Published in final edited form as:

Curr Opin Pharmacol. 2018 October ; 42: 55–61. doi:10.1016/j.coph.2018.07.003.

Will iPSC-cardiomyocytes revolutionize the discovery of drugs for heart disease?

Arne A. N. Bruyneel, Wesley L. McKeithan, Dries A. M. Feyen, and Mark Mercola

The Cardiovascular Institute and Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA

Abstract

Cardiovascular disease remains the largest single cause of mortality in the Western world, despite significant advances in clinical management over the years. Unfortunately, the development of new cardiovascular medicines is stagnating and can in part be attributed to the difficulty of screening for novel therapeutic strategies due to a lack of suitable models. The advent of human induced pluripotent stem cells and the ability to make limitless numbers of cardiomyocytes could revolutionize heart disease modeling and drug discovery. This review summarizes the state of the art in the field, describes the strengths and weaknesses of the technology, and applications where the model system would be most appropriate.

Corresponding author Mark Mercola, PhD, 1651 Page Mill Road, Palo Alto, CA 94305, mmercola@stanford.edu.
Arne A. N. Bruyneel, DPhil

- 1651 Page Mill Road
- Palo Alto, CA 94305
- abruyneel@stanford.edu

Wesley L. McKeithan, PhD

- 1651 Page Mill Road
- Palo Alto, CA 94305
- wlm@stanford.edu

Dries A. M. Feyen, PhD

- 1651 Page Mill Road
- Palo Alto, CA 94305
- dfeyen@stanford.edu

Mark Mercola, PhD

- 1651 Page Mill Road
- Palo Alto, CA 94305
- mmercola@stanford.edu

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

pluripotent stem cells; cardiovascular disease; toxicity screening; drug discovery

Introduction

Cardiovascular disease (CVD) account for 1 out of every 3 deaths in the United States [1]. Despite the need, there have been relatively few new drugs over the past decade and the challenges of developing new CVD drugs have caused several major pharmaceutical companies to refocus their efforts away from the field [2]. A potential explanation for the decline in innovative new medicines is a lack of suitable models to screen for novel therapeutic strategies. An exciting new paradigm in cardiovascular drug discovery is the use of human induced pluripotent stem cell (iPSC)-derived cardiomyocytes (iPSC-CMs) and vascular cells to create in vitro models of human physiology. In addition, improvements in high throughput physiological assay technology make it now feasible to optically quantify action potentials [3,4], calcium transients [5–7], and cell motion [8] at scale. Despite the potential impact of these advances, there remains considerable debate as to whether the combination of these two technologies will revolutionize cardiovascular drug discovery. In this review, we discuss the opportunities and challenges of using iPSCCMs for cardiovascular disease modeling and the implications for drug discovery and safety assessment applications.

Induced Pluripotent Stem Cell-derived Cardiomyocytes

iPSC-CM technology has the potential to transform cardiovascular disease modeling, but important caveats limit applicability for some diseases. A major strength of the technology is that iPSC-CMs can be produced in virtually limitless quantities and, in contrast to rodent models, iPSC-CMs enable the study of human genetics and proteins in a native cellular context. This feature is particularly important when studying mechanisms that involve the interaction between multiple proteins, such as the complex signaling cascades of β -adrenergic signaling [9,10] or the interaction between pore forming α -subunits of ion channels with their ancillary subunits [11]. Additionally, advances in genome editing technologies have enabled the ability to precisely edit or introduce mutations in disease-causing genes to assess the relative contribution of a single mutation on the severity of the disease phenotype [12]. Genome editing is particularly appealing for pharmacogenomic applications as early stage drug candidates can be tested in diverse genetic backgrounds that might confer disease susceptibility.

Despite these advantages, there are clear limitations that hinder the application of iPSC technology in pharmaceutical drug discovery. A major hurdle is that iPSC-CMs are immature relative adult cardiomyocytes. Perhaps most vexing, iPSC-CMs exhibit an immature electrophysiology, morphology, mechanics and metabolism relative to their adult counterparts, despite expressing many of the same proteins and having the same ionic currents, and so more closely resemble fetal rather than adult cardiomyocytes [13–15]. Specifically, iPSC-CMs have a depolarized diastolic membrane potential compared to adult

CMs. Additionally, iPSC-CMs display a circular cell morphology with disorganized sarcomeres and, as a result, the sarcoplasmic reticulum (SR) and transverse tubule (T-tubules) network of iPSC-CMs is underdeveloped. In the absence of these structures, excitation-contraction (EC) coupling does not occur normally and contraction is mediated principally by the influx of extracellular calcium. Fortunately, improving iPSC-CM maturity is an active area of research leading to strategies to improve maturity including long-term culture, culture media alterations [16–19], tissue engineered constructs sometimes with physiological stimulation [20–23], and matrix patterning [24,25]. Interestingly, these studies have shown that altering metabolic or morphologic characteristics (e.g. growing individual cells in a 7:1 rectangular pattern) influences electrophysiological and mechanical maturity, suggesting that development of these diverse physiological processes is interrelated.

