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Perspective

Strengthening cardiac therapy pipelines using human pluripotent stem cell-derived cardiomyocytes

Kavita Raniga,[1,4,*] Aishah Nasir,[1] Nguyen T.N. Vo,[1] Ravi Vaidyanathan,[2] Sarah Dickerson,[2] Simon Hilcove,[2] Diogo Mosqueira,[1] Gary R. Mirams,[3] Peter Clements,[4] Ryan Hicks,[5,6] Amy Pointon,[7] Will Stebbeds,[8] Jo Francis,[9] and Chris Denning[1,*]

[1]The Biodiscovery Institute, University of Nottingham, Nottingham NG7 2RD, UK
[2]FUJIFILM Cellular Dynamics, Inc., Madison, WI, USA
[3]Centre for Mathematical Medicine & Biology, School of Mathematical Sciences, University of Nottingham, Nottingham NG7 2RD, UK
[4]Pathology, Non-Clinical Safety, GlaxoSmithKline R&D, Stevenage SG1 2NY, UK
[5]BioPharmaceuticals R&D Cell Therapy Department, Research and Early Development, Cardiovascular, Renal, and Metabolism (CVRM), BioPharmaceuticals R&D, AstraZeneca, Gothenburg, Sweden
[6]School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London WC2R 2LS, UK
[7]Safety Sciences, Clinical Pharmacology and Safety Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge CB2 0AA, UK
[8]LifeArc, Stevenage SG1 2FX, UK
[9]Mechanistic Biology and Profiling, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge CB2 0AA, UK
*Correspondence: kh.raniga@gmail.com (K.R.), chris.denning@nottingham.ac.uk (C.D.)
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SUMMARY

Advances in hiPSC isolation and reprogramming and hPSC-CM differentiation have prompted their therapeutic application and utilization for evaluating potential cardiovascular safety liabilities. In this perspective, we showcase key efforts toward the large-scale production of hiPSC-CMs, implementation of hiPSC-CMs in industry settings, and recent clinical applications of this technology. The key observations are a need for traceable gender and ethnically diverse hiPSC lines, approaches to reduce cost of scale-up, accessible clinical trial datasets, and transparent guidelines surrounding the safety and efficacy of hiPSC-based therapies.

INTRODUCTION

Developments in human-induced pluripotent stem cell-derived cardiomyocyte (hPSC-CM) differentiation and maturation protocols, coupled with the advancement of more physiologically relevant *in vitro* model systems, have provided a powerful tool for exploring cardiac liabilities, studying cardiogenesis and cardiovascular diseases, and assessing potential transplantation therapies. In this perspective, we showcase key efforts toward the production of research- and clinical-grade hPSC-CMs, implementation of hPSC-CMs in industry settings, and recent clinical applications of this technology. By exploring efforts to harmonize the use of hPSC-CMs, we review areas where standards are needed throughout the process from model development to clinical application.

The production of high-quality stem cell-derived cardiomyocytes

The development of the first human embryonic stem cell (hESC) lines in 1998 and hiPSC lines in 2007, and our ability to control their definitive differentiated state, has prompted the cardiovascular field to evolve at an unprecedented speed.[1,2] Advancements are evident in several key areas, including cellular reprogramming techniques, the differentiation of pluripotent stem cells into cardiovascular cell types, as well as the integration of gene-editing tools in hiPSC-based model systems.

Research into reprogramming techniques has advanced significantly in the last decade. Human chemically induced pluripotent stem cells (hciPSCs) provide a promising path for establishing the groundwork of cell-based therapies. The underlying technology uses small molecules to regulate cell fate with more flexibility and fine-tuning without the need for genetic manipulation and biological materials such as transcription factors.[3,4] Expanded potential stem cells (EPSCs) exhibit unique developmental potency for both embryonic and extra-embryonic cell lineages, a characteristic not found in conventional hESCs and hiPSCs,[5–7] making them a valuable platform for studying embryonic development and developmental disorders. Both hciPSCs and EPSCs can serve as novel sources for hiPSC-CM production, simplifying optimization, standardization, and manufacturing.

Building on this, recent papers highlight the value of using expandable and induced cardiovascular progenitor cells (iCPCs) as a starting material to restore cardiac function and obtain terminally differentiated cardiovascular cell types.[8–11] Notably, hPSC-derived human ventricular progenitors (HVPs) have demonstrated the ability to expand and self-assemble into functional ventricular-like myocardium *in vivo*.[12] Unlike differentiated CMs, further characterization revealed that HVPs harbor the unique capacity to sense and migrate toward the injured myocardium for repair.[13,14] With their self-renewal potential, preserved progenitor phenotype, and tri-lineage-restricted differentiation (cardiomyocytes, endothelial cells, and smooth muscle cells), CPCs are valuable for drug screening, disease modeling, and cardiac regenerative therapies.

For discovery purposes, having control over donor selection based on specific genetic backgrounds or disease mutations, coupled with the utilization of genome-editing tools, such as CRISPR-Cas9, offers a distinct advantage in elucidating the genetic causation of cardiac-inherited disorders. This is especially

true when interrogating common and rare genetic variants across diverse genetic backgrounds.[15–20] Although this level of customization allows for highly targeted studies, it also presents challenges. Differentiation protocols, which span multiple weeks, require both time and technical expertise for assessing off-target effects and ensuring the reproducibility of genotype-phenotype correlations. This holds particular significance for cardiovascular diseases, given their intricate nature as multifaceted, multi-organ systemic disorders characterized by clinical genetic and phenotypic heterogeneity. Conditions such as pulmonary arterial hypertension and hypertrophic cardiomyopathy exemplify this complexity.

In the process of identifying potential drug safety liabilities during early drug discovery, where the priority may not be on donor-matched or gene-edited hiPSC lines, commercially sourced hiPSC-CMs now offer a blend of accessibility, scalability, quality control, and regulatory compliance. These cells are now predominantly integrated into routine cardiac safety assessments during early drug discovery, marking a departure from *ex vivo*-isolated cardiomyocytes or tissue assays sourced from preclinical species.[21–24] Therefore, several investments have been made to develop commercially accessible stem cell banks (Table 1). Challenges around their scale-up, reproducibility, donor diversity, and cost of commercial products can limit their widespread use in both academic institutions and biopharmaceutical companies.

Although challenges remain, it is crucial to garner insights from the various sectors engaged in this field to understand the ongoing endeavors and obstacles. Here, we present viewpoints from a leading stem cell provider, two major pharmaceutical companies, and academic groups working in this field. This exercise reveals several challenges in the field: (1) insufficient traceability for hiPSC lines, especially for donor specifics. (2) High costs and issues with reproducibility impede scale-up for many groups and companies. (3) Suboptimal data sharing and transparent guidelines pertaining to the safety and efficacy of hiPSC-based therapies. Although the focus of the review was on the cardiovascular system, many opportunities and challenges mirror across to other disease models and translation to therapy, such as liver and neurodegenerative diseases.

Commercial hiPSC-CM production: A perspective from FUJIFILM Cellular Dynamics International

In this section, we introduce a perspective from FUJIFILM Cellular Dynamics International (FCDI), a global leader in the production of hiPSC-derived cells, including cardiomyocytes. The hiPSC-CMs (iCell Cardiomyocytes[2]) have been validated in a landmark Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative and are widely published and utilized.[25–31]

This case study outlines the steps required to generate hiPSC-CMs from donor sample acquisition through hiPSC reprogramming, hiPSC banking, and quality control (QC) processes to large-scale manufacture of the hiPSC-CMs and QC of the final product. The criteria and guidelines for each step highlight those broadly accepted in the field while also integrating FCDI's experience and expertise in hiPSC-CM production.[32]

Donor selection and reprogramming guidelines

The utility of hiPSCs derived for clinical and non-clinical use often hinges on the identification of appropriate starting material for reprogramming procedures. Allogeneic and research tool somatic cell donations must pass through a rigorous "Incoming Material Qualification" (iMQ) and suitability assessment before the projects and products progress. Following broadly accepted guidelines published by registries (e.g., Human Pluripotent Stem Cell Registry, hPSCReg), regulatory bodies (e.g., United States Food and Drug Administration, US FDA), and professional organizations (e.g., International Society for Stem Cell Research, ISSCR[33,34]), FCDI uses well-established iMQ procedures for all incoming somatic cell donations (donor cells or donor material).

