NOVEL APPROACHES TO PROARRHYTHMOGENIC RISK TESTING USING AUTOMATED PATCH-CLAMP PLATFORMS

B. AMUZESCU[*,**#], *R. AIRINI*[*,**], *LOREDANA GHICA*[*], *F.B. EPUREANU*[*,**], *A.F. DEFTU*[*,**], *DANIELA MARCELA CUCU*[*,**], *VIOLETA PAULA RISTOIU*[*,**], *D.F.MIHĂILESCU*[*], *BEATRICE MIHAELA RADU*[*,**#]

[*]Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, 91–95 Splaiul Independenței, Bucharest 050095, Romania, e-mail: [#]bogdan@biologie.kappa.ro, [#]beatrice.radu@bio.unibuc.ro
[**]Life, Environmental and Earth Sciences Division, Research Institute of the University of Bucharest (ICUB), 91–95 Splaiul Independenței, Bucharest 050095, Romania

Abstract. This minireview summarizes the evolution of experimental techniques in cardiomyocyte electrophysiology over the last 70 years and progressive development of cardiomyocyte electrophysiology computational models. We emphasize the relationship between hERG (the human Ether-à-go-go-Related Gene) ion channels' susceptibility to block by pharmacological compounds and drug-induced arrhythmias, particularly Torsades-de-Pointes, and expose the already classical clinical and non-clinical, *in vitro* and *in vivo* assays used for cardiac safety testing, included in the S7B and E14 guidelines, constituting the so-called "hERG-centric" paradigm. We further present several in-depth studies that pointed out the limitations of this paradigm, and the requirement for a modern *in vitro* mechanistic approach, combining experimental and *in silico* (modeling) methods, constituting the novel Cardiac *in vitro* Pro-arrhythmia Assay (CiPA) paradigm. We review the most relevant achievements during the past three years in implementing CiPA as a new guideline, the various approaches tested by researchers, particularly for evidencing proarrhythmogenic events like early or delayed afterdepolarizations in human induced pluripotent stem cell-derived cardiomyocyte preparations. We conclude that automated patch-clamp methods, especially those using the third generation CytoPatch™ platforms based on the patented Cytocentering® technology, allow development of complex assays combining all three stages of the CiPA approach in a single experiment, leading to advanced testing methods that will transform CiPA into a robust, highly reliable and reproducible standard for the pharmacological industry.

Key words: cardiomyocyte, human induced pluripotent stem cell-derived cardiomyocyte, hERG, early afterdepolarization, Torsades-de-Pointes, drug-induced arrhythmia, cardiac safety drug testing, Comprehensive *in vitro* Proarrhythmia Assay (CiPA), automated patch-clamp.

INTRODUCTION

The field of cardiomyocyte electrophysiology has progressed steadily over the past seven decades, due to a continuous improvement in experimental

Received: November 2016;
in final form April 2017.