High-throughput physiological screening of cardiomyocytes

New high throughput methods to quantify the kinetics of excitation and contraction make it possible to conduct physiological screening experiments on a large scale (Fig. 1a) [26,27]. Classically, patch clamp electrophysiology is considered the gold standard for measuring action potential (AP) kinetics. High throughput electrophysiology techniques, such as automated planar patch clamp platforms, are routinely used for cell lines [28], but iPSC-CMs present technical challenges, particularly cell dissociation [29,30]. MEAs are routinely used to record extracellular field potentials that report duration of electrical excitation and can distinguish proarrhythmic versus non-proarrhythmic drugs [31]. Additionally, voltage sensitive probes (VSPs) allow for the optical recording of APs from monolayers of iPSC-CMs for both disease modeling and drug testing [3,4]. VSPs coupled to automated microscopy have advantages over MEA of cost-effectiveness and throughput.

Intracellular calcium transients link electrical excitation to contraction, and various methods have been developed to measure the kinetics of both calcium handling and contraction. For calcium handling, small molecule dyes can be used to measure calcium transient kinetics in high throughput [7,32].

To quantify contractile kinetics, optical as well as mechanical methods have been developed to study both the kinetics on contraction as well as contraction force. Contractile kinetics, not to be confused with contractile force, are typically measured with video-microscopy enables the quantification of cellular displacement from either bright-field or fluorescent time series images of single cells or monolayers of iPSC-CMs [33–36]. More complex techniques are required to measure contractile force of individual cells or monolayers including traction force microscopy (TFM) [24] and micro-post deflection assays [37]. Contractile force is the ideal measure, but current methods to quantify force are low throughput and hence cellular displacement is currently used for high-throughput characterization of contractile kinetics.

Applications of iPSC-CMs: Suitability for Purpose?

iPSC-CMs have been proposed as a new technology that might revolutionize cardiovascular drug discovery, yet their limitations have engendered considerable debate as to the

applications for which they can be reliably used. In our opinion, several criteria should be considered when utilizing iPSC-CMs to study CVDs (Fig. 1b). Ideal heart diseases to study with iPSC-CMs **a)** have a cardiomyocyte-autonomous etiology, **b)** are not predicated on three-dimensional architecture or interactions between multiple cell types and **c)** present clinically with a short latency and (if genetic) high penetrance. As a result, congenital heart diseases (CHDs) resulting from mutations in ion channels or contractile proteins are examples of diseases that are readily modeled since clinically relevant phenotypes can be recapitulated in single cardiomyocytes or monolayers of pure cardiomyocytes. Similarly, acquired disease that can be evoked in vitro, such as drug-induced arrhythmia, is possible. On the other hand, anatomical anomalies with unclear cellular etiology, or diseases with long latencies are obviously more challenging.

Heart rhythm disorders

Congenital heart rhythm disorders, such as Long QT Syndrome (LQTS), were the first to be studied using iPSC-CMs [38–42]. Disease phenotypes of other congenital arrhythmogenic disorders, including catecholaminergic polymorphic ventricular tachycardia (CPVT) [43–45] and Brugada syndrome [46,47], have been demonstrated using iPSC-CM technology. While standard iPSC-CM differentiation protocols typically generate predominantly ventricular CMs with few atrial and nodal cells, specialized protocols have been developed to yield purely ventricular or atrial iPSC-CMs by defined factors [48] or by purification [49,50]. As a result, chamber-specific iPSC-CMs can be utilized to study atrial- or ventricular-specific pharmacology [51]. Advances in both cellular maturity and multicellular and/or 3D assay systems will likely improve our ability to gain insight into arrhythmia mechanisms involving structural and conduction abnormalities or even morphological remodeling.

Cardiomyopathies

Common familial forms of cardiomyopathy fall into three major classes: hypertrophic cardiomyopathy (HCM) [18,52,53], dilated cardiomyopathy (DCM) [54–57], and arrhythmogenic right ventricular cardiomyopathy (ARVC) [58–60]. Many of the mutations associated with HCM and DCM patients are located in sarcomeric genes, whereas ARVC mutations cluster predominately in desmosomal proteins [61]. Considering the latency and incomplete penetrance of cardiomyopathies within the patient population, it is likely that evoking robust pathological phenotypes will require physiological maturation of iPSCCMs. Furthermore, additional stressors to mimic environmental factors could exacerbate cardiomyopathy phenotypes in iPSC-CMs. In this regard, several studies have implemented both chemical and mechanical techniques to mature patient-specific iPSCCMs provoke a pathological phenotype in vitro. Birker et al. [18] developed a triiodothyronine hormone (T3)-based method to promote a contractile HCM phenotype in a *MYBPC3*-c.2373dupG iPSC-CM line using TFM. Engineered heart tissues (EHTs) have shown promise in modeling DCM as PLN-R14del mutant iPSC-CMs developed dramatically lower twitch force compared to the isogenic counterparts [57]. To model ARVC, Kim et al [60] treated the iPSC-CMs with a 3-factor cocktail to increase fatty acid oxidation, which is a hallmark of more mature CMs. By activating PPAR- γ with small molecules (5-factor), hallmark traits of ARVC (fat deposits, apoptosis) were induced in PKP2 mutant iPSC-CMs. As illustrated

by these studies, specific culture conditions and maturation-enhancing refinements are key for successful modeling of adult-onset cardiomyopathies.

