Role of induced pluripotent stem cells in diagnostic cardiology

Steven B Karch, Vittorio Fineschi, Pietro Francia, Matteo Scopetti, Martina Padovano, Federico Manetti, Alessandro Santurro, Paola Frati, Massimo Volpe

Abstract

Ethical concerns about stem cell-based research have delayed important advances in many areas of medicine, including cardiology. The introduction of induced pluripotent stem cells (iPSCs) has supplanted the need to use human stem cells for most purposes, thus eliminating all ethical controversies. Since then, many new avenues have been opened in cardiology research, not only in approaches to tissue replacement but also in the design and testing of antiarrhythmic drugs. This methodology has advanced to the point where induced human cardiomyocyte cell lines can now also be obtained from commercial sources or tissue banks. Initial studies with readily available iPSCs have generally confirmed that their behavioral characteristics accurately predict the behavior of beating cardiomyocytes in vitro. As a result, iPSCs can provide new ways to study arrhythmias and heart disease in general, accelerating the development of new, more effective antiarrhythmic drugs, clinical diagnoses, and personalized medical care. The focus on producing cardiomyocytes that can be used to replace damaged heart tissue has somewhat diverted interest in a host of other applications. This manuscript is intended to provide non-specialists with a brief introduction and overview of the research carried out in the field of heart rhythm disorders.
Amiodarone’s most feared side effect is fatal pulmonary interstitial fibrosis, but ventricular tachyarrhythmias and can be life-saving, but can also produce lethal side difficulties of drug animal and human which can pose a danger to patients if unrecognized differences emerge between effective antiarrhythmic drugs; animal-to-human extrapolation is an uncertain process, problematic predictors of arrhythmia occurrence because of anatomic variations that occur during development between animal and human models which can pose a danger to patients if unrecognized differences emerge between animal and human models.

The problems associated with the use of amiodarone and sotalol illustrate the difficulties of drug development. Both drugs are used to treat atrial and ventricular tachyarrhythmias and can be life-saving, but can also produce lethal side effects. Unfortunately, predicting side effects is, at this moment, impossible. Amiodarone’s most feared side effect is fatal pulmonary interstitial fibrosis, but...
hepatitis, hypothyroidism (probably irreversible) and mixed sensorimotor polyneuropathy have all been reported with some regularity. Amiodarone’s most important complications are QT prolongation and TdP [20]. If new and safer replacement drugs are ever to be developed and approved by the United States Food and Drug Administration and the European Medicines Agency, developers will first have to establish that new drugs do not produce predictable untoward side effects or exacerbate the conditions they were designed to treat.

The availability of iPSCs allows researchers to make reasonably accurate predictions about what effect any new drug will have on the heart and its electrical system. Cultured iPSCs, can be used to construct in vitro models of the human cardiac conduction system. The effects observed in vitro can then be used to predict how, and/or whether, a drug will alter electrical conduction, or produce structural alterations in humans. The process is not as simple as it sounds and some knowledge of the subject is crucial to clinicians for the safe use of new drugs.

UNDERLYING PHYSIOLOGY AND CLINICAL MANIFESTATIONS

Cures for cardiac conduction diseases will only be found when their root causes are fully elucidated. Even physicians who have nothing to do with arrhythmia research should retain some knowledge of the molecular biology that underlies cardiac conduction.

The cardiomyocyte repolarization/depolarization cycle begins with a current generated by the outward flow of potassium ions through specific pores or channels. Potassium pores exist in all life forms and many different types have been identified (more than 20). Two types of potassium channels are absolutely critical to the process of cardiac repolarization: The rapid delayed rectifier current (identified as IKr) and the slow delayed rectifier current (identified as IKs). If a drug or a mutation disrupts either of these two currents, the action potential of the cell is prolonged with an increase in the time required for electrical depolarization and repolarization of the ventricles [21]. Prolonged repolarization leads to the occurrence of early after depolarization (EAD) currents. EADs are dangerous because they favor the occurrence of triggered activity (defined as the occurrence of spontaneous action potentials occurring during phase 2 or phase 3 of repolarization, leading to the production of inappropriate action potentials and arrhythmia) [12]. Blockade of the IKr also causes the QT interval to be prolonged, leading to the triggered activity via a slightly different mechanism [22]. Such a situation is likely to occur when a drug molecule interferes with potassium channels as in the case of type III antiarrhythmic drugs. Slowing of the potassium current is associated with a repolarization dispersion, where one area of the myocardium recovers from depolarization faster than an adjoining region, which also makes TdP more likely to occur [23]. Repolarization dispersion is thought to be the reason that myocardial hypertrophy is associated with arrhythmias [24]. The farther the depolarization front has to travel, the greater the interval between depolarization and repolarization. Dispersion is especially likely to occur if the area of abnormal delay and dispersion is located within the Purkinje system or, alternatively, if the area is located in the mid-wall of the left ventricle where the “M cells” are located. These cells have prolonged action potentials that act to further increase the dispersion of repolarization, making the occurrence of TdP even more likely [25]. For a new antiarrhythmic drug to be introduced, it must first be proven that it exerts none of the effects enumerated above.