ROMANIAN J. BIOPHYS., Vol. 27, Nos 1–2, P. 13–22, BUCHAREST, 2017

techniques and equipments, and successive waves of innovative approaches. Thus, the 1950s were dominated by microelectrode impalement experiments in multicellular cardiac tissue preparations such as Purkinje network fragments ("false tendons"). A remarkable study of that epoch is that of Weidmann [35], who demonstrated that the action potential (AP) plateau features a higher membrane resistance compared to the resting state of the fiber. This fact was in contradiction with the first attempts to mathematically model the cardiomyocyte AP starting from the Hodgkin-Huxley model, by slowing down the time constant of K^+ current activation and Na^+ current inactivation [12, 19]. Soon, the experiments of Hall, Hutter and Noble proved the existence of two distinct K^+ current components: a delayed rectifier component, similar to that in the Hodgkin-Huxley model, and an inward rectifier current [13, 15]. These were dubbed I_K and I_{K1}, respectively, in the Purkinje fiber model developed by Sir Denis Noble in 1962 [22]. With the advent of the voltage-clamp technique in 1964, numerous other current components were identified in cardiomyocyte preparations and were progressively incorporated in cardiomyocyte electrophysiology mathematical models, most notably by the group of Noble. Thus, L-type Ca^{2+} currents were discovered by Harald Reuter in 1967 [27], and were incorporated in the Beeler-Reuter ventricular cardiomyocyte model [2], a modification of the MacAllister-Noble-Tsien model [18]. This model represents a turning point, incorporating "slow inward" L-type Ca^{2+} currents, two components of delayed rectifier K^+ currents identified by Noble and Roger Tsien in 1968 [23], known today as I_{Kr} and I_{Ks}, as well as a pacemaker current accounting for automatic rhythmic activity. An improved model of the "funny" (hyperpolarization-activated) pacemaker current I_f was included in the DiFrancesco-Noble model [9], together with a Na^+/Ca^{2+} exchanger (NCX), initially considered electroneutral, the exchange rate of which was updated from 2:1 to 3:1. Inclusion of one exchanger required taking into account the other exchangers and pumps, as well as calcium buffering in different cellular subcompartments, considered in the Hilgemann-Noble atrial cardiomyocyte model [14]. The shape of ventricular action potential and of several ion currents provided by an updated cardiomyocyte electrophysiology model is shown in Figure 1.

I_{KR} INHIBITION BY DRUGS AND TORSADOGENIC RISK

An important consequence of the peculiar I-V plot of the cardiac inward rectifier current I_{K1} is maintenance of a prolonged, several-hundreds-milliseconds depolarization plateau, particularly in ventricular cardiomyocytes, with small inward and outward currents, via a delicate equilibrium, an energy-saving mechanism, but also a "nature's pact with the devil", as Denis Noble named this phenomenon [21]. Repolarizing currents in cardiomyocytes are also faint, while inward Na^+ flow during fast depolarization (phase 0) is robust, resulting in a 100

fold increase in transmembrane conductance. The fragility of repolarization renders it susceptible to the influence of a variety of factors.