Cardiac safety

Maybe the most immediate application of iPSC-CMs is their use for safety assessment of preclinical drug candidates. For example, the major reason for drug failure during development or withdrawal after approval is due to an increased risk for Torsades de Pointes (TdP), ventricular arrhythmia or sudden cardiac death. TdP risk is classically predicted by measuring inhibition of the rapidly-activating component of the delayed rectifier potassium current (I_{Kr}) [62]. However, I_{Kr} inhibition alone is not a robust predictor TdP or lethal arrhythmia. Although the current standard has kept potentially toxic drugs off the market, many safe drugs might have been abandoned [63–67]. The native, albeit immature, electrophysiology and the ability to conduct high throughput assays make iPSC-CM testing attractive to insert early in the drug development pipeline, perhaps at the point of hit to lead medicinal chemistry refinement [8,68–70]. The suitability of iPSCCMs for prediction of arrhythmic cardiotoxicity is currently being investigated by the FDA, pharmaceutical companies and academics as part of the Comprehensive in vitro Proarrhythmia Assay (CiPA) initiative [71–73].

Various drugs also induce cardiomyopathy that can lead to major adverse cardiac events and heart failure [74]. iPSC-CMs might be an appropriate model to assess myopathic liability. For instance, Burrige et al. [75] investigated the mechanism of doxorubicin toxicity by comparing iPSC-CMs derived from patients with differential cardiac susceptibility to doxorubicin. Sharma et al. [8] determined the cardiotoxic propensity of anti-cancer kinase inhibitors in iPSC-CMs, and identified the targeted signaling pathways associated with increased risk. Similarly, Schneider et al. [76] investigated the molecular mechanisms underpinning sorafenib cardiotoxicity, and determined that phosphorylation of phospholamban, a key component of EC coupling, was affected.

Drug discovery and target identification

The drug discovery process typically follows one of two approaches: (1) target-based screening, or (2) phenotypic screening. Phenotypic screening, or screening for modulation of a disease-linked phenotype in a target-agnostic fashion, was historically dominant, but was largely abandoned as a more thorough understanding of biochemistry and physiology sparked a target-driven approach [77,78]. However, phenotypic screens have contributed to about twice as many first-in-class small molecule drugs as solely target-centric approaches [79,80]. Target-based approaches do not take into account the role of a target in the disease setting, and underrepresent potentially useful molecular mechanisms of action. Phenotypic screening also has limitations, primarily a more laborious and technically challenging assay development, and for iPSC-CMs, more complex assay modalities such as high content and kinetic imaging. These constraints lead to a lower throughput than target-driven biochemical or cellular assays, thus limiting the chemical space that can be probed. Moreover, identification of targets of hits obtained from phenotypic screens can be quite challenging and constrain hit advancement, including medicinal chemical optimization of the

compounds or safety profiling. For these reasons, successful programs often combine both target understanding as well as phenotypic cellular assays to derive drug candidates [78].

Vincent et al. [81] described three criteria for the most predictive phenotypic assays: (1) disease relevance of the assay system, (2) disease relevance of the mechanism that evokes the phenotype, and (3) assay readout proximity to the clinical endpoint. A panel of human patient iPSC-CMs is likely to confer disease relevance, provided that the underlying disease mechanisms are sufficiently represented in the simple cultures, and the stimulus is carefully chosen especially for delayed-onset diseases, as discussed above. Using differentiated derivatives, such as iPSC-CMs, to recapitulate a clinically relevant readout in a high throughput assay, it should be possible to use chemical and functional genomics (e.g. siRNA and CRISPR screening) to elucidate disease mechanisms and identify novel therapeutic targets. For the first time, it is possible to functionally scan the proteome to identify proteins that can be inhibited to ameliorate disease manifestations *in vitro*. We expect this approach to reveal a wealth of candidate therapeutic targets that can be validated in animal models and/or clinical genetics. Moreover, funneling the targets into conventional target-centric screens, should generate tool compounds to test the utility of pharmacological inhibition in pre-clinical models.

Conclusions

Phenotypic screening of iPSC-CMs offers the possibility to bring human disease context into the earliest stages of the drug discovery process. We argue that iPSC-CMs are suitable for functional screening in target identification, primary and secondary assays, and cardiac safety. Immaturity, homogeneity, and lack of anatomically appropriate 3D architecture are the main limitations that must be considered in evaluating suitability of iPSC-CMs for particular applications. In the near term, the technology seems most appropriate for modeling diseases with cardiomyocyte autonomous etiology, lack of complex structural presentations, and short latency - consistent with successes in the field in modeling inherited ion channel disorders and myopathies. Maturing the cells to produce more adult-like myocytes is essential and probably linked to the problem of recreating disease-relevant architecture. With more work to address these two issues and reducing them to a screenable setting, the applications of this new technology should expand even further and increase our ability to understand and ultimately treat heart disease.

Acknowledgements

We gratefully acknowledge support from the National Institutes of Health (NIH) (R01HL132225 and R01HL130840 to MM). D.A.M.F. is a Marie Skłodowska-Curie postdoctoral fellow.

References

1. Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, Chiuve SE, Cushman M, Delling FN, Deo R, et al.: Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation* 2018, 137:e67–e492.
2. Fordyce CB, Roe MT, Ahmad T, Libby P, Borer JS, Hiatt WR, Bristow MR, Packer M, Wasserman SM, Braunstein N, et al.: Cardiovascular drug development: is it dead or just hibernating? *J. Am. Coll. Cardiol* 2015, 65:1567–1582. [PubMed: 25881939]

