handling properties[62]. Therefore,

it would be useful to test for positive inotropy induced by the sarco/endoplasmic reticulum Ca2+-ATPase activator CDN1163[64,65].

It is noteworthy that the misidentification of force-modulating drugs might not be solely related to cell type, maturity, or 2-D versus 3-D configuration but rather to a combination of factors that also includes drug incubation time, application method (bolus versus cumulative concentration), stimulation rate, and read-out (e.g., Ca2+ flux versus impedance or capturing multiple parameters simultaneously). The importance of external Ca2+ concentration cannot be overemphasized. At an external Ca2+ concentration of >2 mM, no positive inotropic effects can be expected because hiPSC-CMs (in 3-D) reach their maximal contractile force at ~2 mM[61].

Driven by the successes of the various initiatives above, the need to focus on late-stage structural cardiotoxicity is now being recognized. In particular, the Cardiac Stem Cell Working Group within the US Health and Environmental Sciences Institute has voiced interest in moving in this direction and has started working on a new initiative: The Use of Human Induced Pluripotent Stem–Cell Derived Cardiomyocytes (hiPSC-CMs) to Reliably Assess Acute and Subchronic Cardiotoxicity *In Vitro*. This multidisciplinary project will focus on two issues currently hampering the widespread adoption of hiPSC-CM-based assays: (a) the maturation status of hiPSC- CMs and (b) the lack of rigorous investigation of whether subchronic studies can accurately reflect a range of cardiotoxic effects using phenotypical or biomarker indicators. The ultimate goal is to inform the scientific community and regulatory affairs on using hiPSC-CMs (characterized according to different levels of complexity and maturity) for evaluating (subchronic) cardiotoxicity.

Table 1. Suggested set of 12 drugs with varying modes of action to probe the core pathways that govern cardiomyocyte contraction.

Class	Drug	Predominant mechanism of action in cardiomyocytes
Positive inotropes	Isoprenaline	Non-selective ß adrenergic agonist
	Levosimendan	Calcium sensitizer
	Milrinone	Phosphodiesterase (PDE) III inhibitor
	Bay K 8644	L-type Ca^{2+} channel activator
	Omecamtiv mecarbil	Myosin activator
	CDN1163	SERCA activator
	Ouabain	Na^{+}/K^{+}-ATPase inhibitor
Negative inotropes	Doxorubicin	Impairs Ca^{2+} transport mechanisms in sarcoplasmic reticulum
	Nifedipine	L-type Ca^{2+} channel blocker
	Blebbistatin	Myosin inhibitor
	Sorafenib	Multi tyrosine kinase inhibitor
No effect	Aspirin	n/a

Are we there yet: are hiPSC-CM technologies fully fit-for-purpose?

The validation experiments above show that integration of hiPSC-CMs into various technology platforms is rapidly becoming validated as systems for biomedical discovery and drug evaluation. Nevertheless, as with any model, there are strengths and weaknesses, and there is no single, one- size-fits-all system. Some of the key issues are considered in the sections below, with Table 2 highlighting specific compromises needed between platforms.

Maturity versus complexity

Perhaps the most common question on whether hiPSC-CM technologies are fit for purpose is related to maturity. hiPSC-CMs undergo a finite level of developmental progression and are distinct from their adult counterparts, and this is true of any lineage produced from hiPSCs. For hiPSC- CMs, underdevelopment is recognized by deficiencies in structure, signaling, metabolism, and function, but consensus is emerging on best-in-class approaches to mature hiPSC-CMs (reviewed in 2). Benefits are seen by using T3 hormone, metabolic maturation, 3-D constructs, mechanical load, electrical training, long-term culturing, and auxiliary cell types such as cardiac fibroblasts and cardiac vascular endothelial cells, with combined approaches appearing to be synergistic for maturity.

Various groups have adopted combined approaches, including electrical pacing in 3-D tissues placed under load. In one example[65], cardiac tissues formed from hiPSC-CMs were subjected to intensity training, wherein electrical pacing frequency was increased from 2 Hz to 6 Hz by 0.33 Hz per day over a 2–3-week period. Progression toward features of adult CMs was apparent through various assessments, including physiological responses to isoproterenol and recapitulating pathological hypertrophy. Nevertheless, pacing human cardiac tissue to rates of 6 Hz (360 beats/min) is not physiological. Partial evidence for putative primitive t-tubules was observed, and these structures did not develop to the stage of maturity seen in the adult human myocardium, nor did the electromechanical properties.