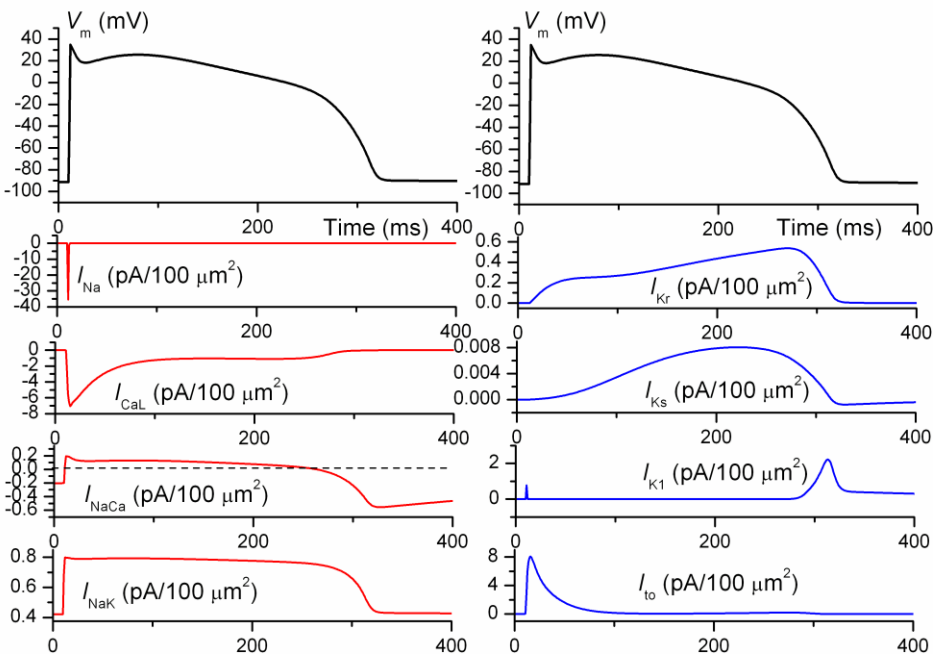

Fig. 1. Waveform of paced action potential and of several cardiac ion currents yielded by a "humanized" Faber-Rudy 2007 [10] model with current densities adapted for mid-myocardial ventricular cardiomyocytes. I_{Na} – fast voltage-dependent Na^+ current; I_{CaL} – L-type Ca^{2+} current; I_{NaCa} – Na^+/Ca^{2+} exchanger current; I_{NaK} – Na^+/K^+ pump current; I_{Kr} and I_{Ks} – rapid and slow delayed rectifier K^+ current; I_{K1} – cardiac inward rectifier K^+ current; I_{to} – transient outward K^+ current.

The hERG1 channel, generating I_{Kr}, the main repolarizing component, has some special features, such as a very flexible glycine-based residue sequence in the C-terminal end of transmembrane helix S6 instead of the Pro-X-Pro sequence, forming a kink, encountered in the majority of other channels. This results in an unusually large inner vestibule, accessible to a variety of drug compounds, the binding of which is stabilized by several aromatic residues [34]. Inhibition of I_{Kr} by pharmacological compounds leads to repolarization impairment and proarrhythmogenic effects such as early afterdepolarizations (EADs), which can in turn trigger by synchronization at ventricular level [29] dangerous life-threatening arrhythmias like Torsades-de-Pointes (TdP), the most common drug-induced arrhythmia. It is estimated that an astounding 40–50% of new drug candidates have to be withdrawn from research pipelines due to proarrhythmogenic liability *via* hERG blockade [20].

Two guidelines for proarrhythmogenic risk assessment of drug candidates are currently in use: S7B for non-clinical tests and E14 for clinical tests. Both were issued in 2005 by the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and are applied by the majority of drug regulatory agencies. The E14 guideline relies on the "thorough QT/QTc (corrected QT interval) study" applied to human patients, assuming marked QT interval prolongation induced by a compound is indicative of torsadogenic risk. The S7B guidelines include *in vitro* testing of hERG inhibitory effects, as well as *in vitro* and *in vivo* cardiac electrophysiology testing. Multiple *in vitro* preparations can be used, such as dissociated cardiomyocytes from different animal species, myocardial tissue preparations including myocardial wedge, papillary muscle, isolated Purkinje fibers or ventricular trabeculae, isolated heart preparations, or *in vivo* approaches such as the "telemeterized conscious dog" with ECG monitoring.

THE "hERG-CENTRIC" PARADIGM

The S7B and E14 guidelines have been tremendously successful, and since their implementing in practice no case of withdrawal from the markets of a commercial compound due to fatal drug-induced arrhythmia has occurred [25]. However, there are concerns that the markers proposed in these guidelines are not accurate enough in predicting arrhythmogenic risk. Lawrence *et al*. [17] analyzed three *in vitro* and three *in vivo* pro-arrhythmia risk prediction models, based on non-standard markers (beyond hERG blockade and APD/QTc prolongation), including early afterdepolarizations (EADs), AP triangulation, instability, reverse use-dependence, transmural dispersion of repolarization, concluding that no one of them shows a clear superiority in prediction efficiency. In a landmark study, Redfern *et al*. [26] correlated for 100 drugs published data on hERG inhibition, APD90 and QT interval prolongation in dogs with QT effects and reports of TdP in humans, as well as with the clinical range of effective therapeutic plasma concentrations for unbound compound ($ETPC_{unbound}$), concluding that torsadogenic compounds in humans broadly exert hERG inhibitory effects within $ETPC_{unbound}$, but interactions with multiple cardiac ion channels may often occur, and thus hERG block or APD/QT prolongation *per se* are not necessarily torsadogenic markers. Starting from this multiplicity of ion channel targets for a drug, Bottino *et al*. [4] developed an original testing method: they adapted parameters of a canine ventricular cardiomyocyte model to experimental canine Purkinje fiber data by rapid non-linear parameter estimation of 14 ion current conductances via training the model to AP data, then they "reverse-engineered" the effects of two compounds on five cardiac ion channels ($I_{Na\ sustained}$, I_{CaL}, I_{Ks}, I_{to1}, I_{NaCa}) beyond I_{Kr} from data in transfected cell lines and effects on APs in canine Purkinje fibers paced at 0.5 and 1 Hz, and further they used the resulting IC_{50} values in "forward" simulations to