- 3*. Paper describing high-throughput calcium and voltage recording of hiPSC-CMs. Bedut S, Seminatore-Nole C, Lamamy V, Caignard S, Boutin JA, Nosjean O, Stephan J-P, Coge F: High-throughput drug profiling with voltage- and calcium-sensitive fluorescent probes in human iPSC-derived cardiomyocytes. *AJP: Heart and Circulatory Physiology* 2016, 311:H44–53. [PubMed: 27199128]
- 4*. Paper describing high-throughput voltage recording of hiPSC-CMs and visualization of arrhythmia phenotypes. McKeithan WL, Savchenko A, Yu MS, Cerignoli F, Bruyneel AAN, Price JH, Colas AR, Miller EW, Cashman JR, Mercola M: An Automated Platform for Assessment of Congenital and Drug-Induced Arrhythmia with hiPSC-Derived Cardiomyocytes. *Front Physiol* 2017, 8:766. [PubMed: 29075196]
5. Cerignoli F, Charlot D, Whittaker R, Ingermanson R, Gehalot P, Savchenko A, Gallacher DJ, Towart R, Price JH, McDonough PM, et al.: High throughput measurement of Ca^{2+} dynamics for drug risk assessment in human stem cell-derived cardiomyocytes by kinetic image cytometry. *Journal of Pharmacological and Toxicological Methods* 2012, 66:246–256. [PubMed: 22926323]
6. Lu HR, Whittaker R, Price JH, Vega R, Pfeiffer ER, Cerignoli F, Towart R, Gallacher DJ: High Throughput Measurement of Ca^{++} Dynamics in Human Stem Cell-Derived Cardiomyocytes by Kinetic Image Cytometry: A Cardiac Risk Assessment Characterization Using a Large Panel of Cardioactive and Inactive Compounds. *Toxicol. Sci* 2015, 148:503–516. [PubMed: 26358003]
7. Daily NJ, Santos R, Vecchi J, Kemanli P, Wakatsuki T: Calcium Transient Assays for Compound Screening with Human iPSC-derived Cardiomyocytes: Evaluating New Tools. *JESR* 2017, 1:1–11. [PubMed: 28966998]
- 8*. Cardiac safety assessment of tyrosine kinase inhibitors as targeted therapeutics for cancer. Many of these drugs are known to cause arrhythmia and cardiomyopathy. This paper utilized a high-throughput contractility assay and patient derived hiPSCCMs as read-out. Sharma A, Burridge PW, McKeithan WL, Serrano R, Shukla P, Sayed N, Churko JM, Kitani T, Wu H, Holmström A, et al.: High-throughput screening of tyrosine kinase inhibitor cardiotoxicity with human induced pluripotent stem cells. *Sci Transl Med* 2017, 9:eaaf2584. [PubMed: 28202772]
9. Lundby A, Andersen MN, Steffensen AB, Horn H, Kelstrup CD, Francavilla C, Jensen LJ, Schmitt N, Thomsen MB, Olsen JV: In vivo phosphoproteomics analysis reveals the cardiac targets of β -adrenergic receptor signaling. *Sci. Signal* 2013, 6:rs11. [PubMed: 23737553]
10. Fuller SJ, Osborne SA, Leonard SJ, Hardyman MA, Vaniotis G, Allen BG, Sugden PH, Clerk A: Cardiac protein kinases: the cardiomyocyte kinome and differential kinase expression in human failing hearts. *Cardiovasc. Res* 2015, 108:87–98. [PubMed: 26260799]
11. Pongs O, Schwarz JR: Ancillary Subunits Associated With Voltage-Dependent K^{+} Channels. *Physiological Reviews* 2010, 90:755–796. [PubMed: 20393197]
12. Termglinchan V, Karakikes I, Seeger T, Wu JC: Current Status of Genome Editing in Cardiovascular Medicine In *Genome Editing*. Springer, Cham; 2016:107–126.
13. Hoekstra M, Mummery CL, Wilde AAM, Bezzina CR, Verkerk AO: Induced pluripotent stem cell derived cardiomyocytes as models for cardiac arrhythmias. *Front Physiol* 2012, 3:346. [PubMed: 23015789]
14. Veerman CC, Kosmidis G, Mummery CL, Casini S, Verkerk AO, Bellin M: Immaturity of human stem-cell-derived cardiomyocytes in culture: fatal flaw or soluble problem? *Stem Cells and Development* 2015, 24:1035–1052. [PubMed: 25583389]
15. Robertson C, Tran DD, George SC: Concise Review: Maturation Phases of Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Stem Cells* 2013, 31:829–837. [PubMed: 23355363]
16. Tohyama S, Hattori F, Sano M, Hishiki T, Nagahata Y, Matsuura T, Hashimoto H, Suzuki T, Yamashita H, Satoh Y, et al.: Distinct metabolic flow enables large-scale purification of mouse and human pluripotent stem cell-derived cardiomyocytes. *Cell Stem Cell* 2013, 12:127–137. [PubMed: 23168164]
17. Yang X, Rodriguez M, Pabon L, Fischer KA, Reinecke H, Regnier M, Sniadecki NJ, Ruohola-Baker H, Murry CE: Tri-iodo-L-thyronine promotes the maturation of human cardiomyocytes-derived from induced pluripotent stem cells. *J Mol Cell Cardiol* 2014, 72:296–304. [PubMed: 24735830]
18. Birket MJ, Ribeiro MC, Kosmidis G, Ward D, Leitguinho AR, van de Pol V, Dambrot C, Devalla HD, Davis RP, Mastroberardino PG, et al.: Contractile Defect Caused by Mutation in MYBPC3

Revealed under Conditions Optimized for Human PSC-Cardiomyocyte Function. *Cell Reports* 2015, 13:733–745. [PubMed: 26489474]