The above study built on earlier work using engineered biowire tissues, which are now providing the stage for next-generation systems. These include elegantly engineered instrumented bioreactors with ventricular-assist capabilities[66] as well as commercial offerings (Table 2) such as Biowire II (TARA Biosystems) and Heart-in-a-Jar (Novoheart)[67]. With Biowire II[68], electrical conditioning appeared to improve sarcomeric organization and promote expression of maturation genes associated with contraction, Ca^{2+} handling, electrical properties, and lipid metabolism. Positive inotropy for one of our suggested reference drugs, milrinone, was also detected. The Heart-in-a-Jar concept is particularly intriguing, as it embeds ventricular-like subtype hiPSC-CMs in collagen-based extracellular matrix hydrogel to create a miniature 3-D, engineered, electromechanically coupled cardiac organoid chamber that is capable of ejecting fluid to mimic pumping action similar to a natural heart, potentially with the ability to vary preload and afterload. This enables clinically relevant functional readouts such as pressure-

volume loop analysis, developed pressure, cardiac output, and ejection fraction. Nevertheless, even the most advanced *in vitro* systems cannot (yet) recapitulate the cellular heterogeneity or structural complexity of the intact heart, where electrocompetent fibroblasts, conducting tissue, innervation, and multidirectional muscle layers serve important functional roles.

There are also outstanding questions regarding the impact of electrical stimulation–induced maturation on both 2-D and 3-D systems. Not only does a 3-week pacing protocol add time and cost to the model, but our submitted work indicates that chronic tachypacing can induce vulnerability to arrhythmias. Applying electrical stimulation may also cause artifacts that interfere with the original signals (e.g., Ca^{2+} fluorescent motion), making it very challenging to analyze the data. In order to reduce movement artifacts, Ronaldson-Bouchard et al.[65] pretreated their cardiac tissues with 5 μM blebbistatin before performing calcium-handling measurements. It may be questionable if and how that influences the eventual pharmacological responses of the tissues to test compounds, and alternative approaches to mitigate motion artifacts may be preferable, such as ratiometric approaches (di-4-ANNEPS for voltage and Fura-2 for Ca^{2+})[69].

Regarding the duration of maturity beyond cessation of the pacing stimulus, anecdotal data suggest that, at least in 2-D monolayers paced for 3 weeks, the hiPSC-CMs revert rapidly (i.e., within 12–24 h) to the immature phenotype; more comprehensive analysis is needed. Similarly, it is not clear how much the apparent improvement in maturity relates to real advances in functionality and predictivity of the model relative to the benefits seen simply by slowing beat rate, which is known to induce positive force-frequency relationships[70,71].

The balance between complexity, throughput, and cost is an important aspect of any model system. Although enticing, many labs struggle to work simultaneously with more than a few organ-on-a-chip devices. Recognizing these issues, some groups have elected to combine multiple cell types into microspheroids, specifically hiPSC-derived endothelial cells and hiPSC-CMs[72]. This has the advantage of only requiring 5,000 cells, with up to 5,000 spheroids being made in each experiment by just one operator. This also makes screening inexpensive[72]. Importantly, the hiPSC-CMs in these microtissues are significantly more mature than in regular 2-D culture when assessed by various structural, functional, and gene expression parameters. Others have also used similar aggregates (or cardiac organoids) containing mixed cardiac and non-CM populations[51,53,73], including hiPSC-derived epicardial cells[94]. These approaches can be combined with simplified measures of contractility, such as using a GoPro video camera to record beating hiPSC-CMs, which can then be analyzed via MUSCLEMOTION[55] freeware, thus making the technology widely available.

Table 2. Comparison of key features between 2D and 3D hiPSC-CM based platforms. Scoring shows "++++" as highly favourable grading to "-" as less desirable. Platforms that require high cell number often associate with higher complexity and potential for greater mechanistic understanding but throughput may be limited by cost. Abbreviations: EHT, engineered heart tissue; PDMS, polydimethylsiloxane.