Sudden cardiac death (SCD) due to ventricular tachycardia (VT) or ventricular fibrillation (VF) accounts for approximately half of all deaths in patients with heart failure (HF) and may be considered a heritable trait [26-32]. Current guidelines [33] recommend an implantable cardioverter-defibrillator (ICD) in patients with symptomatic and severe left ventricular dysfunction of any origin. However, SCD may occur in asymptomatic patients with only mild HF. On the contrary, as many as two-thirds of patients with severe HF implanted with an ICD do not experience device interventions over 3 to 5 years follow-up [34]. A similar clinical scenario leaves unanswered the question of whether selected gene variants may affect the risk of SCD in HF patients. Genomic science provides us with new approaches to identify gene variants or mutations that predispose patients with inherited electrical diseases to SCD. However, a growing body of evidence suggests that DNA changes in the same genes that convey risk in primary electrical diseases may enhance susceptibility to VT/VF even in a polygenic condition such as HF. Sustained VT and VF often occur as a consequence of delayed after-depolarizations triggered by diastolic saccoplasmic...
Gene affect the expression level, ATP affinity, calcium affinity, and processes are critical for the process of converting maturing stem cells back to many species. Whatever the precise role of these diverse factors, other epigenetic and introduced, many other iPSCs, and related transcription factors, have been identified that have been allowed to mature. In 2012, Shinya Yamanaka outlined a method to induce pluripotency by inserting genes that acted as reprogramming factors, also called transduction factors, by attaching them to carrier viruses and inserting the virus into the cells, which eventually causes the cells to express the exogenous genes. The cells are then cultured and finally harvested. Since the technique was first introduced, many other iPSCs, and related transcription factors, have been identified and used, including, miRNAs (a type of non-coding RNA that inhibits translation in many species). Whatever the precise role of these diverse factors, other epigenetic processes are critical for the process of converting maturing stem cells back to inducible pluripotent cells.

Microelectrode array

Microelectrode arrays are used in many fields of study, although the basics of the system are the same no matter what kind of test is being performed; improvements and refinements in this methodology are being reported almost continuously. These tests are performed in wells that look just like those in any clinical laboratory test plate used to observe chemical reactions, however, they differ in one important respect; electrodes are located at the bottom of each well.

When the electrodes and iPSCs are joined together they form the backbone of the system. The idea was derived from earlier networking studies, designed to test neural interactions. Networking electrodes were originally made of titanium salts and gold conductors, but other materials have been used. The system is now so advanced that these wells, indeed, the entire networking system, including software, are all available off the shelf.

iPSCs, can either be studied singly or as part of an integrated network. For most intents and purposes these cells have all the same capabilities as embryonic stem cells that have been allowed to mature. In 2012, Shinya Yamanaka outlined a method to induce pluripotency by inserting genes that acted as reprogramming factors, also called transduction factors, by attaching them to carrier viruses and inserting the virus into the cells, which eventually causes the cells to express the exogenous genes. The cells are then cultured and finally harvested. Since the technique was first introduced, many other iPSCs, and related transcription factors, have been identified and used, including, miRNAs (a type of non-coding RNA that inhibits translation in many species). Whatever the precise role of these diverse factors, other epigenetic processes are critical for the process of converting maturing stem cells back to inducible pluripotent cells.
Once the multiple electrode arrays have been constructed, beating cardiomyocytes, derived from pluripotent stem cells are plated over each well, without the electrodes ever actually penetrating the cells. Such a methodology essentially recreates many aspects of a working myocardium, including the generation of waveforms not very different from those seen on clinical electrocardiograms. Introducing an experimental drug into the system, the probable effect on a beating human heart can be confirmed with a high degree of accuracy.

For example, experimental drugs have been tested in networked iPSCs that alter the duration and shape of the QT interval in almost exactly the same pattern as seen in humans. Not only do drugs produce the same electrocardiographic changes, but physiological stressors also produce changes similar to those that occur in vivo with the same rate and QT interval alterations seen in humans[47,48]. If animal studies suggest that a drug can cause dangerous QT prolongation, it is simple enough to test the drug on networked beating human cardiomyocytes. Another obvious application of this technology is the measurement of calcium transients by using fluorescence microscopy. Calcium indicators are introduced into the cells and the resulting fluorescence can be quantitated noninvasively and used to measure calcium ion flux, which controls inotropy. In the past, such experiments required the use of isolated small animal muscle[49].

The same type of cellular network can be used to study the effect of genetic mutations known to cause cardiac arrhythmias, including channelopathies such as hERG; more than 90 long QT syndrome (LQTS) mutations have been mapped to date. It is possible to measure the effect of mutations on Ikr and Ik1, although debate still exists over the exact mechanism by which some mutations alter potassium flow, answers to at least some of these questions should soon be forthcoming[50]. With the availability of high-throughput networked cardiomyocytes, it is now possible to evaluate a drug’s effects on potassium flow before it is ever given to an animal, let alone evaluated in human clinical trials.

iPSCs from a patient with a novel KCNQ mutation were used by Egashira et al[51] to identify the mutation. The patient had survived VF, thanks to the nearby presence of an automated external defibrillator. Using a slight variation multi-electrode array system (where the electrical activity of clumps of cells, rather than sheets of cells was measured), abnormal repolarization, as manifested by electrical field potential duration, was observed in the spontaneously beating iPSC cardiomyocytes. Egashira et al[51] then added an assortment of potassium ingress and egress blockers to prove that the repolarization abnormality lay within the slow inward potassium channel[51]. At present, the technology is too cumbersome for routine clinical use. In the future, however, it should be possible to use this approach when exome screening fails to identify one of the usual culprits.