19. Parikh SS, Blackwell DJ, Gomez-Hurtado N, Frisk M, Wang L, Kim K, Dahl CP, Fiane A, Tønnessen T, Kryshstal DO, et al.: Thyroid and Glucocorticoid Hormones Promote Functional T-Tubule Development in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Novelty and Significance. *Circulation Research* 2017, 121:1323–1330. [PubMed: 28974554]
20. Hirt MN, Boeddinghaus J, Mitchell A, Schaaf S, Börnchen C, Müller C, Schulz H, Hubner N, Stenzig J, Stoehr A, et al.: Functional improvement and maturation of rat and human engineered heart tissue by chronic electrical stimulation. *J Mol Cell Cardiol* 2014, 74:151–161. [PubMed: 24852842]
21. Liaw NY, Zimmermann W-HH: Mechanical stimulation in the engineering of heart muscle. *Advanced Drug Delivery Reviews* 2016, 96:156–160. [PubMed: 26362920]
22. Lemoine MD, Mannhardt I, Breckwoldt K, Prondzynski M, Flenner F, Ulmer B, Hirt MN, Neuber C, Horváth A, Kloth B, et al.: Human iPSC-derived cardiomyocytes cultured in 3D engineered heart tissue show physiological upstroke velocity and sodium current density. *Sci. Rep* 2017, 7:5464. [PubMed: 28710467]
23. Ronaldson-Bouchard K, Ma SP, Yeager K, Chen T, Song L, Sirabella D, Morikawa K, Teles D, Yazawa M, Vunjak-Novakovic G: Advanced maturation of human cardiac tissue grown from pluripotent stem cells. *Nature* 2018, 13:713.
24. Ribeiro AJS, Ang Y-S, Fu J-D, Rivas RN, Mohamed TMA, Higgs GC, Srivastava D, Pruitt BL: Contractility of single cardiomyocytes differentiated from pluripotent stem cells depends on physiological shape and substrate stiffness. *Proceedings of the National Academy of Sciences* 2015, 112:12705–12710.
25. Herron TJ, Rocha AMD, Campbell KF, Ponce-Balbuena D, Willis BC, Guerrero-Serna G, Liu Q, Klos M, Musa H, Zarzoso M, et al.: Extracellular Matrix-Mediated Maturation of Human Pluripotent Stem Cell-Derived Cardiac Monolayer Structure and Electrophysiological Function. *Circulation: Arrhythmia and Electrophysiology* 2016, 9:e003638.
26. Mercola M, Colas A, Willems E: Induced pluripotent stem cells in cardiovascular drug discovery. *Circulation Research* 2013, 112:534–548. [PubMed: 23371902]
27. del Álamo JC, Lemons D, Serrano R, Savchenko A, Cerignoli F, Bodmer R, Mercola M: High throughput physiological screening of iPSC-derived cardiomyocytes for drug development. *Biochim. Biophys. Acta* 2016, 1863:1717–1727. [PubMed: 26952934]
28. Dunlop J, Bowlby M, Peri R, Vasilyev D, Arias R: High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. *Nat Rev Drug Discov* 2008, 7:358–368. [PubMed: 18356919]
29. Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, Kolaja KL, Swanson BJ, January CT: High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. *Am. J. Physiol. Heart Circ. Physiol* 2011, 301:H2006–17. [PubMed: 21890694]
30. Rajamohan D, Kalra S, Duc Hoang M, George V, Staniforth A, Russell H, Yang X, Denning C: Automated Electrophysiological and Pharmacological Evaluation of Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Stem Cells and Development* 2016, 25:439–452. [PubMed: 26906236]
31. Asakura K, Hayashi S, Ojima A, Taniguchi T, Miyamoto N, Nakamori C, Nagasawa C, Kitamura T, Osada T, Honda Y, et al.: Improvement of acquisition and analysis methods in multi-electrode array experiments with iPS cell-derived cardiomyocytes. *Journal of Pharmacological and Toxicological Methods* 2015, 75:17–26. [PubMed: 25910965]
32. Sirenko O, Crittenden C, Callamaras N, Hesley J, Chen Y-W, Funes C, Rusyn I, Anson B, Cromwell EF: Multiparameter in vitro assessment of compound effects on cardiomyocyte physiology using iPSC cells. *J Biomol Screen* 2013, 18:39–53. [PubMed: 22972846]
33. Hayakawa T, Kunihiro T, Ando T, Kobayashi S, Matsui E, Yada H, Kanda Y, Kurokawa J, Furukawa T: Image-based evaluation of contraction-relaxation kinetics of human-induced pluripotent stem cell-derived cardiomyocytes: Correlation and complementarity with extracellular electrophysiology. *J Mol Cell Cardiol* 2014, 77:178–191. [PubMed: 25257913]