The suitability of donor material is evaluated based on multiple factors, including robust provenance and ethical documentation, such as medical history, consent, and material transfer agreement (MTA). The consent must explicitly permit hiPSC generation, genomic characterization, and data sharing while excluding the retrieval of derived materials. Additional critical donor material attributes include a pathogen-free status, not genetically unstable or mosaic and of sufficient quantity to allow for banking and retention of starting material to ensure the production of hiPSCs that meet evolving standards. Establishment of genetic identity through Single Tandem Repeat (STR) or internally developed single-nucleotide polymorphism (SNP) array is an essential step following somatic cell donation that provides traceability through hiPSC derivation, banking, and differentiation.

FCDI employs an episomal reprogramming methodology that allows for the generation of hiPSCs that:

(1) Do not have reprogramming factors integrated into the genome.
(2) Can be cultured in defined conditions, including animal-free conditions, which enable good manufacturing practice (GMP)-quality cell production.
(3) Maintain genomic fidelity and stability.
(4) Match the donor material SNP/STR profile.
(5) Express pluripotency-associated markers.
(6) Are sterile and mycoplasma free.

To obtain regulatory approval for cell-based therapy products, GMP-established banks undergo additional testing in compliance with FDA guidelines for safety and robustness. Table 2 describes critical attributes and characterization assays for extensive viral testing and QCs.[35,36]

The methods for cardiac differentiation have centered around modulation of Wnt/β-catenin signaling driving cells through mesodermal lineages to form cardiac progenitors, ultimately resulting in terminally differentiated cardiomyocytes. These processes, extensively discussed in recent articles,[37,38] are multistep and often lead to diverse outcomes due to the accumulation of variations. The key factors contributing to this variability include the genetic background of the donor, genetic abnormalities, and aberrations in epigenetic reprogramming.[39] Collectively, these factors impact differentiation efficiency, reproducibility, and the standardization of scaling up. Therefore, to ensure reproducibility in manufacturing and functional performance of the cells produced, it is necessary to establish an iterative banking strategy of mini-bank, seed bank, master cell bank (MCB), and working cell bank (WCB) before carrying out hiPSC-CM production.

Although GMP quality is a requirement for clinical-grade cells and therapeutic products, these attributes remain important for

Table 1. Commercially available hPSCs and their derivatives

Cell type	Gender	Age	Ethnicity	List price
WiCell (hiPSC only): cardiac healthy and diseased				
6 × clinical grade cGMP hESCs	male and female	N/A	unknown	$1,250
70 × hiPSCs—Framingham Heart Study collection	male and female	51–90 years	White	$1,250
70 × hiPSCs—pulmonary artery hypertension study (Dr Marlene Rabinovitch, Stanford University)	male and female	21–100 years	White, European, and Latino	$1,250
97 × hiPSCs—coronary artery disease and myocardial infarction study (Dr. Eric Topol, Scripps Research Institute)	male and female	23–100 years	White, European, and Latino	$1,250
220 × hiPSCs—iPSC Collection for Omics Research (iPSCORE) containing familial and association-based genetic studies	male and female	9–88 years	White, European, Latino, Asian, and Japanese	$1,250
Coriell (hiPSC only): cardiac healthy and diseased				
hiPSC—long QT Syndrome 2, KCNH2-A422T, retroviral reprogramming	female	48 years	Asian Chinese	$1,037–$1,704
hiPSC—isogenic control for Long QT Syndrome 2, gene-edited using CRISPR-Cas9 technology	female	48 years	Asian Chinese	$1,037–$1,704
hiPSC—long QT Syndrome 3, SCN5A-N406K, retroviral reprogramming	female	18 years	Caucasian, Hispanic, and Latino	$1,037–$1,704
Coriell (hiPSC only): fluorescence-tagged genes associated with cardiac phenotypes				
Allen Cell Collection: 56 × certified fluorescently tagged hiPSC lines that target 43 key cellular structures and substructures, including genes associated with cardiac phenotypes (ACTN2, TNNI1, ATP2A2, MYL7, MYL2, TTN, and GJA1)	male	48 years	Asian Chinese	$1,037–$1,704
hiPSC—isogenic control, apparently healthy individual, episomal reprogramming	male	30 years	Asian, Japanese	$750

(*Continued on next page*)

Table 1. ***Continued***

Cell type	Gender	Age	Ethnicity	List price
ATCC (hiPSC only): healthy				
13 × hiPSC lines (from liver, bone marrow, and skin)	male and female	neonate to 72 years	non-Hispanic White African American, Asian, and Hispanic	£170 ($215)
ATCC (hiPSC only): Down syndrome				
1 × hiPSC	male	neonate	White	unavailable
ATCC (hiPSC only): Parkinson's disease				
3 × hiPSCs (vary in reprogramming methods)	male	63 years	White	£1,634 ($2,075)
ATCC (hiPSC only): cystic fibrosis				
1 × hiPSC (homozygous for the Delta 508 mutation in the cystic fibrosis transmembrane conductance regulator gene)	male	16 years	unknown	unavailable
Axol Bioscience: cardiovascular				
healthy ventricular and atrial hiPSC-derived cardiomyocytes	male	74 years	unknown	£337–£925 ($438–$1,175)
Axol Bioscience: neuronal				
hiPSC-derived astrocytes	male	newborn	unknown	£728 ($925)
4 × hiPSC-derived neural stem cells	male and female	31–87 years	White	£506 ($642)
hiPSC-derived microglia	male	40–50 years	unknown	£1,285 ($1,632)
hiPSC-derived sensory neuron progenitors	male	newborn	unknown	£570 ($724)
hiPSC-derived motor neuron progenitors (healthy and ALS)	male and female	62–74 years	unknown	£544 ($691)
hiPSC-derived cortical inhibitory interneuron progenitors	male	newborn	unknown	£660 ($838)
hiPSC-derived cortical excitatory neurons (healthy and Alzheimer's disease)	male and female	newborn to 87 years	unknown	£1,800 ($2,286)
Cellular Dynamics: cardiovascular				
hiPSC-derived cardiac progenitor cells	male, 01279	50–59 years	White	$1,395
2 × hiPSC-derived cardiomyocytes (iCell cardiomyocytes)	female, 01434 and 11713	unknown	White	$695–$1,721

(*Continued on next page*)

Table 1. ***Continued***

Cell type	Gender	Age	Ethnicity	List price
hiPSC-derived cardiomyocytes[2] (iCell cardiomyocytes[2])	female, 01434	under 18 years	White	$1,821
hiPSC-cardiac fibroblasts	isogenic to iCell cardiomyocytes	unknown	White	not currently available (Sept 2023)
Brugada syndrome: hiPSC-derived cardiomyocytes (CACNA1C [G490R] c.1468G>A)	female, 01434	under 18 years	White	$1,045
Catecholaminergic polymorphic ventricular tachycardia: hiPSC-derived cardiomyocytes (RYR2 [E2311D])	female, 01434	under 18 years	White	$2,780
dilated cardiomyopathy: hiPSC-derived cardiomyocytes (LMNA [L35P])	male, 01016	under 18 years	White	$1,045
Cellular Dynamics: neuronal				
hiPSC-derived astrocytes	female, 01434	under 18 years	White	$945
hiPSC-derived dopaminergic neurons (healthy and Parkinson's disease)	male	50–69 years	White	$717–$1,195
hiPSC-derived cerebral cortical neurons	male and female	under 18 years and 50–59 years	White	$1,695
hiPSC-derived glutamatergic cortical neurons and microglial (healthy and Alzheimer's disease)	male	50–69 years	White	$695–$2,445
Cellular Dynamics: vascular				
hiPSC-derived endothelial cells	female, 01434 and 11713	under 18 years and 30–39 years	White	$1,230
Cellular Dynamics: liver				
hiPSC-derived hepatocytes	female	under 18 years	White	$1,745
Greenstone Biosciences: cardiac healthy and diseased				
HiPSC-derived cardiomyocytes (healthy, hypertrophic cardiomyopathy, long QT-syndrome, dilated cardiomyopathy); also provide hiPSC-derived cardiac fibroblasts, endothelial cells, smooth muscle cells and pericytes	male and female	16–60 years	White	$599–$999

Pricing details are current as of October 2023, as per the manufacturer's website. Cell quantities per vial can vary among manufacturers, and certain products may include appropriate cell culture media and supplements.