The recent discovery of the TECRL gene, an arrhythmia-inducing gene that produces features of catecholaminergic VT (CPVT) and LQTS, was accomplished using much the same technology[52]. Three patients were studied; two with a history of cardiac arrest and one with an episode of recorded CPVT. Once iPSCs had been produced and the mutation identified and sequenced, electrophysiological studies were then performed. These demonstrated exactly the same features (catecholamine sensitivity, triggered activity, delayed afterdepolarizations as had been seen in the patients. The abnormalities were all reversed by the addition of flecainide, a class 1c antiarrhythmic drug. Had iPSCs not been available, finding a remedy would have been purely by empiric trial and error. However, the real significance of the study is that there is now a reliable methodology with which to screen drugs for effectiveness.

Even without going to the effort of creating an entire iPSCs network, it is still possible to clinically diagnose some disorders from the electrical behavior of a single iPSC. A very recent report describes two patients with known Brugada Syndrome. When compared to the findings in two healthy controls, it was observed that each of the Brugada Syndrome patients carried one of two different sodium voltage-gated channel alpha and subunit 5 variants. The electrical characteristics of iPSCs produced from the patient’s own skin fibroblasts were studied. The studies showed reductions in inward sodium current density and reduced maximal upstroke velocity of action potential when compared with healthy controls. Furthermore, iPSC cardiomyocytes from the Brugada Syndrome patients demonstrated increased triggered activity, abnormal calcium (Ca2+) transients, and beating interval variation, the very same abnormalities previously reported in other studies, using different methodologies[53]. Late in 2016, a study using individual iPSCs was used to confirm results observed in a previous knockout mouse study. The studies had suggested the existence of a new cardiac regulatory mechanism that appeared to play a key role in the association between arrhythmias and myocardial hypertrophy. When the mouse studies were
repeated in human iPSCs, it was possible to confirm that the same stress-activated kinase was operative in human cells[54].

**Heart disease screening**

Another obvious application for iPSCs is screening for suspected heart disease, and for determining the significance of a mutation once it has been identified. Hypertrophic cardiomyopathy (HCM) is a very good example. The clinical diagnosis can be difficult to make (left ventricular hypertrophy with wall thickness > 15 mm, in the absence of ventricular dilation or any apparent disease that could cause hypertrophy)[55]. Unfortunately, it is not uncommon for there to be a complete disconnect between phenotype and genotype: Abnormal genes may be present but symptoms and signs absent.

Both sarcomeric mutations and non-sarcomeric mutations in HCM can be identified by whole-exome sequencing, and these studies demonstrate that the same genotype may be responsible for sudden death in one individual, but remain asymptomatic in another[56]. Multiple mutations have been detected in patients with HCM: Nine sarcomeric genes are known to carry most HCM-related mutations and encode sarcomeric mutations, while an additional nine mutations code for sarcomeric Z-disc proteins such as muscle LIM protein, α-actinin, or telethonin[57,58].

Since iPSCs cardiomyocytes became available, the pathogenic effects of some mutations (MYH7 and MYBPC3) associated with HCM have already been identified[59], and calcium blockade has been found to be an effective treatment for another HCM mutation (MYH7-R663H)[60]. Whole-exome sequencing almost never yields the identity of a single culprit gene, but rather detects multiple mutations, some of which may be relevant and some not. If one single mutation is responsible for the obvious phenotype of HCM, it has yet to be identified. It hardly needs saying, but exactly the same methodology used to identify culprit genes could be applied to genomic studies of countless other disorders, just by inducing the required cell type from transformed fibroblasts.

**DISCUSSION**

Overcoming the ethical problems related to the use of stem cells through the introduction of iPSCs opens up an interesting scenario on the study of the cellular basis of diseases[61,62]. The use of pluripotent cells makes it possible to reproduce models for the study of cardiological pathologies which frequently cause SCD and are often diagnosed post-mortem such as structural cardiomyopathies and channelopathies[63-66].

Furthermore, iPSCs can be exploited in the personalization of therapies in relation to the possibility of carrying out pharmacological tests on cells derived from the patient[67-70].

Although the principles are easy to understand, at present there are some important caveats. One is that fibroblast generated iPSCs demonstrate an immature phenotype so that they more closely resemble mid-gestation human fetal hearts[71-73]. These differences may well alter final experimental and clinical results, depending on the stage of development of the iPSCs being used. When used in other fields, the same caveat applies. Now that this difference has been recognized, finding ways to make sure the cells are organized and function as adult cells is the object of intense research, which has already begun to generate results. Recent reports indicate that iPSCs can be stimulated and made to mature by a combination of pacing and increasing mechanical stress[74-77].

Another issue that had been delaying progress is that protocols used to produce iPSCs do not produce just one kind of cell, but rather yield a mixed population of cardiomyocyte subtypes including ventricular-, atrial- and pacemaker-like cells[78-81]. Birket and colleagues[82] made the early observation that even though the iPSCs can behave like normal human cardiomyocytes, the production process leads to unequal numbers of each of the subtypes. Obviously, different results will be generated depending on which type of cell predominates. Many laboratories are working on effective cell separation methods and standardized methods should soon be available.
Induced pluripotent stem cells can provide new ways to study arrhythmias and heart disease in general, accelerating the development of new, more effective antiarrhythmic drugs, clinical diagnoses, and personalized medical care. iPSCs: Induced pluripotent stem cells. Figure created with BioRender (https://biorender.com).