34. Czirok A, Isai DG, Kosa E, Rajasingh S, Kinsey W, Neufeld Z, Rajasingh J: Optical-flow based non-invasive analysis of cardiomyocyte contractility. *Sci. Rep* 2017, 7:10404. [PubMed: 28871207]
35. Sala L, van Meer BJ, Tertoolen LGJ, Bakkers J, Bellin M, Davis RP, Denning C, Dieben MAE, Eschenhagen T, Giacomelli E, et al.: MUSCLEMOTION: A Versatile Open Software Tool to Quantify Cardiomyocyte and Cardiac Muscle Contraction In Vitro and In Vivo. *Circulation Research* 2018, 122:e5–e16. [PubMed: 29282212]
36. Huebsch N, Loskill P, Mandegar MA, Marks NC, Sheehan AS, Ma Z, Mathur A, Nguyen TN, Yoo JC, Judge LM, et al.: Automated Video-Based Analysis of Contractility and Calcium Flux in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes Cultured over Different Spatial Scales. *Tissue Engineering Part C: Methods* 2015, 21:467–479. [PubMed: 25333967]
37. Beussman KM, Rodriguez ML, Leonard A, Taparia N, Thompson CR, Sniadecki NJ: Micropost arrays for measuring stem cell-derived cardiomyocyte contractility. *Methods* 2016, 94:43–50. [PubMed: 26344757]
38. Moretti A, Bellin M, Welling A, Jung CB, Lam JT, Bott-Flügel L, Dorn T, Goedel A, Höhnke C, Hofmann F, et al.: Patient-specific induced pluripotent stem-cell models for long-QT syndrome. *N. Engl. J. Med* 2010, 363:1397–1409. [PubMed: 20660394]
39. Matsa E, Rajamohan D, Dick E, Young L, Mellor I, Staniforth A, Denning C: Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation. *European Heart Journal* 2011, 32:952–962. [PubMed: 21367833]
40. Itzhaki I, Maizels L, Huber I, Zwi-Dantsis L, Caspi O, Winterstern A, Feldman O, Gepstein A, Arbel G, Hammerman H, et al.: Modelling the long QT syndrome with induced pluripotent stem cells. *Nature* 2011, 471:225–229. [PubMed: 21240260]
41. Yazawa M, Hsueh B, Jia X, Pasca AM, Bernstein JA, Hallmayer J, Dolmetsch RE: Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature* 2011, 471:230–234. [PubMed: 21307850]
42. Lahti AL, Kujala VJ, Chapman H, Koivisto AP, Pekkanen-Mattila M, Kerkela E, Hyttinen J, Kontula K, Swan H, Conklin BR, et al.: Model for long QT syndrome type 2 using human iPSC cells demonstrates arrhythmogenic characteristics in cell culture. *Disease Models and Mechanisms* 2012, 5:220–230. [PubMed: 22052944]
43. Jung CB, Moretti A, Mederos y Schnitzler M, Iop L, Storch U, Bellin M, Dorn T, Ruppenthal S, Pfeiffer S, Goedel A, et al.: Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia. *EMBO Mol Med* 2012, 4:180–191. [PubMed: 22174035]
44. Di Pasquale E, Lodola F, Miragoli M, Denegri M, Avelino-Cruz JE, Buonocore M, Nakahama H, Portararo P, Bloise R, Napolitano C, et al.: CaMKII inhibition rectifies arrhythmic phenotype in a patient-specific model of catecholaminergic polymorphic ventricular tachycardia. *Cell Death Dis* 2013, 4:e843. [PubMed: 24113177]
45. Penttinen K, Swan H, Vanninen S, Paavola J, Lahtinen AM, Kontula K, Aalto-Setälä K: Antiarrhythmic Effects of Dantrolene in Patients with Catecholaminergic Polymorphic Ventricular Tachycardia and Replication of the Responses Using iPSC Models. *PLoS ONE* 2015, 10:e0125366–17. [PubMed: 25955245]
46. Liang P, Sallam K, Wu H, Li Y, Itzhaki I, Garg P, Zhang Y, Vermglinchan V, Lan F, Gu M, et al.: Patient-Specific and Genome-Edited Induced Pluripotent Stem Cell-Derived Cardiomyocytes Elucidate Single-Cell Phenotype of Brugada Syndrome. *J. Am. Coll. Cardiol* 2016, 68:2086–2096. [PubMed: 27810048]
47. Selga E, Sendfeld F, Martinez-Moreno R, Medine CN, Tura-Ceide O, Wilmut SI, Pérez GJ, Scornik FS, Brugada R, Mills NL: Sodium channel current loss of function in induced pluripotent stem cell-derived cardiomyocytes from a Brugada syndrome patient. *J Mol Cell Cardiol* 2018, 114:10–19. [PubMed: 29024690]
48. Zhang Q, Jiang J, Han P, Yuan Q, Zhang J, Zhang X, Xu Y, Cao H, Meng Q, Chen L, et al.: Direct differentiation of atrial and ventricular myocytes from human embryonic stem cells by alternating retinoid signals. *Cell Res* 2010, 21:579–587. [PubMed: 21102549]