hiPSC-CM technology	Format	Substrate complexity		Protocol complexity		Typical cell number required		Clinically relevant readout	
Monolayers	2D	++++	Matrix coating (e.g. fibronectin)	++++	Direct seeding of hiPSC-CMs with/without other cell types	+++	3 x 10^4 / well	-	Twitch movement
Micro-spheroids	3D	+++	Self-assembly in standard culture plate	+++	Mixing different cell types	++++	5 x 10^3 / spheroid	-	Fractional shortening or isotonic contraction
EHT	3D	++	Engineered PDMS substrate in culture plate	++	Mixing different cell types in hydrogel	++	1 x 10^6 / construct	++	Twitch tension or auxotonic contraction
Biowire II	3D	+	Engineered PDMS substrate in culture plate	-	Mixing different cell types in hydrogel; electrical pacing for 3 weeks	+	2 x 10^6 / construct	++	Twitch tension or auxotonic contraction
Heart-in-jar	3D	-	Organoid chamber	+	Mixing different cell types in 3D shaped hydrogel	-	1 x 10^7 / construct	++++	Developed pressure / ejection fraction

The spheroid approach potentially simplifies the addition of other auxiliary cell types such as neural lineages to enable evaluation of drugs that work via the neurohormonal system. For instance, in the CRACK IT work described above, the functional consequences of clonidine were predicted incorrectly. This was because no adrenergic neurons were present and the mode of action for clonidine is through alpha-adrenoceptors in the brain stem, with consequences that include reduced heart rate. Similarly, connection of primary liver cells to hiPSC-CMs within microfluidic human-on-a-chip systems has the potential to investigate the effect of hepatic metabolism on off-target cardiotoxicity[54]. This is the case for the prodrug dolasetron, a selective serotonin receptor antagonist that is metabolized by the liver into its active form, hydrodolasteron, which may block sodium channels and cause electrophysiological changes in the heart. Finally, engineering vascular networks into hiPSC-CM-derived constructs would allow for the investigation of drug effect on hemodynamics, an area for which there are currently no satisfactory *in vitro* models.

Consistency, quality, and cost

Whichever technology is adopted, there are still many issues and unanswered questions. Often, published protocols fail to translate between labs. Differences in the (epi)genetic background are intrinsic to the biology of hiPSC-CMs and contribute to both the strength and weakness of this model system. No simple explanation has been found for differences between lines. The differences may be due to minor genetic changes that occur during the reprogramming process itself. Krijger et al.[74] reported that the genome topology dynamics changed massively during somatic cell reprogramming, establishing an embryonic stem cell–like 3-D genome, irrespective of the somatic cell source. However, they found that early-passage hiPSCs carry topological hallmarks that enable recognition of their cell of origin.

Parameters that could be improved by the unification of protocols include culture, differentiation, and cryopreservation, while a standardized set of quality control criteria would improve reproducibility and signal-to-noise ratios. In this regard, commercial cell lines offer an advantage because they are produced to good laboratory practice standards and subjected to considerable quality control, although there is still batch-to-batch variation, and the number of genotypes offered is still very limited. Only recently did Cellular Dynamics International expand its portfolio to include hiPSC-CMs that carry genetic mutations associated with HCM (e.g., MyCell MYH7 with mutation-causing R403Q change). Also, Ncardia now offers a hiPSC-CM-based screening service for novel drug candidates against diseases that include HCM *(Figure 3)*. This means that there are opportunities for personalized medicine in commercial and noncommercial laboratories, although it should be noted that a strict code of conduct is required to gain appropriate informed consent, licenses, and so on.

An additional consideration is cost. For most vendors, the list price (as of 2019) is approximately $1,000 per million hiPSC-CMs, and each Heart-in-a-Jar uses up to 10 million cells, thus limiting this technology to questions with high-value returns (Table 2). Ncardia now offers hiPSC-CMs in preseeded, quality-controlled, and ready-to-use assay plates (CardioPlate™) for a variety

of different commercial platforms. The cost of such assays will be relatively high, since they combine specialized plates with commercial hiPSC-CMs, but successful implementation of such resources into predictive and translational systems could be offset by reducing costs at later stages in drug development.

This said, continual improvements are being made in production methods for hiPSC-CMs, with an expectation that costs will be decreased by both technology development (e.g., bioreactors) and economies of scale. Looking forward, the amplification of cells at multiple stages of the differentiation process may be helpful. Complex but elegant approaches have been developed to differentiate hiPSCs toward cardiovascular progenitor cells, which could be isolated and proliferated for over 40 population doublings, while also being able to switch conditions to produce pacemaker, atrial, and ventricular CMs[75]. Unpublished data suggest that modulation of WNT signaling can temporarily inhibit the transition of proliferating cardiovascular progenitors into quiescent CMs, which can be exploited to further expand hiPSC-CM populations by a hundred-fold. Similarly, it may be possible to induce limited proliferation of differentiated hiPSC-CMs, surprisingly, by ion channel modulators[76]. A task for future studies will be to confirm the functionality of the expanded populations of hiPSC-CMs.