CONCLUSION

In summary, the main applications of stem cells include disease modeling, cell diagnostics, and therapy personalization (Figure 1). Such tasks involve molecular profiling, the identification of biomarkers of the expression of the pathological phenotype, as well as the identification and testing of targeted therapies. The availability of pluripotent cardiac stem cells, especially networked beating cardiomyocytes, is likely to revolutionize our understanding of many cardiac rhythm disorders and diseases, provide a rational testing method for the development of drugs, permit clinicians to assess effectiveness before drug administration and, most importantly, save lives.

REFERENCES

1 Sasaki K, Makiyama T, Yoshida Y, Wuriyanghai Y, Kamakura T, Nishiuchi S, Hayano M, Harita T, Yamamoto Y, Kohjiti H, Hirose S, Chen J, Kawamura M, Ohno S, Itoh H, Takeuchi A, Matsuoka S, Miura M, Sumitomo N, Horie M, Yamanaka S, Kimura T. Patient-Specific Human Induced Pluripotent Stem Cell Model Assessed with Electrical Pacing Validates S107 as a Potential Therapeutic Agent for Catecholaminergic Polymorphic Ventricular Tachycardia. *PLoS One* 2016; 11: e0164795 [PMID: 27764147 DOI: 10.1371/journal.pone.0164795]

2 Staerk J, Dawlaty MM, Gao Q, Maetzel D, Hanna J, Sommer CA, Mostoslavsky G, Jaenisch R. Reprogramming of human peripheral blood cells to induced pluripotent stem cells. *Cell Stem Cell* 2010; 7: 20-24 [PMID: 20621045 DOI: 10.1016/j.stem.2010.06.002]

3 Peerani R, Rao BM, Bauwens C, Yin T, Wood GA, Nagy A, Kumacheva E, Zandstra PW. Niche-mediated control of human embryonic stem cell self-renewal and differentiation. *EMBO J* 2007; 26: 4744-4755 [PMID: 17948051 DOI: 10.1038/sj.emboj.7601896]

4 Radisic M, Christman KL. Materials science and tissue engineering: repairing the heart. *Mayo Clin Proc* 2013; 88: 884-898 [PMID: 23910415 DOI: 10.1016/j.mayocp.2013.05.003]

5 Marion MH, Box NA, Spreeuwel AC, van der Schaft DW, Bouten CV. Material-based engineering strategies for cardiac regeneration. *Curr Pharm Des* 2014; 20: 2057-2068 [PMID: 23886381 DOI: 10.2174/13816128113199990582]

6 Venugopal JR, Prabhakaran MP, Mukherjee S, Ravichandran R, Dan K, Ramakrishna S. Biomaterial strategies for alleviation of myocardial infarction. *J R Soc Interface* 2012; 9: 1-19 [PMID: 21900319 DOI: 10.1098/rsif.2011.0301]

7 Wu J, Zeng F, Weisel RD, Li RK. Stem cells for cardiac regeneration by cell therapy and myocardial tissue engineering. *Adv Biochem Eng Biotechnol* 2009; 114: 107-128 [PMID: 19543706 DOI: 10.1007/10_2008_37]

8 Finosh GT, Jayabalan M. Regenerative therapy and tissue engineering for the treatment of end-stage cardiac failure: new developments and challenges. *Biomatter* 2012; 2: 1-14 [PMID: 23507781 DOI: ]
Karch SB et al. iPSCs in diagnostic cardiology

10.4161/biom.19429)

9 Rocco M, Goumans MJ, Sluijter JP, Doevendans PA. Stem cell sources for cardiac regeneration. Panminerva Med 2008; 50: 19-30 [PMID: 18427385]

10 Talibabi M, Aghdami N, Baharvand H. Human cardiomyocyte generation from pluripotent stem cells: A state-of-art. Life Sci 2016; 145: 98-113 [PMID: 26682938 DOI: 10.1016/j.lfs.2015.12.023]

11 Lu HR, Gallagher DJ, Yan GX. Assessment of drug-induced proarrhythmia: The importance of study design in the rabbit left ventricular wedge model. J Pharmacol Toxicol Methods 2016; 81: 151-160 [PMID: 27374776 DOI: 10.1016/j.vascn.2016.06.006]

12 Hundahl LA, Teitle-Hansen J, Jespersen T. Rat Models of Ventricular Fibrillation Following Acute Myocardial Infarction. J Cardiovasc Pharmacol Ther 2017; 22: 514-528 [PMID: 28381093 DOI: 10.1177/1074248417702894]

13 Dobevo D, Wehrens XHT. Mouse Models of Cardiac Arrhythmias. Circ Res 2018; 123: 322-334 [PMID: 30026380 DOI: 10.1161/CIRCRESAHA.118.313406]

14 Yong SL, Wang QK. Animal models for cardiac arrhythmias. Methods Mol Med 2006; 129: 127-148 [PMID: 17085809 DOI: 10.1385/1-59745-213-0:127]

15 Schwartz PJ. Do animal models have clinical value? Am J Cardiol 1998; 81: 14D-20D [PMID: 9537218 DOI: 10.1016/s0002-9149(98)00148-9]