49. Bizio A, Guerrero-Serna G, Hu B, Ponce-Balbuena D, Willis BC, Zarzoso M, Ramirez RJ, Sener MF, Mundada LV, Klos M, et al.: Myosin light chain 2-based selection of human iPSC-derived early ventricular cardiac myocytes. *Stem Cell Res* 2013, 11:1335–1347. [PubMed: 24095945]
50. Josowitz R, Lu J, Falce C, D'Souza SL, Wu M, Cohen N, Dubois NC, Zhao Y, Sobie EA, Fishman GI, et al.: Identification and purification of human induced pluripotent stem cell-derived atrial-like cardiomyocytes based on sarcolipin expression. *PLoS ONE* 2014, 9:e101316. [PubMed: 25010565]
51. Devalla HD, Schwach V, Ford JW, Milnes JT, El-Haou S, Jackson C, Gkatzis K, Elliott DA, Chuva de Sousa Lopes SM, Mummery CL, et al.: Atrial-like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial-selective pharmacology. *EMBO Mol Med* 2015, 7:394–410. [PubMed: 25700171]
52. Lan F, Lee AS, Liang P, Sanchez-Freire V, Nguyen PK, Wang L, Han L, Yen M, Wang Y, Sun N, et al.: Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. *Cell Stem Cell* 2013, 12:101–113. [PubMed: 23290139]
53. Han L, Li Y, Tchao J, Kaplan AD, Lin B, Li Y, Mich-Basso J, Lis A, Hassan N, London B, et al.: Study familial hypertrophic cardiomyopathy using patient-specific induced pluripotent stem cells. *Cardiovasc. Res* 2014, 104:258–269. [PubMed: 25209314]
54. Sun N, Yazawa M, Liu J, Han L, Sanchez-Freire V, Abilez OJ, Navarrete EG, Hu S, Wang L, Lee A, et al.: Patient-Specific Induced Pluripotent Stem Cells as a Model for Familial Dilated Cardiomyopathy. 2012, 4:130ra47–130ra47.
55. Streckfuss-Bömeke K, Tiburcy M, Fomin A, Luo X, Li W, Fischer C, Özcelik C, Perrot A, Sossalla S, Haas J, et al.: Severe DCM phenotype of patient harboring RBM20 mutation S635A can be modeled by patient-specific induced pluripotent stem cell-derived cardiomyocytes. *J Mol Cell Cardiol* 2017, 113:9–21. [PubMed: 28941705]
56. Wyles SP, Hrstka SC, Reyes S, Terzic A, Olson TM, Nelson TJ: Pharmacological Modulation of Calcium Homeostasis in Familial Dilated Cardiomyopathy: An In Vitro Analysis From an RBM20 Patient-Derived iPSC Model. *Clinical And Translational Science* 2016, 9:158–167. [PubMed: 27105042]
57. Stillitano F, Turnbull IC, Karakikes I, Nonnenmacher M, Backeris P, Hulot JS, Kranias EG, Hajjar RJ, Costa KD: Genomic correction of familial cardiomyopathy in human engineered cardiac tissues. *European Heart Journal* 2016, 37:3282–3284. [PubMed: 27450564]
58. Ma D, Wei H, Lu J, Ho S, Zhang G, Sun X, Oh Y, Tan SH, Ng ML, Shim W, et al.: Generation of patient-specific induced pluripotent stem cell-derived cardiomyocytes as a cellular model of arrhythmogenic right ventricular cardiomyopathy. *European Heart Journal* 2013, 34:1122–1133. [PubMed: 22798562]
59. Caspi O, Huber I, Gepstein A, Arbel G, Maizels L, Boulos M, Gepstein L: Modeling of arrhythmogenic right ventricular cardiomyopathy with human induced pluripotent stem cells. *Circulation: Cardiovascular Genetics* 2013, 6:557–568. [PubMed: 24200905]
60. Kim C, Wong J, Wen J, Wang S, Wang C, Spiering S, Kan NG, Forcales S, Puri PL, Leone TC, et al.: Studying arrhythmogenic right ventricular dysplasia with patient-specific iPSCs. *Nature* 2013, 494:105–110. [PubMed: 23354045]
61. Hershberger RE, Hedges DJ, Morales A: Dilated cardiomyopathy: the complexity of a diverse genetic architecture. *Nat Rev Cardiol* 2013, 10:531–547. [PubMed: 23900355]
62. Bowes J, Brown AJ, Hamon J, Jarolimek W, Sridhar A, Waldron G, Whitebread S: Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. *Nat Rev Drug Discov* 2012, 11:909–922. [PubMed: 23197038]
63. Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PKS, Strang I, Sullivan AT, Wallis R, et al.: Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc. Res* 2003, 58:32–45. [PubMed: 12667944]
64. Kramer J, Obejero-Paz CA, Myatt G, Kuryshv YA, Bruening-Wright A, Verducci JS, Brown AM: MICE models: superior to the HERG model in predicting Torsade de Pointes. *Sci. Rep* 2013, 3:2100. [PubMed: 23812503]