Table 2. Quality control methods

Key critical attributes	Common methods
Footprint-free reprogramming	episomal plasmids, Sendai virus, mRNA
Derived in and expanded under defined conditions, feeder-free	E8 medium and vitronectin
Genomic fidelity and stability	G-band KT, SNP arrays, WES/WGS
Identity match to donor material	SNP/STR profile
Express pluripotency-associated markers	Flow cytometry: OCT4, Tra-1-60, SSEA4. Gene expression: panels (Pluritest) or RNA-seq
Sterile, endo-, and myco-free	routine mycoplasma testing

Abbreviations: KT, karyotyping; WES/WGS, whole-exome sequencing/whole-genome sequencing; SNP/STR, single nucleotide polymorphism/short tandem repeats.

researchers who may not directly work with or prioritize GMP standard culture. Adherence to donor selection and reprogramming guidelines ensures data integrity and reproducibility of results and comprehensive tracking, which subsequently allows the research community to draw meaningful comparisons and share a reliable pool of resources. Moreover, even if researchers may not initially intend to pursue clinical applications, opting for GMP-grade lines may facilitate the eventual transition into therapeutic development, aligning with UK and US standards, such as procurement, testing, processing, storage, distribution, and import/export.[40] These factors mean that there are considerable logistical, cost, and time implications associated with any changes to the production pipeline. For example, there is caution in departing from established episomal pluripotency reprogramming approaches to newer chemical induction pluripotency methods within commercial or regulatory settings.

After reprogramming, and as soon as the selected hiPSCs have been expanded to several wells of multi-well culture plates or small flasks (typically around passage 5), the lines should be cryopreserved as minibanks (MBs). The bank size at this time point is typically small (10–20 million cells). QC of each bank has several shared assays, but the expansiveness of the assays is bank level- and bank use-dependent. Figure 1 shows a workflow summary. For example, the need for regular monitoring of genomic stability and integrity in hiPSCs is essential due to the well-recognized accumulation of growth-promoting mutations overtime,[41,42] whereas extensive viral testing is required for donor material and MCBs within the clinical pipeline.

Most hiPSC or hiPSC-derived product providers perform standard QC procedures, which test for normal karyotype, sterile cultures, mycoplasma-free, endotoxin-free, and viability after freeze-thaw cycles. FCDI also conducts identity confirmation by SNP analysis, comparing the DNA fingerprint of the product with that of the original tissue sample. The functional QC testing of hiPSC-CMs can be achieved via multiple platforms/methods depending on the readout (e.g., electrical activity, contractility, or metabolic). For example, as routine functional assays used by FCDI include measuring calcium transients in response to known inotropic compounds (e.g., isoprenaline), multi-electrode arrays (MEA), and impedance-based measurements for 2D monoculture CMs and 3D microtissues. Irrespective of the platform used, baseline beat rate is reported by most commercial providers in their Certificate of Analysis.

Challenges in hiPSC-CM production for research and cell-based therapies

In the past decade, numerous clinical studies involving hPSCs have emerged. By the end of 2019, there were at least 54 studies targeting 22 different diseases, with a notable shift toward using hiPSCs over hESCs.[43,44] Although small-scale hiPSC culture and differentiation are adequate for academic and research purposes, they prove inadequate when attempting to scale up for cellular therapies. In the context of cell-based therapy for heart failure, it is projected that at least 1 billion cells will be needed per patient. Given the potential target population necessitating thousands of doses per year, the total demand could range from 10^{11} to 10^{14} cells. Establishing well-defined, cost-efficient differentiation processes capable of generating cGMP-compliant cells at scale for clinical development and commercialization faces significant challenges.

Characterization of the starting material

One significant challenge stems from the reliance on research-grade raw materials in cardiac differentiation protocols, which were not originally designed for use in cell therapy applications. Early hPSC culture systems initially depended on feeder layers of mouse embryonic fibroblasts to support cell growth. Substantial advancements occurred in shifting from feeder layers to extracellular matrix (ECM) coating, such as Matrigel, and further progressing to defined ECM molecules like fibronectin and vitronectin.[45] Even in cases where the research is initially geared toward non-clinical applications, it is important to anticipate the possibility of future translational needs and consider the relevant regulatory guidelines, which can differ significantly from one country to another. Typically, all raw materials that come into contact with hPSCs (e.g., human serum/proteins and affinity purified proteins) need to be qualified.[46] For example, when using hPSCs transitioned to feeder-free conditions, there remains a potential risk of murine virus contamination, necessitating additional testing.

Human iPSCs and their derived cell types, including both healthy and disease-specific types, are widely accessible and can be obtained from numerous companies or biobank facilities, often government funded [e.g., the European Bank for induced pluripotent stem cells (EBiSC)]. QC involves assessing purity of derivatives (e.g., hiPSC-CMs) and infectious disease testing (HBV, HCV, HIV-1, and HIV-2). Differences in the performance of seemingly identical cell lines have been well-described in the literature.[47] Differences between multiple clones from a single donor are known as "clone-to-clone variation," whereas fluctuations in yield and purity among differentiation batches of a single cell line are referred to as "batch-to-batch variation." There is also variability between hiPSC-CMs produced by different individuals. To illustrate this, action potential duration data from 18 lines available academically and commercially were compared. This showed variation of ~400% between lines, far exceeding the 50% variability in the normal QT interval range for human hearts.[48] Metabolically, there was a 5-fold variation in oxygen consumption rates between hiPSC-CMs from different individuals, which exceeded the level of impact caused by