16 Claus S, Bleyer C, Schüttler D, Tomsits P, Renner S, Klymiuk N, Wakili R, Massberg S, Wolf E, Käßb S. Animal models of arrhythmia: classic electrophysiology to genetically modified large animals. Nat Rev Cardiol 2019; 16: 457-475 [PMID: 30894679 DOI: 10.1038/s41569-019-0179-0]

17 Dubois VF, Casarotto E, Danhof M, Della Pasqua O. Pharmacokinetic-pharmacodynamic modelling of drug-induced QTc interval prolongation in man: prediction from in vitro human ether-à-go-go-related gene binding and functional inhibition assays and conscious dog studies. Br J Pharmacol 2016; 173: 2819-2832 [PMID: 27427789 DOI: 10.1111/bph.13556]

18 van Opstal JM, Schoenmakers M, Verdagens SC, de Groot SH, Leunissen JD, van Der Hulst FF, Molenschot MM, Wellens HJ, Vos MA. Chronic amiodarone evokes no torsade de pointes arrhythmias despite QT lengthening in an animal model of acquired long-QT syndrome. Circulation 2001; 104: 2722-2727 [PMID: 11723026 DOI: 10.1161/hc4701.099579]

19 Semasinghe Bandaralage SP, Nirthanan S, Niransan S. Does Sotalol Still Have a Role in the Management of Arrhythmias? Am J Ther 2019; 26: e161-e169 [PMID: 27759583 DOI: 10.1097/MJT.0000000000000507]

20 Tran HT, Chow MS, Kluger J. Amiodarone induced torsades de pointes with excessive QT dispersion following quinidine induced polymorphic ventricular tachycardia. Pacing Clin Electrophysiol 1997; 20: 2275-2278 [PMID: 9309756 DOI: 10.1111/j.1540-8159.1997.tb04249.x]

21 Yap YG, Camm AJ. Drug induced QT prolongation and torsades de pointes. Heart 2003; 89: 1363-1372 [PMID: 14594906 DOI: 10.1136/heart.89.11.1363]

22 Zhou YY, Liu TF. The ionomic mechanisms of early after depolarization in mouse ventricular myocytes: the role of IK1. Methods Find Exp Clin Pharmacol 1997; 19: 443-453 [PMID: 9413827]

23 Burton FL, Cobbe SM. Dispersion of ventricular repolarization and refractory period. Cardiovasc Res 2001; 50: 10-23 [PMID: 11282074 DOI: 10.1097/00007636-200101000-00197-3]

24 Kang YJ. Cardiac hypertrophy: a risk factor for QT-prolongation and cardiac sudden death. Toxicol Pathol 2006; 34: 58-66 [PMID: 16507545 DOI: 10.1080/01926230500419421]

25 Henry H, Rappel WJ. The role of M cells and the long QT syndrome in cardiac arrhythmias: simulation studies of reentrant excitations using a detailed electrophysiological model. Chaos 2004; 14: 172-182 [PMID: 15003058 DOI: 10.1063/1.1636272]

26 Darbar D. Genomics, heart failure and sudden cardiac death. Heart Fail Rev 2010; 15: 229-238 [PMID: 18437561 DOI: 10.1007/s10741-008-9905-9]

27 Gollob MH. Genetic profiling as a marker for heart failure and sudden cardiac death. Curr Opin Cardiol 2006; 21: 42-46 [PMID: 16355028 DOI: 10.1097/01.hco.0000198982.86131.64]

28 Osman J, Tan SC, Lee PY, Low TY, Jamal R. Sudden Cardiac Death (SCD) - risk stratification and prediction with molecular biomarkers. J Biomed Sci 2019; 26: 39 [PMID: 31118017 DOI: 10.1186/s12929-019-0535-8]

29 Backer J, Braverman AC. Heart failure and sudden cardiac death in heritable thoracic aortic disease caused by pathogenic variants in the SMAD3 gene. Mol Genet Genomic Med 2018 [PMID: 29715556 DOI: 10.1002/mgg3.396]

30 Wei D, Tao L, Huang M. Genetic variations involved in sudden cardiac death and their associations and interactions. Heart Fail Rev 2016; 21: 410-414 [PMID: 27241195 DOI: 10.1007/s10741-016-9636-6]

31 Arking DE, Chugh SS, Chakravarti A, Spooner PM. Genomics in sudden cardiac death. Circ Res 2004; 94: 712-723 [PMID: 15059941 DOI: 10.1161/01.RES.0000123861.16082.95]

32 Kolder IC, Tanck MW, Bezissa CR. Common genetic variation modulating cardiac ECG parameters and susceptibility to sudden cardiac death. J Mol Cell Cardiol 2012; 52: 620-629 [PMID: 22248531 DOI: 10.1016/j.yjmcc.2011.12.014]

33 Yancey CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Dzau MR, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kaspr EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines. 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. J
M, Schlaepfer J, Mummery CL, Stevenson B, Kutalik Z, de Vries AA, Rivard L, Wilde AA, Talajic H, Le Béchec A, Monshouwer-Kloots JJ, Zwetsloot T, Kosmidis G, Latour F, Alikashani A, Hoekstra Devalla HD

Cardiovasc Res

Ogawa S, Fukuda K. Disease characterization using LQTS-specific induced pluripotent stem cells. Murata M, Kurokawa J, Furukawa T, Makita N, Aiba T, Shimizu W, Horie M, Kamiya K, Kodama I, Seki T, Kuroda Y, Yae K, Hashimoto H, Tanaka T, Hattori F, Sato T, Miyoshi S, Takatsuki S, Nozaki Y