predict effects on isolated cardiomyocytes, individual cardiomyocytes in a myocardial wedge, and the combined electrocardiogram of that wedge model. For the two tested compounds, the model predicted no QT interval prolongation or increased dispersion of repolarization, in spite of the presence of hERG inhibitory effects. Another important study is that of a group at ChanTest led by Arthur M. Brown (Kramer *et al*., [16]), who proved that hERG inhibition alone is not predictive of torsadogenic risk. Using automated patch-clamp platforms on heterologous expression cell lines, they obtained IC_{50} values for hERG, the L-type Ca^{2+} channel Cav1.2, and the cardiac voltage-dependent Na^{+} channel Nav1.5 for an impressive list of compounds. Further, they studied five multiple logistic regression models, showing that combined models that include inhibition data for multiple channels yield better prediction of torsadogenic risk compared to hERG inhibition alone. Illustrative examples of lack of effectiveness of classical markers to predict torsadogenic risk are verapamil and amiodarone. Verapamil is a potent hERG blocker (IC_{50} 0.25 μM at room temperature [16]), yet it does not feature torsadogenic risks, because it also blocks Cav1.2 with similar potency (IC_{50} 0.2 μM), and the effects of blocking an outward and an inward current compensate and cancel each other. Amiodarone is also virtually non-torsadogenic (inducing very unfrequently TdP episodes), despite its marked effect on QTc (prolongation beyond 550 ms) due to effects on multiple calcium and sodium cardiac currents that, again, compensate each other [28].

THE CIPA PARADIGM

This lack of efficiency and robustness in predicting torsadogenic risk of classical proarrhythmogenic markers, both *in vitro* and *in vivo*, related to hERG block and QTc prolongation, which can therefore be associated with a so-called "hERG-centric" paradigm, led to the development of a new proarrhythmogenic risk testing paradigm named the "CiPA paradigm" (Cardiac *in vitro* Pro-arrhythmia Asssay). The proposal was first discussed at a "think tank" meeting held at the FDA headquarters on July 23, 2013, and its rationale and methods were described in Sager *et al*. 2014 [28]. The "CiPA initiative" considers an advanced mechanistic *in vitro* approach as an alternative to the tests comprising the "hERG-centric" paradigm, composed of three stages: stage 1 – assessment of inhibitory effects of a compound on multiple human cardiac ion channels (five or seven distinct channels) expressed in heterologous cell lines, preferably via automated patch-clamp; stage 2 – use of inhibition data derived in previous stage with an advanced humanized ventricular cardiomyocyte model, like the O'Hara-Rudy 2011 model [24], to assess via *in silico* simulations triggering of pro-arrhytmogenic events like early or delayed afterdepolarizations (EADs or DADs) by the tested compound; stage 3 –

validation of *in silico* predictions by real experiments on human cardiomyocytes derived from induced pluripotent stem cells (hiPSC-CM), in various experimental settings. This stepwise approach may result into an in-depth characterization of each tested compound, and may rescue some drug candidates that were previously rejected based on "hERG-centric" criteria, but which are in fact devoid of torsadogenic risks. This explains the large support for the CiPA initiative by a number of drug regulatory agencies and high profile international research institutes, like US Food and Drug Administration (FDA), European Medicines Agency (EMA), Pharmaceuticals and Medical Devices Agency (PMDA-Japan), Health Canada, Japan National Institute of Health Sciences (NIHS), Safety Pharmacology Society (SPS), International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI), Cardiac Safety Research Consortium (CSRC), etc. (http://cipaproject.org).