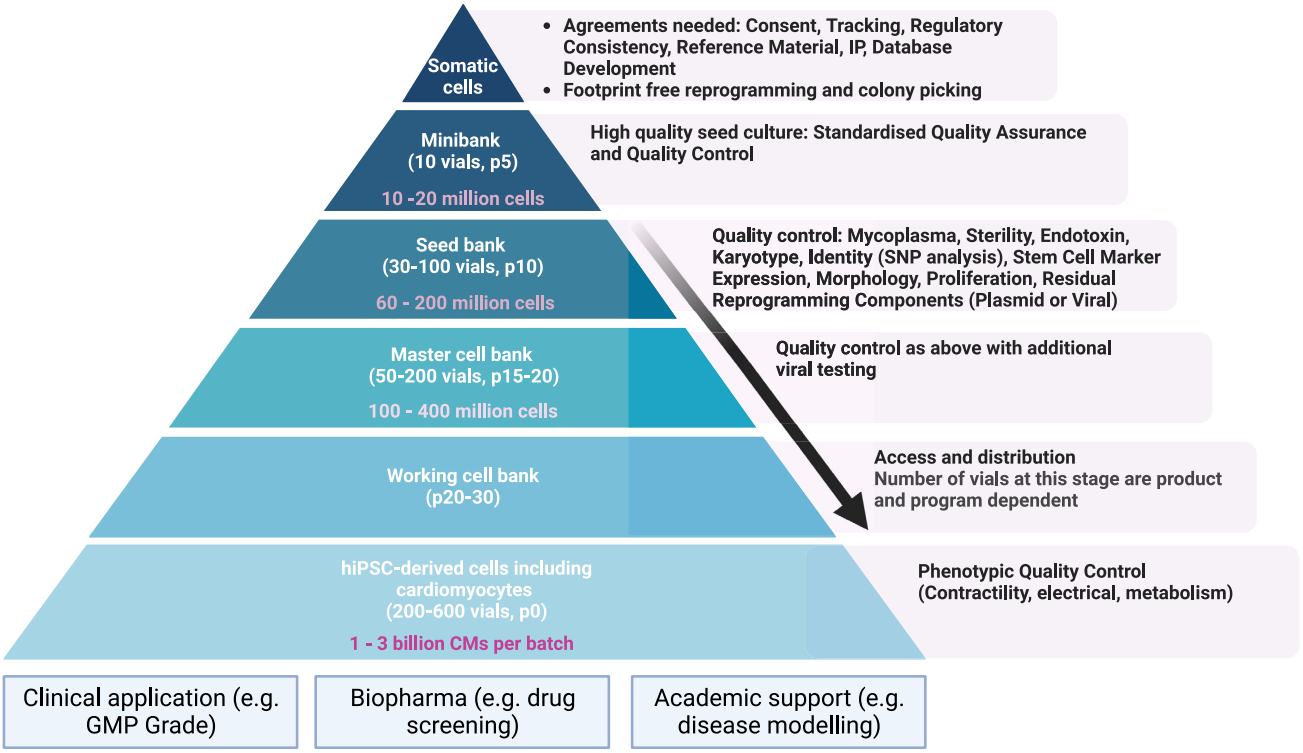

Figure 1. Large-scale hiPSC production and differentiation workflow

sarcomeric mutations associated with conditions such as hypertrophic cardiomyopathy.[49] Another study showed large baseline differences in contractile force and kinetics within 5 commercial and 5 academic hPSC-CM lines, supporting the need for careful consideration of "predictive" cell lines and routine testing and QC with multiple cell lines.[50]

FCDI's iCell Cardiomyocytes and iCell Cardiomyocytes[2] are mixed populations of hiPSC-CMs consisting of atrial, ventricular (>50%), and nodal cell types, characterized by expression of cardiac-specific markers (e.g., alpha-actinin and cardiac troponin) and electrophysiology. Certain cardiac conditions, such as atrial fibrillation and septic cardiomyopathy, require the isolation of specific subsets (atrial, ventricular, and nodal) of cardiomyocytes. Recently, companies like Axol Bioscience offer chamber-specific cardiomyocytes, which undergo additional QC to determine their origin via expression levels of ventricular-specific (e.g., MLC2v and HAND1) or atrial-specific markers (e.g., MLC2a, atrial natriuretic peptide, Kv1.5, and Kir3.1/3.4), alongside functional testing (e.g., multi-electrode array and contractility assays).[51–53]

Lately, there have been concentrated efforts toward the development and optimization of chemically defined and integrated xeno-free hiPSC-CM platforms, resulting in cardiac purities exceeding 80%.[54–58] Although these reports are encouraging, anecdotal evidence suggests that the absence of growth factors lowers the reproducibility of differentiation and purity, which will need to be addressed. It is crucial to note that even animal-free protocols and/or materials and cells produced in GMP-compliant facilities may not automatically align with the requirements of regulatory bodies (e.g., FDA and EMA) for clinical purposes. Therefore, due diligence needs to be conducted to identify what testing has been done on components for the induction, expansion, and differentiation of hiPSCs.

Manufacturing large-scale hiPSC types

The process of producing, storing, and efficiently distributing high-quality hiPSCs and their derived lineages to research institutions and clients demands a wealth of expertise, time, and careful oversight. There are several challenges surrounding large-scale manufacturing.

Traditional 2D differentiation processes are heavily operator dependent, lack in-process monitoring, and are not scalable in part due to inefficient cell growth surface area-to-volume ratio.[59] Bioreactor-based manufacturing and the use of automated robotic systems have been shown to improve quality control, productivity, robustness, and standardization, addressing many of these shortcomings.[60] Additionally, organizations such as Cell and Gene Therapy Catapult provide services to generate robust scale-up strategies for stem cell manufacturing, partnering with universities, biopharma, and government bodies. However, these scale-out strategies can have a substantial impact on the cost of goods throughout the process. Managing expenses is a vital factor in establishing an economically sustainable supply chain for cell-based therapies. A recent workshop report from the International Stem Cell Banking Initiative (ISCBI) examined five case studies from four different countries. Primary manufacturing costs were driven by factors such as the requirement for skilled personnel, specialized cell culture materials, clean-room facilities, QC, and safety testing, as well as expenses associated with validating manufacturing processes.[61]

There is a need to develop appropriate methods for cryopreservation and transportation of hiPSC-derived cells.[62] Current protocols report approximately 80% hPSC-CM viability following cryopreservation after 5 days of culture in CryoStor CS10 and STEMdiff CM freezing medium, which are two readily available, xeno- and serum-free cryoprotectant formulations.[63] How reproducible these approaches are between labs is yet to be seen, and the long-term impact of cryopreservation needs to be completely understood. A recent study found that although there was no significant difference in cell survival after thawing, variations were observed in force generation and calcium handling in xeno-free cryopreserved hiPSC-CMs.[64]

Finally, traceability plays a crucial role in the regulatory review and approval processes. Although hiPSC repositories provide the basic information relating to cell source, essential information pertaining to donor specifics, reprogramming techniques, and infectious disease testing is often lacking in transparency and proves challenging to obtain. This is illustrated by a report from Kobold and colleagues. Clinical studies, to date, have used only 11 hESC lines and 1 hiPSC line, and however, 2 lines were missing in the Human Pluripotent Stem Cell Registry (hPSCreg; https://hpscreg.eu), with only 10 hESC and no hiPSC lines registered.[44] Regulatory agencies require comprehensive information to assess the quality and safety of these therapies, and incomplete traceability can hinder this evaluation and translation.

Gender-balanced, ethnically diverse hiPSCs

The primary challenges in creating the ideal panel of healthy normal donors with racial diversity or disease-specific cohorts have revolved around demand.

Analysis of major hiPSC repositories across the US and Europe have revealed limited representation of ethnic diversity (primarily Caucasian) and disease models. There is also uneven distribution of male and female hiPSC lines, coupled with a lack of proper identification during banking.[65] Efforts aimed at creating inclusive large-scale repositories internationally have been initiated by academics partnering with funding bodies, including the UK Medical Research Council (MRC) and Wellcome Trust. Collaborative partnerships including Coriell Institute and FCDI developed high-quality, well-characterized stem cell bank California Institute for Regenerative Medicine (CIRM), which utilized the commercial standards described in this case study. In addition, the International Stem Cell Banking Initiative (founded in 2007) aims to coordinate best practices around stem cell production and biobanking as well as regulations and policies through their consortium with academic and industrial partners across more than 28 countries.[66–68]

Social determinants of health including economic, social, environmental, and psychosocial factors including sex- and ethnic-related disparities have been shown to impact risk and outcome of cardiovascular diseases.[69–71] This has prompted the development of academically led cohorts such as the Framingham Heart study (Table 1) that created 68 hiPSC lines from participants to investigate the effect of genetic variants on cardiometabolic disease phenotypes. FCDI also produced ethnically diverse iCell Cardiomyocytes available as part of the diversity panel. However, due to limited uptake and demand, as of March 2022, these diverse donor cell lines are now available under custom services only, which are usually tailored to meet the specific needs and hence typically an expensive option.

Drug-induced cardiotoxicity screening with hiPSC-CMs

Over the last 20 years, we have seen a huge advancement in the application of hiPSC-CMs impacting cellular models used by industry and academia. At the turn of the century, research most commonly used primary rodent cells, surrogate cell lines, and primary human cell lines when accessible. For drug discovery, this resulted in a dependency on *ex vivo* and *in vivo* models for critical path decision-making and assessment of likely risk benefit. Alongside human ether-a-go-go-related gene (hERG) screening or safety pharmacology primary cell and tissue-based studies, rabbit ventricular wedge or papillary muscle preparations from other species (e.g., guinea pig) were often the earliest model used to predict impaired cardiac function.[72–74]

Although these low-throughput models provided valuable data, the timing of their deployment in routine screening paradigms identified liabilities relatively late in the drug discovery cycle. Moreover, these assays were limited to testing only a handful of molecules and would not detect human-specific liabilities, highlighting an opportunity for earlier decision-making and higher-throughput assays, which may be more predictive of clinical effect. Since the evolution of hiPSC technologies, there has been a dramatic shift in the balance toward using *in vitro* assays as frontline systems to quickly flag any potential impacts of cardiac function and structure and to gain a real insight into the mechanism of action.