Biophys Acta

Laurila E

[PMID: 215-223] [PMID: 8951248]

Koop A, Goldmann P, Chen SR, Thieleczek R, Varsányi M. ARVC-related mutations in divergent region 3 alter functional properties of the cardiac ryanodine receptor. Biophy J 2008; 94: 4668-4677 [PMID: 18326664 DOI: 10.1529/biophysj.107.122382]

Milting H, Lukas N, Klaube B, Körfer R, Perrot A, Osterziel KJ, Vogt J, Peters S, Thieleczek R, Varsányi M. Composite polymorphisms in the ryanoide receptor 2 gene associated with arrhythmogenic right ventricular cardiomyopathy. Cardiovasc Res 2006; 71: 496-505 [PMID: 16769042 DOI: 10.1016/j.cardiores.2006.04.004]

Francia P, Adduci C, Semprini L, Stanzione R, Sendoz A, Caprinozzi M, Santini D, Cotugno M, Palano F, Musumeci MB, Ruibatto S, Volpe M. RyR2 Common Gene Variant G1886S and the Risk of Ventricular Arrhythmias in ICD Patients with Heart Failure. J Cardiovasc Electrophysiol 2015; 26: 656-661 [PMID: 25773045 DOI: 10.1111/jce.12658]

Sakuntabhai A, Burge S, Monk S, Hovnanian A. Spectrum of novel ATP2A2 mutations in patients with Darier's disease. Hum Mol Genet 1999; 8: 1611-1619 [PMID: 10441323 DOI: 10.1093/hmg/8.9.1611]

Lelakowski J, Piekarz J, Rydlewiska A, Majewski J, Senderek T, Ząbek A, Malecka B. Factors predisposing to ventricular tachyarrrhythmia leading to appropriate ICD intervention in patients with coronary artery disease or non-ischaemic dilated cardiomyopathy. Kardiol Pol 2012; 70: 1264-1275 [PMID: 23264245]

Asai Y, Tada M, Otsuji TG, Nakatsuji N. Combination of functional cardiomyocytes derived from human stem cells and a highly-efficient microelectrode array system: an ideal hybrid model assay for drug development. Curr Stem Cell Res Ther 2010; 5: 227-232 [PMID: 20214558 DOI: 10.2174/1574888010798214502]

Shi Y, Desponts C, Do JT, Hahm HS, Schöler HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. Cell Stem Cell 2008; 3: 568-574 [PMID: 18983970 DOI: 10.1016/j.stem.2008.10.004]

Lakshmipathy U, Davila J, Hart RP. miRNA in pluripotent stem cells. Regen Med 2010; 5: 545-555 [PMID: 20632858 DOI: 10.2217/reme.10.34]

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 [PMID: 24812011 DOI: 10.1093/toxsci/kfu084]

Nozaki Y, Honda Y, Watanabe H, Saiki S, Koyabu K, Itoh T, Nakamura C, Nakamori C, Nakayama I, Iwasaki S, Suzuki S, Washio I, Takahashi E, Miyamoto K, Yamanishi A, Endo H, Shinozaki J, Nagasawa C, Nakayama C, Okata M, Kuroki M, Shinozaki J, Nagawa H, Kunimatsu T. CSAHi study: Validation of multi-electrode array systems (MEA60/2100) for prediction of drug-induced proarrhythmia using human iPS cell-derived cardiomyocytes - assessment of inter-facility and cells lot-to-lot-variability. Regul Toxicol Pharmacol 2016; 77: 75-86 [PMID: 26884090 DOI: 10.1016/j.yrtvp.2016.02.007]

Laurila E, Ahola A, Hyttinen J, Aalto-Sellälä K. Methods for in vitro functional analysis of iPSC derived cardiomyocytes - Special focus on analyzing the mechanical beating behavior. Biochim Biophys Acta 2016; 1863: 1864-1872 [PMID: 26707468 DOI: 10.1016/j.bbcan.2015.12.013]

Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. Cell 2001; 104: 569-580 [PMID: 11239413 DOI: 10.1016/s0092-8674(01)00243-4]

Egashira T, Yusu S, Suzuki T, Aizawa Y, Yamakawa H, Matsuhashi T, Ohno Y, Tohyama S, Okata S, Seiki T, Kuroda Y, Yae K, Hashimoto H, Tanaka T, Hattori F, Sato T, Miyoshi S, Takatsuki S, Murata M, Kurokawa J, Furukawa T, Makita N, Aiba T, Shimizu W, Horie M, Kamiya M, Kadowa I, Ogawa S, Fukuda K. Disease characterization using LQTS-specific induced pluripotent stem cells. Cardiovasc Res 2012; 95: 419-429 [PMID: 22739199 DOI: 10.1093/cvr/cvs206]
Karch SB et al. iPSCs in diagnostic cardiology

EMBO Mol Med 2016; 8: 1390-1408 [PMID: 27861123 DOI: 10.15252/emmm.201505719]

53 Liang P, Sallam K, Wu H, Li Y, Izthaki I, Garg P, Zhang Y, Vermeulian V, Lan F, Gu M, Gong T, Zhuge Y, He C, Ebert AD, Sanchez-Freire V, Churko J, Hu S, Sharma A, Lam CK, Scheinman MM, Bers DM, Wu JC. 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 [PMID: 27870448 DOI: 10.1016/j.jacc.2016.07.779]