IMPLEMENTING CIPA ASSAYS

To study feasibility of approaches included in the CiPA initiative and to develop novel CiPA guidelines for cardiac safety drug testing, SPS has settled three working groups, including top experts from pharmaceutical companies, contract research organizations (CROs), and academia, each of them addressing one stage of the CiPA protocol [11]. Thus, the Ion Channel Working Group (ICWG) develops standardized testing protocols for a variety of cardiac ion channels expressed in cell lines, including I_{Kr} (hERG), I_{CaL} (Cav1.2), $I_{Na\ peak}$ and $I_{Na\ late}$ (Nav1.5 peak and late current), $I_{to\ 1\ fast}$ (Kv4.3), I_{Ks} (KCNQ1), and I_{K1} ($K_{IR}2.1$) [7]. A panel of 28 test compounds was defined, including drugs with high, intermediate, and low torsadogenic risk. High risk compounds are: azimilide, bepridil*, dofetilide*, ibutilide, quinidine*, vandetanib, disopyramide, (±)-sotalol*; intermediate risk compounds: astemizole, chlorpromazine*, cisapride*, clarithromycin, clozapine, domperidone, droperidol, terfenadine*, pimozide, risperidone, ondansetron*; low risk compounds: diltiazem*, loratadine, metoprolol, mexiletine*, nifedipine*, nitrendipine, ranolazine*, tamoxifen, verapamil* (compounds marked with * represent the test set, used to develop robust voltage-clamp protocols for the CiPA ion channel panel, while dofetilide and nifedipine are cardiomyocyte calibration compounds). The compounds should be ideally tested both at room temperature (RT) and physiological temperature (PT) (35–37ºC), using different automated patch-clamp platforms, or by manual patch-clamp. In a recent study, Crumb *et al*. provided inhibition data against the CiPA panel of ion channels for 30 compounds, tested by manual patch-clamp [8]. In this study, $I_{Na\ late}$ was tested upon application of veratridine 50 μM in the bath solution, a method that yields large late Na^+ currents, driving many Na^+ channels in the sustained burst mode due to failure to inactivate, but is prone to experimental errors because tested

drugs may interact with veratridine [6]. The In Silico Working Group (ISWG), acting under FDA direction, seeks to apply inhibition data derived by ICWG for the 12 compounds of the test set to the O'Hara-Rudy 2011 humanized ventricular cardiomyocyte electrophysiology model. For the first attempts, the group utilized the inhibition data obtained by Crumb *et al.* [8], combined with dynamic hERG inhibition data generated also by manual patch-clamp at RT and PT, evaluating the usefulness of classical proarrhythmogenic markers such as APD90, triangulation, and EAD occurrence. A part of ICWG transformed recently into a Rapid Response Team (RRT) to mediate interactions between the ion channel and modeling groups. Largely, by the end of 2016, the analysis performed by these two groups for the CiPA panel of drugs was completed. The third group, the Cardiac Stem Cell Working Group (SCWG), sponsored by HESI, attempts to validate modeling predictions by experiments on hiPSC-CM preparations. A large variety of methods are employed and there is still no consensus upon which of them is the best. Thus, multielectrode array (MEA) extracellular AP recordings can be obtained from 2D layers of cardiomyocytes or 3D artificial myocardium constructs, impalement intracellular microelectrodes such as MEA nanopillars can be used [5], optically recorded APs using voltage-sensitive dyes can be obtained from isolated cardiomyocytes or CM tissue layers using fluorescence plate readers or fluorescence microscopy setups. A recent study assessed 26 drugs and 3 drug combinations on two popular commercial hiPSC-CM ventricular cardiomyocyte preparations (Cor.4U®, Axiogenesis, Cologne, DE, and iCell® Cardiomyocytes, CDI, Madison, WI) via voltage-sensitive dyes optical AP recordings and electrical MEA recordings, along with manual patch-clamp on cell lines expressing hERG, Nav1.5, and Cav1.2, concluding that hiPSC-CM represent an adequate *in vitro* test system for pro-arrhythmia [3]. The authors demonstrated that hiPSC-CM respond well to hERG and Cav1.2 blockers, and to a lesser extent to $I_{Na\ late}$ blockers. They also assessed by RT-qPCR the levels of expression of SCN5A (main subunit on Nav1.5), CACNA1C (main subunit of Cav1.2), KCNH2 (main subunit of Kv11.1 – hERG1 – I_{Kr}), and KCNQ1 (main subunit of KvLQT – I_{Ks}) gene transcripts in these commercial cardiomyocyte preparations compared to adult ventricular cardiomyocytes.