For pharmaceutical drug discovery, these systems are now fundamental and amenable to high-throughput 96-, 384-, and 1,536-well plate formats. The use of technologies, such as MEA,[75,76] impedance,[77,78] fluorescence imaging plate reader (FLIPR),[79,80] and live-cell brightfield imaging,[22,81,82] is now routinely used alongside others that assess changes in derived waveform data such as peak frequency, peak amplitude, and peak widths. The potential utility and impact of hiPSC-CMs have been well discussed in other articles.[83,84] They have demonstrated their effectiveness in several crucial areas, including (1) investigating both short-term and long-term cellular responses to compounds that cannot be achieved in other systems such as adult human cardiac tissue,[85] (2) elucidating structural cardiotoxicity mechanisms, for example, anthracycline-induced cardiotoxicity,[86] and (3) capturing both structural and functional cardiotoxicity.[22]

Numerous studies have consistently demonstrated that hiPSC-CMs and hESC-CMs present a significant advantage over traditionally used non-human and/or non-cardiac cell lines for evaluating cardiotoxicities.[83,87] This encompasses a variety of end points including contractility and indicators of cell health, such as endoplasmic reticulum integrity and mitochondrial health measured by membrane potential. Further outputs include calcium transients and electrophysiological parameters.

Earlier research by Pointon and colleagues showed that hESC-CMs have better predictive value than rat myoblastic H9c2 cells when compared with known *in vivo* cardiotoxic effects (Table 3). Subsequent high-throughput hiPSC-CM studies using unpaced (chronicity) or paced (to physiological ranges) conditions and therapeutically relevant drug concentrations demonstrated sensitivities and specificities that surpassed 70%. We developed new CardioMotion software to analyze contractility responses to 136 drugs of diverse modes of action. This provided ~73% sensitivity and ~83% specificity, which not

Table 3. Human PSC-CMs for the identification of cardiovascular liabilities

Project	Assay platform	Model system	Measurements and readouts	Number of compounds	Main outcomes	Ref
Comprehensive *in vitro* Proarrhythmia Assay (CiPA)	MEA and VSD	2D	arrhythmia events, delayed repolarization, and repolarization prolongation	28	CiPA adoption as a drug development tool for evaluating the efficacy of drugs	Cavero et al.,[89] Li et al.,[90] Lu et al.[91]
The Japan iPS cardiac safety assessment (JiCSA)	MEA	2D	field potential waveform, field potential duration, incidence of after depolarization, and triggered activity	60	MEA protocol for assessing the torsadogenic potential of compounds	Kanda et al.[92]
InPulse CRACK-IT Challenge	CellOPTIQ, TTM, EHT	2D and 3D EHT	contractility, electrophysiology, and/or calcium handling	28	2D monolayers (85% predictive accuracy) and 3D EHTs (93% predictive accuracy)	Saleem et al.[93]
The Tox21 program	FLIPR, TetraCycler Microplate Handler, and ImageXpress Micro XL	2D	intracellular calcium flux assay, cell viability (Calcein AM, Hoechst), and mitochondrial depolarization (JC-10)	69 representative environmental chemicals and drugs	demonstrate the concentration-response data for *in vitro* bioactivity phenotypes by Toxicological Prioritization Index (ToxPi)	Sirenko et al.[94]
Ncardia's smart library	Ncardia's DiscoverHIT Platform	2D	calcium transient assays, cell viability, NT-proBNP AlphaLISA assay, AlphaLISATruHits assay, and high-content-imaging secondary BNP assay	3,600	10,000 data points in cardiomyopathy disease-relevant phenotypic screen using a fully annotated drug/drug-like "smart" library	De Korte et al.[95]
AstraZeneca	IonOptix and FLIPR Tetra	2D	calcium transient, cardiac contraction, and cell viability (ATP depletion)	31 inotropic and 20 non-inotropic	87% sensitivity and 70% specificity	Pointon et al.[23]
	IonOptix and ImageXpress	3D microtissue	cardiac contraction and cell viability (ATP depletion)	29 inotropic and 13 non-inotropic	80% sensitivity and 91% specificity	Pointon et al.[24]
GlaxoSmithKline	CardioMotion	2D	cardiac contractility metrics	136 compounds (incl. 14 structural cardiotoxicants)	72.9% sensitivity (86% for structural toxicants) and 82.5% specificity	Stebbeds et al.[22]

(*Continued on next page*)

Table 3. *Continued*

Project	Assay platform	Model system	Measurements and readouts	Number of compounds	Main outcomes	Ref
AstraZeneca-GlaxoSmithKline-University of Cambridge collaboration*	FLIPR Tetra	2D	dynamic calcium transients	63	machine learning tools: open-source Python toolkit (38 parameters from calcium transient waveform data, 0.86 accuracy) and random forest model (25 parameters) for predicting cardiac activity	Yang et al.[96,97]
A population-based hiPSC drug screening platform for toxicity assessment	Opera Phenix High-Content Screening System and Maestro Edge	2D	live/dead percentage (Hoechst & propidium iodide), cell viability assay, and electrophysiology	2,374	population-based hiPSC cell bank representing > 477 million people based on HLA-A, HLA-B and DRB1	Huang et al.[98]
Resolving cardiotoxicity induced by COVID-19 treatments	IonOptix Calcium and Contractility System, Operetta CLS High-Content Analysis System, Zeiss LSM 710 confocal microscope, and Tecan Freedom EVO 150 liquid handler	2D and 3D EHT	cell death (calcein-AM/ propidium iodide/Hoechst), sarcomere disarray, calcium handling, mitochondrial membrane potential, mitochondrial-associated ROS, intracellular ROS, and cardiac contraction	2,464 small molecules and 21 COVID-19 repurposed drugs	human ESC-CMs screened on EHT platform identified FDA approved protective drugs for alleviating Remdesivir-induced cardiotoxicity	Xu et al.[99]
Drugs induce cardiomyocyte proliferation	Operetta CLS high-content imaging system	2D	DNA synthesis assay (EdU), live/fixed proliferation assay (Ki67, Hoechst)	5,094	novel automated hybrid live/fixed assay for cardiomyocyte proliferation, which distinguishes proliferation from multinucleation and polyploidization, to identify 30 compounds that enhance proliferation of hPSC-CM; the clinically approved L-type calcium channel blockers were the most active compounds from the phenotypic screen to induce cardiomyocyte proliferation	Woo et al.[100]
Cardiotoxicity screening of tyrosine kinase inhibitors	IC200 Kinetic Imaging Cytometer, Zeiss LSM 510 Meta confocal microscope and EPC 10 patch clamp amplifier	2D	viability, contractility, calcium transients, electrophysiology, kinase phosphorylation profiling, and gene expression	21	a cardiac safety index for TKI toxicity; emphasis on deriving hiPSCs from a broader cohort of individuals to establish a population-level, prospective, preclinical toxicity assessment to better inform drug development	Sharma et al.[101]

Abbreviations: MEA, multi-electrode array; VSD, voltage-sensitive dye; TTM, triple transient measurement; EHT, engineered heart tissue; BNP, B-type natriuretic peptide; TKI, tyrosine kinase inhibitor; ROS, reactive oxygen species; FLIPR, fluorometric imaging plate reader.

only exceeded outcomes from a well-established calcium flux assay but also correctly identified toxicants previously detected using *in vivo* models.[22] Such studies further affirm the reliability and potential of these cells for predicting *in vivo* effects,[22,23,88] and the expansive list of cardiotoxicity studies that have used hPSC-CMs is provided in Table 3.