54 Chowdhury SK, Liu W, Zl M, Li Y, Wang S, Tsui H, Prebar S, Castro S, Zhang H, Ji Y, Zhang X, Xiao R, Zhang R, Lei M, Cganek L, Guan K, Miller CB, Liao X, Jain MK, Boyett MR, Cartwright EJ, Shelds HA, Wang X. Stress-Activated Kinase Mitogen-Activated Kinase-7 Governs Epigenetics of Cardiac Repolarization for Arrhythmia Prevention. Circulation 2017; 135: 683-699 [PMID: 27899394 DOI: 10.1161/CIRCULATIONAHA.116.022941]

55 Gersh BJ, Maron BJ, Bonow RO, Dearani JA, Fifer MA, Link MS, Naidu SS, Nishimura RA, Omman SR, Rakowski H, Seidman CE, Towbin JA, Udelson JE, Yancy CW; American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Developed in collaboration with the American Association for Thoracic Surgery, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. J Am Coll Cardiol 2011; 58: e212-e260 [PMID: 22075469 DOI: 10.1016/j.jacc.2011.06.011]

56 McNally EM, Barefield DY, Puckelwitz MJ. The genetic landscape of cardiomyopathy and its role in heart failure. Cell Metab 2015; 21: 174-182 [PMID: 25851172 DOI: 10.1016/j.cmet.2015.01.013]

57 Knöll R, Kostin S, Klee S, Savvatis K, Klinge L, Stelke I, Gunkel S, Kötter S, Babicz K, Sohns M, Möhr T, Didé M, Knoll G, Zimmermann WH, Zimmermann U, Thewes P, Beelckhöller H, Maier J, Schaper W, Schaper J, Kraft T, Tschöpe C, Linke WA, Chien KR. A common MLP (muscle-LIM protein) variant is associated with cardiomyopathy. Circ Res 2010; 106: 695-704 [PMID: 20944516 DOI: 10.1161/CIRCRESAHA.109.206243]

58 Kraker J, Viswanathan SK, Knöll R, Sadyayappan S. Recent Advances in the Molecular Genetics of Familial Hypertrophic Cardiomyopathy in South Asian Descendants. Front Physiol 2016; 7: 499 [PMID: 28740609 DOI: 10.3389/fphys.2016.00499]

59 Ross SB, Fraser ST, Sensmian C. Induced pluripotent stem cells in the inherited cardiomyopathies: From disease mechanisms to novel therapies. Trends Cardiovasc Med 2016; 26: 663-672 [PMID: 27906521 DOI: 10.1016/j.tcm.2016.05.001]

60 Lan F, Lee AS, Liang P, Sanchez-Freire V, Nguyen PK, Wang L, Han L, Yen M, Wang Y, Sun N, Abilez OJ, Hu S, Ebert AD, Navarette EG, Simmons CS, Wheeler M, Pruitt B, Lewis R, Yamaguchi Y, Ashley EA, Bers DM, Robbins RC, Longaker MT, Wu JC. Abnormal calcium handling properties underlie familial hypertrophic cardiomyopathy pathology in patient-specific induced pluripotent stem cells. Cell Stem Cell 2013; 12: 101-113 [PMID: 23290139 DOI: 10.1016/j.stem.2012.10.010]

61 Frati P, Scopetti M, Santurro A, Gatto V, Fineschi V. Stem Cell Research and Clinical Translation: A Roadmap about Good Clinical Practice and Patient Care. Stem Cells Int 2017; 2017: 5080259 [DOI: 10.1155/2017/5080259]

62 Scopetti M, Santurro A, Gatto V, La Russa R, Manetti F, D'Errico S, Frati P, Fineschi V. Mesenchymal stem cells in neurodegenerative diseases: Opinion review on ethical dilemmas. World J Stem Cells 2020; 12: 168-177 [PMID: 32260049 DOI: 10.4245/wjsc.v12.i3.168]

63 De Marco E, Vacchiano G, Frati P, La Russa R, Santurro A, Scopetti M, Guglielmi G, Fineschi V. The genetic landscape of cardiomyopathy and its role in heart failure. Cell Metab 2015; 21: 174-182 [PMID: 25851172 DOI: 10.1016/j.cmet.2015.01.013]

64 Frati P, Santurro A, Gatto V, Fineschi V. Evolution of post-mortem coronary imaging: from selective coronary arteriography to post-mortem CT-angiography and beyond. Radiol Med 2018; 123: 351-358 [PMID: 29357039 DOI: 10.1007/s11547-018-0855-x]

65 De Marco E, Vacchiano G, Frati P, La Russa R, Santurro A, Scopetti M, Guglielmi G, Fineschi V. Detection and differentiation of early acute and following age stages of myocardial infarction with quantitative post-mortem cardiac LCT MR. Forensic Sci Int 2017; 270: 248-254 [PMID: 27836412 DOI: 10.1016/j.forsciint.2016.10.014]

66 Schwenkner N, Jackowski C, Persson A, Warnstje MJ, Schuster F, Riva F, Zech WD. Detection and differentiation of early acute and following age stages of myocardial infarction with quantitative post-mortem cardiac LCT MR. Forensic Sci Int 2017; 270: 248-254 [PMID: 27836412 DOI: 10.1016/j.forsciint.2016.10.014]