CONCLUSIONS AND PERSPECTIVES

Therefore, to date there is no consensus about the optimal method to be employed for stage 3 of the CiPA protocol, and different research groups worldwide eagerly test a large variety of solutions [32].

However, our opinion is that automated patch-clamp platforms, and particularly the CytoPatch™ platforms relying on the patented Cytocentering® technology [31, 33], *via* their high quality and stability of gigaseal whole-cell

configuration, offer the best testing method, allowing accurate recording of resting and action potential, as well as of several cardiac ion channel components, in the same cardiomyocyte [1].

In preliminary studies we tested the feasibility of mixed current-clamp and voltage-clamp recordings in commercial hiPSC-CM preparations on the CytoPatch™ platform, with application of pharmacological compounds [30].

Within a EU-funded project for Regional Development (POC no. 146/2016, acronym CiPA3), we are attempting to combine all three stages of the CiPA paradigm in a single experiment performed on enhanced hiPSC-derived ventricular cardiomyocytes, in order to provide a robust and reproducible industrial standard for cardiac safety drug testing.

Acknowledgements. The authors gratefully acknowledge Dr. Thomas Knott and Dr. William Crumb for instructive comments. This study was funded from Competitiveness Operational Programme 2014–2020 project P_37_675 (contract no. 146/2016), Priority Axis 1, Action 1.1.4, co-financed by the European Funds for Regional Development and Romanian Government funds. The contents of this publication does not necessarily reflect the official position of the European Union or Romanian Government.

REFERENCES

1. AMUZESCU, B., O. SCHEEL, T. KNOTT, Novel automated patch-clamp assays on stem cell-derived cardiomyocytes: Will they standardize *in vitro* pharmacology and arrhythmia research?, *J. Phys. Chem. Biophys.*, 2014, **4**, 1–4, http://dx.doi.org/10.4172/2161–0398.1000153.
2. BEELER, G.W., H. REUTER, Reconstruction of the action potential of ventricular myocardial fibres, *J. Physiol.*, 1977, **268**, 177–210.
3. BLINOVA, K., J. STOHLMAN, J. VICENTE, D. CHAN, L. JOHANNESEN, M.P. HORTIGON-VINAGRE, V. ZAMORA, G. SMITH, W.J. CRUMB, L. PANG, B. LYN-COOK, J. ROSS, M. BROCK, S. CHVATAL, D. MILLARD, L. GALEOTTI, N. STOCKBRIDGE, D.G. STRAUSS, Comprehensive translational assessment of human-induced pluripotent stem cell derived cardiomyocytes for evaluating drug-induced arrhythmias, *Toxicol. Sci.*, 2016, **155**, 234–247.
4. BOTTINO, D., R.C. PENLAND, A. STAMPS, M. TRAEBERT, B. DUMOTIER, A. GEORGIVA, G. HELMLINGER, G.S. LETT, Preclinical cardiac safety assessment of pharmaceutical compounds using an integrated systems-based computer model of the heart, *Prog. Biophys. Mol. Biol.*, 2006, **90**, 414–443.
5. BURRIDGE, P.W., E. MATSA, P. SHUKLA, Z.C. LIN, J.M. CHURKO, A.D. EBERT, F. LAN, S. DIECKE, B. HUBER, N.M. MORDWINKIN, J.R. PLEWS, O.J. ABILEZ, B. CUI, J.D. GOLD, J.C. WU, Chemically defined generation of human cardiomyocytes, *Nat. Methods*, 2014, **11**, 855–860.
6. CHEVALIER, M., B. AMUZESCU, V. GAWALI, H. TODT, T. KNOTT, O. SCHEEL, H. ABRIEL, Late cardiac sodium current can be assessed using automated patch-clamp, *F1000Res.*, 2014, **3**, 245.
7. COLATSKY, T., B. FERMINI, G. GINTANT, J.B. PIERSON, P. SAGER, Y. SEKINO, D.G. STRAUSS, N. STOCKBRIDGE, The comprehensive *in vitro* proarrhythmia assay (CiPA) initiative – Update on progress, *J. Pharmacol. Toxicol. Methods*, 2016, **81**, 15–20.