Due to the quality and scale of the data, this has opened seemingly endless possibilities including the use of mechanistic electrophysiology modeling—for instance, using a mathematical model to predict changes to hiPSC-CM electrophysiology[102,103] based on ion channel screening data[104] and then assessing what we know about a drug's mechanism of action by how well the simulations match experiments.[105,106] If there is confidence in understanding a drug's action (*why* it has its observed effects on hiPSC-CM electrophysiology/contraction), we can make more confident translation predictions to human *in vivo* electrophysiology. One of the challenges here is dealing with clone-to-clone and batch-to-batch variability,[107,108] and tailored mathematical models for each batch/preparation may be one solution.[109,110] Machine learning may also assist in terms of detecting features of experimental hiPSC-CM recordings that indicate a cardiotoxicity risk.[111] All of these *in silico* techniques will enable improved and quantitative decision-making in assessing cardiotoxicity liabilities.[96,112,113]

Complex *in vitro* model development

The 2D monoculture models described above are already mainstream across the pharmaceutical industry and academia. However, as discussed, they do present limitations regarding their translatability to the clinic. Increasing the complexity of *in vitro* model systems is generally considered to increase the physiological relevance and, consequently, the translational power of these models.

Various hiPSC-CM models aim to bridge the translational gap between the simple 2D monoculture assays and the clinic. This has provided academic and commercial demand for other hiPSC-derived lineages, including cardiac endothelial cells (CECs) and cardiac fibroblasts (CFs), including isogenic variants. Various combinations of hiPSC-CMs, CECs, and CFs are being used to produce 3D microtissues, engineered heart tissues (EHTs), and "heart-on-a-chip" models.[114,115] Such approaches are superseding the use of primary cell lines, which display considerable batch-to-batch variability due to being harvested from a pool of different donors.

Improved hiPSC-CM maturity can be achieved in 3D.[116] Multicultures of hiPSC-CMs with CFs, CECs, and, in some cases, immune cells (resident and circulating macrophages) can produce complex microtissues that form a synchronous syncytium, high connexin-43 levels, and stronger electromechanical signal conduction, which improve predictivity.[88,117–119] The multi-lineage complexity is more physiologically relevant than 2D monocultures, not least because cardiomyocytes constitute only approximately 30% of the cells in the human heart. These 3D models are already in use and are a target for optimization to enable high-throughput screening of more translational models in this space, although obstacles around throughput and reproducibility are still to be addressed.

EHTs, including the Biowire II platform, have been well characterized for both *in vitro* and *in vivo* cardiac studies.[30,50,93,120,121] The hybrid of cells and materials used to create EHTs combine the advantages discussed above with the ability to directly measure force continuously. In contrast, high-throughput techniques used such as calcium imaging and CardioMotion provide data on contractility, which is distinct and only a surrogate for force of contraction. Additionally, electrical pacing is often incorporated into 3D models, enabling control of beat rate, which allows greater data standardization than spontaneous beating. Over time, pacing has been suggested to increase the maturity of hiPSC-CMs.[93,122]

As an example of EHTs being used in practice, Saleem and colleagues demonstrated a predictive accuracy of 93% in detecting drug-induced changes in contractility, along with a positive force-frequency relationship that is not observable in 2D monocultures.[93] Recent advances in EHTs using ring-shaped models, with separate chambers for atrial and ventricular hiPSC-CMs, which more closely resemble the physiological reality, could further increase the physiological relevance of these models, particularly with regards to atrial fibrillation.[123]

Heart-on-a-chip models are the apogee of complex hiPSC-CM-based models. They generally consist of relatively large microtissues containing up to 10 million cells (hiPSC-CMs, -CFs, and -CECs) per tissue. Culture conditions (such as oxygenation and shear stress) are controllable, and biosensors are capable of continuously monitoring physiological response in a 3D format.[124,125] As reviewed by Andrysiak et al.[126] and Cho et al.,[127] there are several similar models emerging, including those as commercialize enterprises. Data are showing improved maturity of hiPSC-CMs, predictive power for safety testing, and physiological relevance for disease understanding and target validation. Novoheart, a stem cell biotechnology company, provides various bioengineered human heart constructs, including the first human heart-in-a-jar for toxicity and efficacy testing.[128] Currently, these systems are early in their developmental stage, offering limited readouts such as contractility. They are yet to achieve neuro-hormonal integration of an intact mammalian system and do not include homeostatic mechanisms. Nevertheless, there is clear potential for evaluating pathophysiologically relevant myocardial safety liabilities *in vitro*.

Despite the promise of these methods, complexity comes at a price; although 2D monoculture hiPSC-CM models are routinely used in industry for high-throughput safety assessment,[22,23] screening up to hundreds of putative therapeutics at an early stage in a single run, complex models are invariably more expensive, lower throughput, and less reproducible (Table 4; example costing and scalability). This presents an obstacle for their utility in either high-throughput screening for target validation or early-stage safety screening. Currently, complex models of hiPSC-CMs are more often used for later stage, preclinical safety testing and investigative toxicology.

With the use of hiPSC-CM models becoming more widespread, there is a clear opportunity for further evolution as tools to integrate into high-throughput screening platforms for drug development and early safety screening (Figure 2). Continuous consideration will be needed to ensure high reproducibility and high throughput at an appropriate cost point while minimizing the level of operator training required.

Table 4. Approximate example cost and scalability comparison between 3D microtissues (cardiomyocytes, cardiac fibroblasts, and cardiac endothelial cells) and the 2D monoculture cardiomyocyte assay

Criteria	3D microtissue assay	2D FLIPR assay
Experimental and technical requirements	high content imaging and analytics, automated sterile liquid handling	automated calcium flux: FLIPR screening system (molecular devices); integrated analysis: ScreenWorks Peak Pro
Advantages	(1) 3D microtissue demonstrated to be more predictive than 2D FLIPR assay (hiPSC-CMs, hiPSC-ECs, and hiPSC-CFs) (2) reduced cell usage vs. 2D system (3) able to detect chronic effects (48 h endpoint)	ease of use and rapid readout, kinetic information on rate of calcium flux
Limitations	scalability, user time, cost of multiple cell lines	calcium dye toxicity;limited to acute drug responses; cost of cellular reagents
Consumable cost per compound, $	71	159
Total cost per compound, $	437	311
Time from ordering compound to data delivery, weeks	4	2
Scalability max compounds per year based on FTE requirements	260	20,000

Estimated costs as of November 2023. Abbreviations: FTE, full-time equivalent; FLIPR, fluorometric imaging plate reader.

FUTURE PERSPECTIVES

In this section, we offer perspectives on where and how the field is evolving relating to three main areas. These include continuing challenges of 2D and 3D systems, unmet needs, and gaps in translating preclinical models to human benefit and the developing area of regenerative stem cell therapies.

2D and 3D model systems: what is next?

Challenges with these simple 2D and 3D systems remain:

- They are unable to describe compound-mediated changes in *in vivo* organ physiology, such as effects of blood pressure on cardiac function.
- Many platforms are limited in their recapitulation of different cell types/associations found in natural myocardium.
- Cardiomyocytes are relatively immature structurally (e.g., absence of t-tubules) and functionally when compared with adult human cardiomyocytes, likely related to the poor mechanical stimulation/loading in simple culture systems. It is well reported that hiPSC-CMs exhibit the spontaneous beating typically representative of embryonic cardiomyocytes, which is not normally exhibited by mature atrial or ventricular myocytes. This is generally attributed to the presence of the pace-making current If and related reduction of the hyperpolarizing current associated with reduced I_{K1} functional expression.[87,129,130]
- Most commercially available lines depict the healthy state, although therapeutics are often administered to patients who may have cardiovascular comorbidities and already exhibit symptoms affecting cardiac function, such as diabetes, that may sensitize them to additional risks. Human iPSC-CMs obtained from individuals with genetic cardiomyopathies, featuring specific genetic alterations for testing mechanistic hypotheses or potentially sourced from patients with acquired diseases (including models with abnormal tissue loading), offer valuable avenues for exploring cardiac safety within the patient population.[131] Moreover, they contribute to enhancing our mechanistic understanding of the intricate interplay within cardiomyocytes.