Selection of platform is based on needs and the biology question being asked

As technology offerings progress, there will be a need for guidance on what the attributes of each platform are and how they might be best used. This can be a complex issue and is best illustrated by considering two platforms: the xCELLigence® RTCA CardioECR and CellOPTIQ®. These platforms are designed to use hiPSC-CMs at medium throughput (i.e., hundreds of assays a day), but they exemplify two distinct approaches to recording electrical and mechanical activity. The RTCA CardioECR is an example of a solid-state device that measures extracellular voltage (via MEA) and/or resistance (via impedance)[77]. The CellOPTIQ® is an example of an optical device that uses fluorescent dyes to measure transmembrane voltage and/or image analysis to measure contractility[69,78]. Both have their strengths and weaknesses, and the choice of approach depends critically on the experimental questions and constraints.

Solid-state devices are capable of very high sampling rates and can measure continuously for indefinite periods, limited only by data storage. This makes it relatively easy to achieve long-term assessment of contractility, which can be useful for studying processes such as chronic drug toxicity and disease or developmental progression. The integration of electrodes also means that electrical stimulation can be performed while maintaining sterility and cell viability; this is a challenge for optical systems, which rely on external electrodes. Optogenetic stimulation may be used on optical platforms to circumvent this issue, provided any effects of light-induced cell damage can be avoided.

Nevertheless, the specialist requirements of solid-state platforms, such as the RTCA CardioECR, mean that each 48-well plate costs about £300 (based on 2019 list prices),

considerably more than for optical platforms, which are usually compatible standard tissue culture plates (about £1 per 96-well plate). Solid-state platforms cannot be applied routinely to single isolated cells or discrete microtissues. This is in contrast to optical platforms, which can use these cell configurations in addition to cell sheets. Solid-state devices use one site per well for indirect measurement of electrical and contractile events by assessing extracellular voltage and impedance changes, which can be a limitation, especially if cell coverage is patchy or if cell-electrode contact is poor. Also, plates compatible with solid-state devices tend to have a small viewing area, which limits approaches based on high-content imaging.

Optical methods yield more direct measures of transmembrane potential and cell contraction, but voltage-sensitive dyes are currently problematic for continuous measurements, which necessitates protocols that involve intermittent, short-duration (30–60 sec) measurements. In addition, chemical probes for voltage analysis can cause undesirable side effects on cell function and are not compatible with some media (e.g., poor signal-to-noise ratios in certain protein-containing media). Care also needs to be taken with chemical probes to measure Ca2+ handling, though it is noteworthy that this parameter is easily integrated into optical platforms but less so into solid-state platforms.

Unlike 3-D EHTs, neither solid-state nor optical platforms can measure absolute force of contraction, which may make the detection of positive inotropic effects more challenging (see above discussion). Another consideration is how changes in cell-substrate adhesion can give the impression of increased amplitude of contraction because the cells move more freely due to fewer anchor points with the substratum. We have seen this in our own work examining cardiotoxicity induced by the anticancer drug doxorubicin over a 24-h time course. Relative to baseline, contraction amplitude increased initially but then decreased at later stages. Artifacts can be mitigated on the RTCA CardioECR by combining amplitude and cell health outputs, which show that cell death is occurring.

Auxiliary solutions may be needed to complement hiPSC-CM platforms

The discussions above show that increasing maturation of hiPSC-CMs is possible and that important considerations include the appropriate technology platform, the biological question, the throughput needed, and the price per data point. Currently, there are no single, one-size-fits-all platforms and no fully matured hiPSC-CMs in culture; these may not be realistic expectations. The pharmaceutical industry already uses multiple assays to build a picture of efficacy and safety, and so the same is likely to be true for hiPSC-CMs. For example, cardiotoxic effects of tyrosine kinase inhibitors have been evaluated[79] using hiPSC-CMs in a high-throughput 2-D platform for provisional screening, followed by a 3-D platform to seek more detailed mechanistic insights. Alternatively, Gong & Sobie[80] used compensatory in silico approaches. Action potentials and calcium transients were evaluated in hiPSC-CMs or in adult rabbit or guinea pig CMs to determine whether there were measurable differences across 13 ion channels, pumps, and transporters that are common to both models. Corrective algorithms were then created to partially compensate for deficiencies in the hiPSC-CM system.