67 Borro M, Gentile G, Cipolloni L, Folds-Papp Z, Frati P, Santurro A, Lionetto L, Simmaco M. Personalised Healthcare: the DiMA CLinic Model. Curr Pharm Biotechnol 2017; 18: 242-252 [PMID: 28183244 DOI: 10.2174/13892010196666170208125131]

68 Stratakis CA. Genetics and the New (Precision) Medicine and Endocrinology: In Medias Res or Ab Initio? Endocrinol Metab Clin North Am 2017; 46: xv-xvi [PMID: 28476238 DOI: 10.1016/j.ecl.2017.03.001]

69 La Russa R, Fineschi V, Di Sanzo M, Gatto V, Santurro A, Martini G, Scopetti M, Frati P. Personalized Medicine and Adverse Drug Reactions: The Experience of an Italian Teaching Hospital.
Maeda H, Ishikawa T, Michiue T. Forensic molecular pathology: its impacts on routine work, education and training. *Leg Med (Tokyo)* 2014; 16: 61-69 [PMID: 24480586 DOI: 10.1016/j.legalmed.2014.01.002]

Rajamohan D, Matsa E, Crutchley J, Patel A, George V, Denning C. Current status of drug screening and disease modelling in human pluripotent stem cells. *Bioessays* 2013; 35: 281-298 [PMID: 22886688 DOI: 10.1002/bies.201200053]

Fonoudi H, Ansari H, Abbasalizadeh S, Larijani MR, Kiani S, Hashemizadeh S, Zarchi AS, Blue GM, Pahlavan S, Hu X, Lloyd SG, Ge Y, Zhang J. A Universal and Robust Integrated Platform for the Scalable Production of Human Cardiomyocytes From Pluripotent Stem Cells. *Stem Cells Transl Med* 2015; 4: 1482-1494 [PMID: 26511653 DOI: 10.5966/sctm.2014-0275]

Geng L, Kong CW, Shum AM, Chow MZY, Che H, Zhang C, Yau KL, Chan CW, Keung W, Li RA. Probing flecainide block of $I_{Na}$ using human pluripotent stem cell-derived ventricular cardiomyocytes adapted to automated patch-clamping and 2D monolayers. *Toxicol Lett* 2018; 294: 61-72 [PMID: 29738359 DOI: 10.1016/j.toxlet.2018.05.006]

Ruan JL, Tulloch NL, Razumova MV, Saiget M, Muskheili V, Pabon L, Reinecke H, Regnier M, Murry CE. Mechanical Stress Conditioning and Electrical Stimulation Promote Contractility and Force Maturation of Induced Pluripotent Stem Cell-Derived Human Cardiac Tissue. *Circulation* 2016; 134: 1557-1567 [PMID: 27737958 DOI: 10.1161/circulationaha.114.014998]

Gao L, Gregorich ZR, Zhou W, Mattapally S, Oudak Y, Lou X, Kannappan R, Borovjagin AV, Walcott GP, Pollard AE, Fast VG, Hu X, Lloyd SG, Ge Y, Zhang J. Large Cardiac Muscle Patches Engineered From Human Induced-Pluripotent Stem Cell-Derived Cardiac Cells Improve Recovery From Myocardial Infarction in Swine. *Circulation* 2018; 137: 1712-1730 [PMID: 29233823 DOI: 10.1161/CIRCUITRAHA.117.030785]

Ruan JL, Tulloch NL, Saiget M, Paige SL, Razumova MV, Regnier M, Tung KC, Keller G, Pabon L, Reinecke H, Murry CE. Mechanical Stress Promotes Maturation of Human Myocardium From Pluripotent Stem Cell-Derived Progenitors. *Stem Cells* 2015; 33: 2148-2157 [PMID: 25865043 DOI: 10.1002/stem.2036]

Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cells-derived cardiomyocytes. *Circ Res* 2014; 114: 511-523 [PMID: 24481842 DOI: 10.1161/CIRCRESAHA.114.300558]

Denning C, BordoFF V, Crutchley J, Firth KS, George V, Kalra S, Kondrashov A, Hoang MD, Mosqueira D, Patel A, Prodanov L, Rajamohan D, Skarnes WC, Smith JG, Young LE. Cardiomyocytes from human pluripotent stem cells: From laboratory curiosity to industrial biomedical platform. *Biochim Biophys Acta* 2016; 1863: 1726-1748 [PMID: 26524115 DOI: 10.1016/j.bbamcr.2015.10.014]

Shaheen N, Shiti A, Gepstein L. Pluripotent Stem Cell-Based Platforms in Cardiac Disease Modeling and Drug Testing. *Clin Pharmacol Ther* 2017; 102: 203-208 [PMID: 28718902 DOI: 10.1002/cpt.722]

Tan SH, Ye L. Maturation of Pluripotent Stem Cell-Derived Cardiomyocytes: a Critical Step for Drug Development and Cell Therapy. *J Cardiovasc Transl Res* 2018; 11: 375-392 [PMID: 29557052 DOI: 10.1007/s12265-018-9801-5]

Del Alamo 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 [PMID: 26952934 DOI: 10.1016/j.bbamcr.2016.03.003]

Birket MJ, Mummery CL. Pluripotent stem cell derived cardiovascular progenitors—a developmental perspective. *Dev Biol* 2015; 400: 169-179 [PMID: 25624264 DOI: 10.1016/j.ydbio.2015.01.012]