Despite these associated caveats, which restrict the ability of hiPSC-CM-based platforms for modeling some phenotype/drug responses, there is sufficient "fitness for purpose" to allow pharmaceutical and biotechnology companies alike to use data generated from these models in early decision-making on molecule progression and even to screen out liabilities while still optimizing the chemistry. In the field of cardiac ion channel/electrophysiological effects, the impact of these models is evident in the CiPA initiative, which has actively encouraged the use of hiPSC-CMs to improve the prediction of proarrhythmic risk in new drug candidates.[21]

The bulleted points above are inter-related in terms of effects on mechanical loading. This means that there is potential to drive maturity in monoculture systems by culturing cells on hyperelastic silicone membranes, in muscular thin films, or in a high-throughput 96-well plate format. Such approaches add mechanical stress to stimulate cardiomyocyte development and allow chronic testing under loading conditions over several days.[132,133] Current research efforts on developing advanced maturation techniques, such as 3D tissue engineering,[30,134,135] electrical stimulation,[136] and metabolic modulation[137] are important for both drug discovery platforms and the functional integration of these cells upon transplantation.[138]

Unmet needs and gaps in translating preclinical models to human benefit

Availability of hiPSC-derived lineages representative of cells present within the myocardium and vascular network has

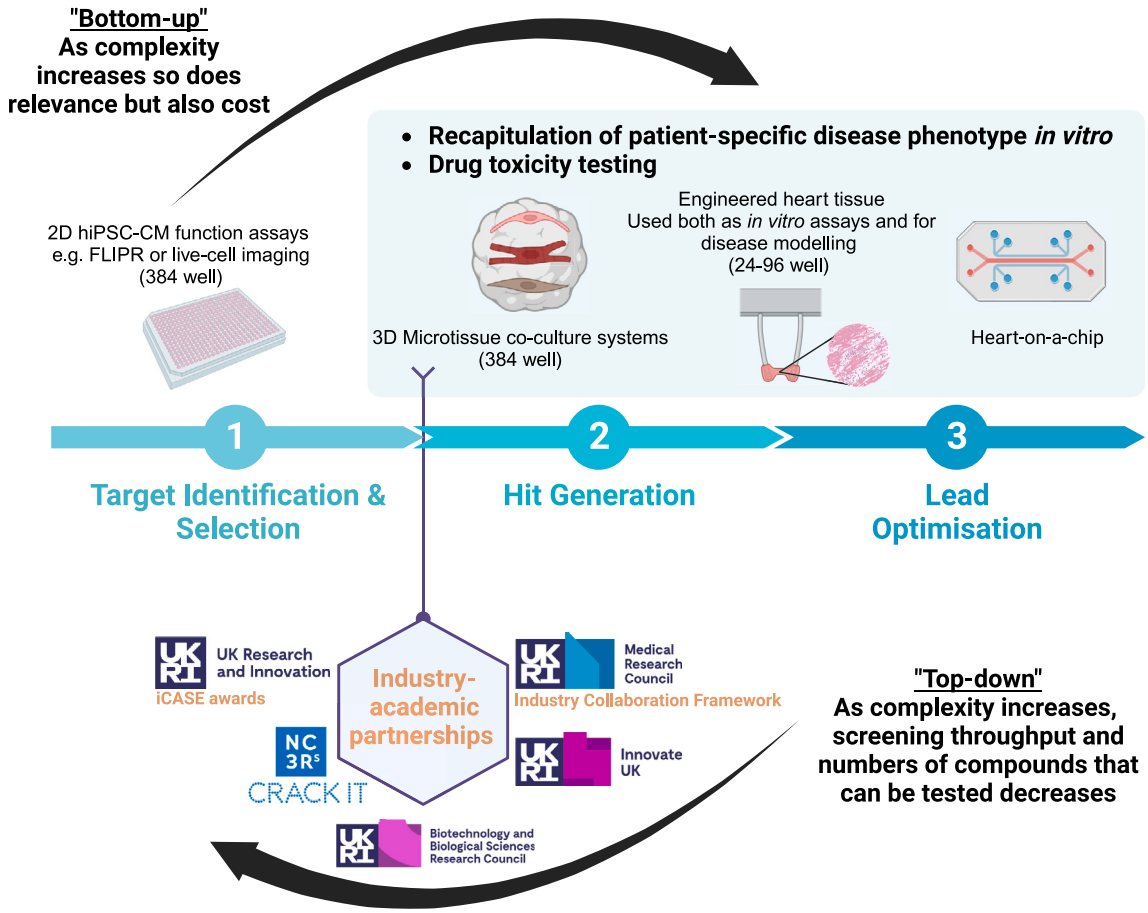

Figure 2. Overview of the use of commercially derived and/or in-house hiPSC-CMs within biopharma and academia

The challenge in transitioning from 2D to 3D model systems involves optimizing and choosing the ideal the model system for the biological question, addressing imaging complexities, managing compound screening, and improving data analysis. Novel approaches, including more early interdisciplinary collaborations, can enhance the evaluation of complex *in vitro* models for disease modeling and cardiotoxicity screening, promoting their widespread adoption.

accelerated progress in several applications. This includes *in vitro* model development to support drug discovery (efficacy and toxicology) and unveiling new mechanistic understanding at the molecular and cellular levels.

Plate-based drug screening using hiPSC derivatives has significantly enhanced compound selection decisions, enabling early identification of risk of cardiovascular liabilities and selection of compounds with no or reduced risk.[22,24] This has the potential to assist the development and early identification of novel therapies that show efficacy without unwanted cardiovascular liabilities. Recent advancements, including scaffold-free, self-organizing cardiac organoids that encompass all three main cardiac lineages through solely relying on developmental mechanisms with the ability to control the level of complexity, offer exciting potential for integration into drug discovery platforms, potentially enhancing the translatability of findings.[139] Similarly, heart-on-a-chip technology has started to enable tissue-level insights and decision-making in drug discovery.[124,140] The next natural stage of evolution of the technology will be to replace some or all aspects of *in vivo* models with *in vitro* models, both in healthy and disease settings. This is already happening. For example, field potential (safety) data from an hiPSC-CM model, in tandem with *in silico* model predictions was a factor in GlaxoSmithKline's decision to move away from using the rabbit ventricular wedge model as a primary screen, leveraging this technology to impact decision-making and advance the 3Rs of animal use.

These advances are important since they underpin the main purpose of safety assessment, namely hazard identification and risk assessment prior to exposing healthy volunteers and/or patients to a therapeutic agent at the right dose to elicit the desired benefit without overt side effects. Therefore, multiple *in vitro* and *in vivo* approaches are required for safety, efficacy, and clinical translatability and to account for systemic effects, including interactions with various cell types, organs, and systems.[141,142] When using *in vitro* models, it is easy to forget that tissue- and body-level pathophysiology, toxicities, and disease mechanisms are governed by the interplay between different organ systems, all in concert with individual genetic make-up and unique physiology.

To address this complexity, various approaches are being undertaken

Body-on-a-chip: Several reports have shown models that connect different organ systems including the liver, kidneys, and immune and nervous system. This is required to incorporate drug

metabolism, peripheral resistance, immune response, and autonomic control, respectively.[143–147] A heart-liver multi-organ system was recently developed in a study aimed at investigating cardiotoxicity upon hepatic metabolism.[148] This is particularly valuable for compounds that do not exhibit direct toxicity to the heart. For instance, cyclophosphamide itself is not cardiotoxic, rather the cardiac damage is attributed to the metabolites it produces.[149]

Hormonal control: Autonomic and hormonal mechanisms modulate significant mechanical/loading effects via the vasculature, mediating both acute and chronic myocardial effects of drugs and different disease states, including myocardial remodeling, which involves multiple cell types. The development of such approaches would enable an expanded repertoire of cardiovascular liabilities that could be detected *in vitro*, for example, the incorporation of a functional immune component could enable immune-driven toxicities (myocarditis/pericarditis) to be detected and the incorporation of autonomic control and peripheral resistance could enable hemodynamic-driven cardiac and vascular pathologies to be detected.