65. Shah RR, Hondeghem LM: Refining detection of drug-induced proarrhythmia: QT interval and TRIaD. *Heart Rhythm* 2005, 2:758–772. [PubMed: 15992736]
66. Roden DM: Mechanisms and management of proarrhythmia. *Am. J. Cardiol* 1998, 82:49I–57I.
67. Lacerda AE, Kuryshv YA, Chen Y, Renganathan M, Eng H, Danthi SJ, Kramer JW, Yang T, Brown AM: Alfuzosin delays cardiac repolarization by a novel mechanism. *Journal of Pharmacology and Experimental Therapeutics* 2008, 324:427–433. [PubMed: 17986649]
68. Clements M, Thomas N: High-Throughput Multi-Parameter Profiling of Electrophysiological Drug Effects in Human Embryonic Stem Cell Derived Cardiomyocytes Using Multi-Electrode Arrays. *Toxicol. Sci* 2014, 140:445–461. [PubMed: 24812011]
69. Matsa E, BurrIDGE PW, Yu K-H, Ahrens JH, Termglinchan V, Wu H, Liu C, Shukla P, Sayed N, Churko JM, et al.: Transcriptome Profiling of Patient-Specific Human iPSC-Cardiomyocytes Predicts Individual Drug Safety and Efficacy Responses In Vitro. *Cell Stem Cell* 2016, 19:311–325. [PubMed: 27545504]
70. Liang P, Lan F, Lee AS, Gong T, Sanchez-Freire V, Wang Y, Diecke S, Sallam K, Knowles JW, Wang PJ, et al.: Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 2013, 127:1677–1691. [PubMed: 23519760]
- 71*. CiPA initiative paper exploring the suitability of hiPSC-CM for drug safety testing. Blinova K, Stohlman J, Vicente J, Chan D, Johannesen L, Hortigon-Vinagre MP, Zamora V, Smith G, Crumb WJ, Pang L, et al.: Comprehensive Translational Assessment of Human-Induced Pluripotent Stem Cell Derived Cardiomyocytes for Evaluating Drug-Induced Arrhythmias. *Toxicol. Sci* 2017, 155:234–247. [PubMed: 27701120]
- 72*. CiPA initiative paper exploring the suitability of hiPSC-CM for drug safety testing. Ando H, Yoshinaga T, Yamamoto W, Asakura K, Uda T, Taniguchi T, Ojima A, Shinkyo R, Kikuchi K, Osada T, et al.: A new paradigm for drug-induced torsadogenic risk assessment using human iPSC cell-derived cardiomyocytes. *Journal of Pharmacological and Toxicological Methods* 2017, 84:111–127. [PubMed: 27956204]
73. Huang H, Pugsley MK, Fermini B, Curtis MJ, Koerner J, Accardi M, Authier S: Cardiac voltage-gated ion channels in safety pharmacology: Review of the landscape leading to the CiPA initiative. *Journal of Pharmacological and Toxicological Methods* 2017, 87:11–23. [PubMed: 28408211]
74. Yeh ETH, Bickford CL: Cardiovascular complications of cancer therapy: incidence, pathogenesis, diagnosis, and management. *J. Am. Coll. Cardiol* 2009, 53:2231–2247. [PubMed: 19520246]
75. BurrIDGE PW, Li YF, Matsa E, Wu H, Ong S-G, Sharma A, Holmström A, Chang AC, Coronado MJ, Ebert AD, et al.: Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. *Nat Med* 2016, 22:547–556. [PubMed: 27089514]
76. Schneider C, Wallner M, Kolesnik E, Herbst V, Mächler H, Pichler M, Lewinski von D, Sedej S, Rainer PP: The Anti-Cancer Multikinase Inhibitor Sorafenib Impairs Cardiac Contractility by Reducing Phospholamban Phosphorylation and Sarcoplasmic Calcium Transients. *Sci. Rep* 2018, 8:5295. [PubMed: 29593308]
77. Terstappen GC, Schlüpen C, Raggiaschi R, Gaviraghi G: Target deconvolution strategies in drug discovery. *Nat Rev Drug Discov* 2007, 6:891–903. [PubMed: 17917669]
- 78*. Review on the use of phenotypic assays in drug discovery. Moffat JG, Vincent F, Lee JA, Eder J, Prunotto M: Opportunities and challenges in phenotypic drug discovery: an industry perspective. *Nat Rev Drug Discov* 2017, 16:531–543. [PubMed: 28685762]
- 79*. Seminal analysis of phenotypic versus target-centric drug screening. Swinney DC, Anthony J: How were new medicines discovered? *Nat Rev Drug Discov* 2011, 10:507–519. [PubMed: 21701501]
80. Swinney DC: Phenotypic vs. target-based drug discovery for first-in-class medicines. *Clinical Pharmacology & Therapeutics* 2013, 93:299–301. [PubMed: 23511784]
81. Vincent F, Loria P, Pregel M, Stanton R, Kitching L, Nocka K, Doyonnas R, Stepan C, Gilbert A, Schroeter T, et al.: Developing predictive assays: The phenotypic screening “rule of 3.” *Sci Transl Med* 2015, 7:293ps15–293ps15.
82. Green EM, Wakimoto H, Anderson RL, Evanchik MJ, Gorham JM, Harrison BC, Henze M, Kawas R, Oslob JD, Rodriguez HM, et al.: A small-molecule inhibitor of sarcomere contractility

suppresses hypertrophic cardiomyopathy in mice. *Science* 2016, 351:617–621. [PubMed: 26912705]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights:

- Physiological screening of iPSC-CMs is a powerful new tool for drug discovery
- Advantages include human genetics, cellular context and throughput
- Caveats include immature metabolic, mechanical and electrical physiology
- The platform might revolutionize some, but clearly not all, applications

Figure 1: Physiological screening of iPSC-CMs – assay readouts and applications.

(a) Induced pluripotent stem cells can be derived from patients and differentiated to virtually all cell types of the body, including cardiomyocytes (iPSC-CMs). By utilizing fluorescent dyes excitation-contraction coupling of cardiomyocytes can be measured in high throughput using automated microscopy platforms. Ibutilide is a class III anti-arrhythmic that induces a dose-dependent prolongation of APD and early after depolarizations. Isoproterenol is a non-selective β -adrenoceptor agonist that causes increased beat rate and fastened the decay kinetics of the calcium transients in iPSCCMs. Mavacamten (MYK-461) is an inhibitor of the cardiac myosin heavy chain ATPase [82] that reduces contractile motion of iPSC-CMs.

(b) iPSC-CMs have advantages and limitations that govern their utility of the model for specific applications. While iPSC-CMs mimic the human context and can be used in high throughput assays, immature physiology and simple cultures are limitations. As a result, disease manifestations need to be CM autonomous and have early onset or can be provoked are most appropriate for study with iPSC-CMs. Examples include channelopathies, cardiomyopathies, and cardiac safety testing.