8. CRUMB, W.J. JR., J. VICENTE, L. JOHANNESEN, D.G. STRAUSS, An evaluation of 30 clinical drugs against the comprehensive *in vitro* proarrhythmia assay (CiPA) proposed ion channel panel, *J. Pharmacol. Toxicol. Methods*, 2016, **81**, 251–262.
9. DI FRANCESCO, D., D. NOBLE, A model of cardiac electrical activity incorporating ionic pumps and concentration changes, *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 1985, **307**, 353–398.
10. FABER, G.M., J. SILVA, L. LIVSHITZ, Y. RUDY, Kinetic properties of the cardiac L-type Ca^{2+} channel and its role in myocyte electrophysiology: a theoretical investigation, *Biophys. J.*, 2007, **92**, 1522–1543, Epub 2006 Dec 1528.
11. FERMINI, B., J.C. HANCOX, N. ABI-GERGES, M. BRIDGLAND-TAYLOR, K.W. CHAUDHARY, T. COLATSKY, K. CORRELL, W. CRUMB, B. DAMIANO, G. ERDEMLI, G. GINTANT, J. IMREDY, J. KOERNER, J. KRAMER, P. LEVESQUE, Z. LI, A. LINDQVIST, C.A. OBEJERO-PAZ, D. RAMPE, K. SAWADA, D.G. STRAUSS, J.I. VANDENBERG, A new perspective in the field of cardiac safety testing through the comprehensive *in vitro* proarrhythmia assay paradigm, *J. Biomol. Screen.*, 2016, **21**, 1–11.
12. FITZHUGH, R., Thresholds and plateaus in the Hodgkin-Huxley nerve equations, *J. Gen. Physiol.*, 1960, **43**, 867–896.
13. HALL, A.E., O.F. HUTTER, D. NOBLE, Current-voltage relations of Purkinje fibres in sodium-deficient solutions, *J. Physiol.*, 1963, **166**, 225–240.
14. HILGEMANN, D.W., D. NOBLE, Excitation-contraction coupling and extracellular calcium transients in rabbit atrium: reconstruction of basic cellular mechanisms, *Proc. R. Soc. Lond. B Biol. Sci.*, 1987, **230**, 163–205.
15. HUTTER, O.F., D. NOBLE, Rectifying properties of heart muscle, *Nature*, 1960, 188, 495.
16. KRAMER, J., C.A. OBEJERO-PAZ, G. MYATT, Y.A. KURYSHEV, A. BRUENING-WRIGHT, J.S. VERDUCCI, A.M. BROWN, MICE models: superior to the HERG model in predicting Torsade de Pointes, *Sci. Rep.*, 2013, **3**, 2100.
17. LAWRENCE, C.L., C.E. POLLARD, T.G. HAMMOND, J.P. VALENTIN, Nonclinical proarrhythmia models: predicting Torsades de Pointes, *J. Pharmacol. Toxicol. Methods*, 2005, **52**, 46–59.
18. MCALLISTER, R.E., D. NOBLE, R.W. TSIEN, Reconstruction of the electrical activity of cardiac Purkinje fibres, *J. Physiol.*, 1975, **251**, 1–59.
19. NOBLE, D., Cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations, *Nature*, 1960, **188**, 495–497.
20. NOBLE, D., Computational models of the heart and their use in assessing the actions of drugs, *J. Pharmacol. Sci.*, 2008, **107**, 107–117.
21. NOBLE, D., Modeling the heart, *Physiology* (Bethesda), 2004, **19**, 191–197.
22. NOBLE, D., A modification of the Hodgkin-Huxley equations applicable to Purkinje fibre action and pace-maker potentials, *J. Physiol.*, 1962, **160**, 317–352.
23. NOBLE, D., R.W. TSIEN, The kinetics and rectifier properties of the slow potassium current in cardiac Purkinje fibres, *J. Physiol.*, 1968, **195**, 185–214.
24. O'HARA, T., L. VIRÁG, A. VARRÓ, Y. RUDY, Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation, *PLoS Comput. Biol.*, 2011, **7**, e1002061.
25. POLAK, S., M.K. PUGSLEY, N. STOCKBRIDGE, C. GARNETT, B. WIŚNIOWSKA, Early drug discovery prediction of proarrhythmia potential and its covariates, *AAPS J*, 2015, **17**, 1025–1032.
26. REDFERN, W.S., L. CARLSSON, A.S. DAVIS, W.G. LYNCH, I. MACKENZIE, S. PALETHORPE, P.K. SIEGL, I. STRANG, A.T. SULLIVAN, R. WALLIS, A.J. CAMM, T.G. HAMMOND, 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.
27. REUTER, H., The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration, *J. Physiol.*, 1967, **192**, 479–492.