This hybrid approach achieved enhanced predictivity of a panel of 30 drugs and incorporated an estimate of the impact of genetic changes associated with heart failure. Collectively, this suggests that it will be advantageous to use best-in-class, quality-controlled, hiPSC-CM platforms with sequential testing and intelligent compensatory algorithms to identify the most informative parameters, risk scores, and assessments of pathogenesis.

Where could hiPSC-CMs take us next?

As a technology, hiPSC-CMs are becoming valuable for drug discovery, safety evaluation, and both drug and patient risk stratification. They have been used to inform decisions on advancing drugs into phase I testing[81] and in developing suitable treatment regimens for patients with complex LQTS, furthering the aim of personalized medicine[82]. More recently, hiPSC-CMs have been used in functional assessment of the cardiotoxicity of cosmetic compounds[83], showing that the utility of these cells is expanding to new fields.

Future use of hiPSC-CMs in other areas of pharmacological testing is expected, for example, in defining pharmacokinetic (PK) and pharmacodynamic relationships to mimic the PK profile seen *in vivo* or in assessing effects of chronic exposure and structural toxicity. Compounds such as casopitant require long exposure to manifest a cardiotoxic phenotype[84], so it is conceivable that suitable experimental designs will address pharmacological/toxicological effects as a function of concentration, time, and tissue levels, thereby improving translation relevance to the clinic. Defining structural toxicity is also an area of need. It is possible to have functional changes that do not result in damage to tissues, and these may even be beneficial depending upon the nature and extent of the change and the patient context, for example, negative inotropes (e.g., L-type calcium channel blockers) used for positive therapeutic benefit in hypertension. Therefore, when testing compounds with no known effects, it may be important to incorporate end points[53] that reflect structural or metabolic changes (e.g., mitochondrial membrane potential, sarcomeric organization, endoplasmic reticulum integrity) and biomarker release into culture medium (e.g., cardiac troponin, fatty acid–binding protein-3, myocardial isoform of creatine kinase). Indeed, an evaluation of four anticancer tyrosine kinase inhibitors showed that sorafenib caused a profound defect in mitochondrial metabolism[85], with the authors suggesting that these cells can also be used for biomarker discovery.

Subtleties in functional consequences are not always obvious in hiPSC-CMs harboring heterozygous mutations, which could limit the ability to risk stratify or treat patients from whom hiPSCs have been derived. An interesting approach to this is being adopted by Joseph C. Wu's lab at Stanford. Biopsies from patients with heterozygous mutations in genes associated with LQTS are being converted into hiPSCs. The healthy allele is then disrupted by CRISPR-mediated targeting to create hemizygous cells, wherein only the mutant allele is expressed. This approach appears to unveil the impact of a particular mutation within the context of the patient's genome, where different modifier genes or other mutations

can change the penetrance of the condition. The data produced are used to risk stratify or determine treatment strategy in a completely patient-specific manner[86]. This notion extends to drug-induced cardiotoxicity. Human iPSC-CMs de-rived from breast cancer patients with known susceptibility to doxorubicin-induced cardiotoxicity were consistently more sensitive to doxorubicin than hiPSC-CMs from nonsusceptible patients, with decreased cell viability, impaired mitochondrial and metabolic function, impaired calcium handling, decreased antioxidant pathway activity, and increased reactive oxygen species production[87]. Work by the same group showed that hiPSC-CMs could be used in transcriptome-based toxicology analysis to predict and risk stratify patient-specific susceptibility to cardiotoxicity[88].

By extension, hiPSC technologies are also likely to be used to develop new medicines for cardiovascular disease, including by repurposing existing drugs currently used for other indications. There is evidence that this can work from other areas of human stem cell biology. Studies with hESCs carrying the causal mutation of myotonic dystrophy (DM1) were used to show that metformin, a drug usually used to treat diabetic patients, could correct defective alternative splicing. This led to a phase I clinical trial that showed that repurposing metformin in DM1 patients led to improved mobility[89].