Patient characteristics: Incorporating patient characteristics like genetics, age, sex, ethnicity, comorbidities, disease features, past medications, and lifestyle factors will improve predictivity to meet patient needs. Developments in this area are emerging via the application of genetic manipulation via patient cell sourcing or knowledge of appropriate chemical stimuli to enable the development of specific phenotypes. These different approaches to incorporate patient characteristics have their advantages and disadvantages, for example, the use of CRISPR technology to induce a specific mutation enables one element of the disease phenotype to be altered at the genetic level.[150–152]

Multi-modal approaches: Future challenges to overcome include the incorporation of multiple patient characteristics into one model system; however, their value will be determined by their specific needs. In conjunction with cellular model advancements, progress in terms of endpoints and the ability to assess translatable biomarkers in a non-destructive temporal manner will be required to enable humanized drug discovery and quantitative decision-making.[153] To realize this potential, the specific context of use needs to be clearly decided before embarking on future model development. In addition, reproducibility and robustness of models need to be optimized to ensure reliable decisions can be made. Advancements in this manner will enable the development and application of model systems for drug discovery as stem cell-derived cardiomyocytes have over the past 10–15 years.

Regenerative stem cell therapies

Cell-based therapies have the potential to halt, reverse, and even cure failing hearts, with multiple different cell lines, cell types, delivery formats, and mechanistic approaches currently being investigated for their therapeutic potential. One such approach is the application of hPSC-CMs for the regeneration of lost cardiac tissue.[44,154,155] Heart failure, both ischemic and non-ischemic, is a global epidemic, with prevalence of ~2% throughout Europe and a prognosis worse than many common cancers. Chronic development of heart failure with reduced ejection fraction (HFrEF) is a significant concern, affecting >50% of patients and increasing due to population growth and aging. Patients are exposed to long-term risks: (1) reduced pump function and (2) disrupted electrical conduction leading to arrhythmic sudden cardiac death. The need to address HFrEF has led to industrial and/or academic partnerships to explore the use of various configurations, with hiPSC-CMs at their core. Thus, investigations include: (1) the state of maturity of the differentiated cells, from early progenitors to more mature ventricular cardiomyocytes. (2) Cell format, from single cells to 3D organoids. (3) Cell type composition in addition to cardiomyocytes, including lineages such epicardial, endocardial, CECs, CFs, and/or smooth muscle cells. (4) The formulation and delivery of the cell therapy, from cell suspensions injected directly into the infarcted area through to engineered cell sheets placed on the outside of the heart, with or without carrier materials.

In recent years, there has been a notable rise in *in vivo* transplantation studies conducted in rats,[124] guinea pigs,[156] porcine,[157,158] and non-human primates,[159,160] recently reviewed in Menasché[154] and Selvakumar et al.[161]. A key challenge pertaining to translatability is graft cell survival. Cardiomyocyte (CM) survival is only ~10% upon grafting into the heart of large animals, irrespective of whether delivery is via direct injection or hydrogel patches.[87] Reasons postulated include myocardial ischemia, inflammation, extensive local scarring, immune response, and lack of electromechanical coupling. Clarity is also lacking regarding the natural history of transplanted cells at a cellular level, the degree of clonality of surviving populations, and the factors that regulate cell selection and preferential growth or integration *in vivo*.

Nevertheless, promising techniques involving pharmacology and gene editing are being developed to address these challenges. For example, Marciano et al. simultaneously knocked down three depolarization-associated genes (*HCN4*, *CACNA1H*, and *SLC8A1*) and upregulated hyperpolarization-associated *KCNJ2* gene in hPSC-CMs, eliminating automaticity *in vitro*, and when transplanted *in vivo* into porcine hearts, the quadruple-gene-edited cells coupled electromechanically with host cardiomyocytes without causing engraftment arrhythmias.[150] In another encouraging study, researchers demonstrated that co-transplantation of hiPSC-CMs and hiPSC-ECs in mice and non-human primates resulted in the restoration of cardiac function and reduced arrhythmic events compared with hiPSC-CM transplantation alone.[162]

Another major challenge is graft immunogenicity. Most small animal studies use immunosuppressed rodents, lack of clarity of level of immunosuppression necessary in humans (even for allografts), and lack of humanized models. Addressing these issues with advanced models is required concurrently with approaches, such as the continued development of hypoimmune "universal" human hPSCs, novel immuno-modulators, and scaffolds that elute immunosuppressant drugs. Other challenges include the ability to track the health and function of transplanted cells *in vivo*. Since there are likely to be significant differences between animal models and humans, it is essential that we are equipped to track grafted cells in patients long term. Improved imaging modalities and novel proteomics are vital to understanding transplanted cell-host interactions in early-stage clinical trials. This is coupled with ever-advancing technologies that can augment hPSC-derived cell therapies, such as gene editing to enhance efficacy or mitigate safety.[163,164]

Concurrently with addressing these challenges *in vivo*, there has been an increasing number of first-in-human and early-stage clinical trials. In 2018, the first human trial using clinical-grade hiPSC-CMs for heart regeneration was approved.[165] Following this, several clinical studies involving hiPSC-CMs have been emerging. Notably, an academically led spin-out, Heartseed, in collaboration with the pharmaceutical company Novo Nordisk has started a first-in-human clinical study to evaluate the safety and efficacy of hiPSC-CM spheroids injected into patients with severe heart failure over a period of 26 weeks post-transplantation (clinicaltrials.gov ID: NCT04945018).[158,166] Although this study uses allogenic cells, resulting in patients requiring prolonged immunosuppressants to mitigate the risk of immunological rejection, the consideration of using autologous cells would avoid this concern. However, the substantial costs and logistics surrounding production, stability and testing, and timing involved in patient enrollment and availability of the biological material present significant challenges. Nevertheless, this study highlights an important step forward, shedding light on the safety and efficacy of cellular models for future clinical therapies.

Translating basic research into commercially viable therapies faces challenges related to the hPSC line, the processes, reagents, costs, and facilities needed for GMP manufacturing, clinical trial design, route and practical administration, and delivery of the therapy. Further considerations are determining the right commercial path for these new advanced therapy medicinal products (ATMPs),[167] the patent landscape, and the evolving requirements of regulators. Different territories have their own guidelines,[168,169] such as the European Medicines Agency (EMA) in Europe, Medicines and Healthcare products Regulatory Agency (MHRA) in the UK, MHLW in Japan, and the Food and Drug Administration (FDA) in the US, with the latter channeled via the Center for Biologics Evaluation and Research (CBER) (https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products).[170,171] This increasing landscape of novel cellular therapies, coupled with advancing models and assays in which their safety and efficacy can be assessed, offers hope to address the unmet medical need in heart disease.

Conclusions

Over the past decade, large-scale hPSC initiatives have facilitated global accessibility to stem cell banks and a range of different hPSC-derived cell types. In this perspective, we have discussed the processes and applications of hPSC-CMs for drug development and cellular therapy. To expedite their translatability, integration with other cell types and organ systems in a physiologically relevant manner necessitates collaborative efforts between research institutions, biopharmaceutical companies, research funders, and regulatory bodies. This also extends to effective communication with the public, particularly in the context of stem cell therapies. We have discussed the application of hPSC-CMs, with a particular focus on drug discovery pipelines where integration into high-throughput and automated screening platforms with multiple readouts is critical. Emergence of complex and more physiologically relevant hPSC-CM models has led to the re-evaluation of their application in an industrial and commercial setting that will ultimately determine their impact on the clinical prognosis of heart disease.

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AUTHOR CONTRIBUTIONS

Conceptualization, K.R., A.N., N.T.N.V., and C.D.; writing—original draft, all authors; visualization, K.R., A.N., and N.T.N.V.; project administration, K.R.; writing—review & editing, K.R. and C.D.; supervision, C.D. and K.R.; funding acquisition, W.S., J.F., and C.D.

DECLARATION OF INTERESTS

R.V., S.D., and S.H. are employees of FUJIFILM Cellular Dynamics International. Peter Clements is an employee of GlaxoSmithKline. R.H., A.P., and J.F. are employees of AstraZeneca. W.S. is an employee of LifeArc.

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