28. SAGER, P.T., G. GINTANT, J.R. TURNER, S. PETTIT, N. STOCKBRIDGE, Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium, *Am. Heart J.*, 2014, **167**, 292–300.
29. SATO, D., L.H. XIE, A.A. SOVARI, D.X. TRAN, N. MORITA, F. XIE, H. KARAGUEUZIAN, A. GARFINKEL, J.N. WEISS, Z. QU, Synchronization of chaotic early afterdepolarizations in the genesis of cardiac arrhythmias, *Proc. Natl. Acad. Sci. USA.*, 2009, **106**, 2983–2988, Epub 2009 Feb 2913.
30. SCHEEL, O., S. FRECH, B. AMUZESCU, J. EISFELD, K.H. LIN, T. KNOTT, Action potential characterization of human induced pluripotent stem cell-derived cardiomyocytes using automated patch-clamp technology, *Assay Drug Dev. Technol.*, 2014, **12**, 457–469.
31. SCHEEL, O., H. HIMMEL, G. RASCHER-EGGSTEIN, T. KNOTT, Introduction of a modular automated voltage-clamp platform and its correlation with manual human ether-à-go-go related gene voltage-clamp data, *Assay and Drug Development Technologies*, 2011, **9**, 600–607.
32. SERVICK, K., A painstaking overhaul for cardiac safety testing, *Science*, 2016, **353**, 976–977.
33. STETT, A., C. BURKHARDT, U. WEBER, P. VAN STIPHOUT, T. KNOTT, Cytocentering: a novel technique enabling automated cell-by-cell patch clamping with the CYTOPATCH chip, *Receptors Channels*, 2003, **9**, 59–66.
34. THOMAS, D., C.A. KARLE, J. KIEHN, The cardiac hERG/IKr potassium channel as pharmacological target: structure, function, regulation, and clinical applications, *Curr. Pharm. Des.*, 2006, **12**, 2271–2283.
35. WEIDMANN, S., Effect of current flow on the membrane potential of cardiac muscle, *J. Physiol.*, 1951, **115**, 227–236.