Another key area for the use of hiPSC-CM technologies could be in assisting in the assignation of ADRs and black box warnings. Of the top 200 prescribed drugs, 82 carry ADRs[17]. Additional complexity is added by contraindications with other drugs or disease states. For example, the antifungal agent ketoconazole is cardiotoxic if used with terfenadine because it strongly increases its plasma concentration. Similarly, the use of the anticancer tyrosine kinase inhibitor sunitinib could be of concern in patients with preexisting impairment to heart function arising from, e.g., HCM. It is therefore conceivable that, in the future, large panels of genotypes could be captured by reprogramming patient cells (patients in a dish) or by Cas9/CRISPR engineering (disease genotypes in a dish), with hiPSC-CM-based assays then being used to assist in evaluating off-target drug risk (*Figure 2*). Progressing this concept further would allow hiPSC derivatives to be used for *in vitro* clinical trials. Careful selection of panels of hiPSC lines could provide a resource that reflects the patient population more accurately than current clinical trials, where numbers of participants are low and inclusion of individuals who have comorbidities is rare. It is therefore encouraging that various large-scale banking initiatives have been undertaken to create many thousands of healthy and diseased hiPSC lines, including by the Human Induced Pluripotent Stem Cells Initiative, StemBANCC/IMI, California Institute for Regenerative Medicine, New York Stem Cell Foundation, and the Precise Medicine Initiative[27,90].

An area of active investigation is the use of hiPSC-CMs in identifying new pathogenic loci and modifier genes, which could be used as diagnostics or therapeutic targets. It is well established that, in many diseases, the impact of the primary mutation on pathophysiology differs between patients[91]. We derived hiPSC-CMs from a family harboring a mutation known to cause HCM but found that the phenotype differed considerably between individuals. Cas9/CRISPR gene

correction in hiPSC-CMs from the father, the most severely affected family member, only allowed partial rescue of the phenotype[21]. Conversely, introduction of the mutation into hiPSC-CMs from the healthy son caused limited phenotypic change. This indicates that additional mutations or gene modifiers were involved, which is consistent with anecdotal evidence from large-scale genetic screening programs that predicted that HCM should occur in 1:200 people, yet the condition presents clinically in only 1:500. The concept of combining hiPSC-CM and Cas9/CRISPR technologies to show whether variants of uncertain significance are pathogenic has now been established. Clinical genetic testing has emerged as the standard of care to identify genetic variants in patients suspected of having LQTS. Phenotypic analysis of hiPSC-CMs harboring a T983I substitution in the KCNH2 gene (LQTS2) showed a higher proarrhythmia risk than isogenic cells in which the mutation had been corrected, and hence the authors suggested that this variant should be classed as potentially pathogenic[92]. Such studies pave the way to deciphering the impact of variants identified through genome-wide association studies.

Conclusions and perspectives

hiPSC-CMs are unequivocally becoming an established tool in biomedicine. This is reflected by a recent report from BCC Research[93], which estimated the 2018 value of hiPSC-CMs to be $1.3 billion and growing at a compounded annual growth rate of 20.3%. Exemplars are in the areas of discovery, safety, diagnosis, risk stratification, and mechanistic insight. Nevertheless, expecting hiPSC-CM systems to be perfect is unrealistic because a model is, by definition, a simplification of reality. It is important to play to the strengths and adapt to the limitations, particularly incomplete maturation. Approaches that couple advanced hiPSC-CM multicellular gene-edited systems with smart corrective algorithms and risk scorecards are likely to be productive. There is also a balance to be achieved between complexity, throughput biology, and price per data point. Currently, simpler 2-D and 3-D systems can be used in (high-throughput) primary screens and discovery work, while complex organ-or human-on-a-chip technologies can give greater insight into detailed mechanisms. Deciphering the functional consequences of novel variants and modifier genes to guide patient risk stratification and treatment is challenging because correcting polymorphic variants is a costly, labor-intensive process that takes time, and the same is true for high-resolution phenotyping. Nevertheless, the first tantalizing studies in this space are now appearing. Advancing this further will require international collaboration between regulatory agencies, academia, and industry, and so it is encouraging to see the level of cooperation that has occurred via initiatives such as CiPA, JiCSA, and CRACK IT.

Disclosure statement

The following interests are declared as factors that might be viewed as biasing this review: T.d.K. is an employee of Ncardia, and some of the content in the section titled Where Could hiPSC-CMs Take Us Next? describes company product development; B.J.v.M. and C.L.M.

are codevelopers of the Triple Transient Measurement System; G.L.S. is cofounder and chief scientific officer of Clyde Biosciences; P.C. is an employee of GlaxoSmithKline Research & Development; and T.E. and A.H. are cofounders of EHT Technologies, and A.H. is chief executive officer.

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