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SM Journal of Cardiology and Cardiovascular Diseases

Review Article

Cardiotoxicity Testing in Drug Development

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Article Information

Received date: Aug 19, 2016
Accepted date: Aug 20, 2016
Published date: Sep 23, 2016

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Keywords Cardiotoxicity; hSC-CMs; Proarrhythmic; Potassium channel; Repolarization

Abstract

The average cost to develop and gain marketing approval for a new drug is estimated to be $2.558 billion according to the most recent analysis by the Tufts Center for the Study of Drug Development. Among solutions to rein in the rising development costs, early and efficient assessment of a drug's cardiotoxicity is essential to reduce drug attrition in late phases of development or drug withdrawal after approval. This article aims to provide a basic understanding of different types of cardiotoxicity followed by an overview of current and new methodologies in cardiotoxicity testing. Emphasis is placed on emerging technologies for the evaluation of proarrhythmia which include the use of human stem cell derived cardiomyocytes and *in silico* modeling. These developments represent an evolving paradigm shift which laid the foundation for the CiPA initiative (Comprehensive *in vitro* Proarrhythmia Assay), a global effort to establish a mechanistically based new system for cardiac safety testing. This is a shift from the current approach which relies on over simplified *in vitro* assays that measure blockade of a single heterologously expressed potassium ion channel (the K_v11.1 or hERG channel). Although increasing levels of complexity of the new system pose new challenges, substantial progress has been made and regulatory implementation is not far away.

Introduction

Cardiotoxicity is one of the leading causes of drug attrition during development [1], and accounts for 22-28% of US post-marketing drug withdrawal [2,3]. In the past decade, driven by rapid advancement of stem cell technologies and deepened knowledge of cardiac pathophysiology, a shift of testing paradigm is undergoing to more accurately predict the risk of drug-induced cardiotoxicity in preclinical and early clinical stages in order to avoid drug candidates being pursued or abandoned erroneously. After a brief introduction of cardiotoxicity, this article aims to provide an overview of current testing methods, their drawbacks and new technologies, with an emphasis on *in vitro* methods.

Cardiotoxicity, when considered as a spectrum of drug-induced adverse effects on cardiovascular system, involves both direct damage to the heart and indirect effects due to alteration of haemodynamic environment or thrombotic events. A plethora of reviews have categorized the drug induced cardiotoxic effects at the physiological level [4-6]. An effort is made here to divide drug-induced cardiotoxicity at the molecular level into three categories including 1) direct damage to mitochondria, 2) disruption of kinase signaling pathways and 3) inhibition of cardiac ion channels. Among various pathways that can lead to mitochondrial dysfunction, generation of reactive oxygen species by chemotherapeutic agents anthracyclines is the most extensively studied [6-8]. Other anti-cancer drugs, usually targeted, fall into the second category. Trastuzumab is one example which inhibits erb-2 pathways [9], resulting in ATP depletion and contractile dysfunction [10]. Bevacizumab is another example which inhibits VEGF signaling pathways leading to arterial thrombotic events [11,12]. Considerable attention has been given to agents that are proarrhythmic. It's now well-understood that prolonging electrical depolarization and repolarization of the ventricles (i.e. QT intervals) is associated with torsades de pointes (TdP), a form of polymorphic ventricular tachycardia that can lead to ventricular fibrillation and sudden cardiac death [1,13,14]. The underlying mechanism of QT prolongation is believed to be the blockade of the rapid component of the delayed rectifier potassium current (I_{Kr}) conducted by the potassium channel (K_v11.1) that's encoded by the human Ether-à-go-go-Related Gene *(hERG or KCNH2)* [15,16].

Current Regulatory Frame Work and Methodologies

The early 1990s witnessed an exponentially increased reporting rate of TdP caused by non-cardiac drugs [17]. Subsequent studies led to the first description of hERG channel and the recognition that the blockade of I_{Kr} is the predominant mechanism responsible for the drug-induced delayed repolarization that is linked to TdP [16]. As part of a global response from drug regulators, the International Committee on Harmonization (ICH) in 2005 introduced guidelines outlining the evaluation of the potential of new chemical entities to delay ventricular repolarization at non-clinical (S7B) [18] and clinical (E14) [19] stages of drug development. In ICH S7B, both *in vitro* and *in vivo* tests are required at four functional levels including the I_{Kr} in cardiomyocytes or heterologous expression systems (*in vitro*) and action potential, Electro Cardio Gram (ECG) and proarrhythmic

effects measured in isolated cardiac preparations or animals (*in vivo/ex vivo*) [18]. The clinical guidance ICH E14 establishes a clinical thorough QT (TQT) study, a carefully controlled clinical test to assess drug-induced QTc (the QT interval corrected for changes in heart rate) prolongation [19].

For *in vitro* tests, Human Embryonic Kidney (HEK) and Chinese Hamster Ovary (CHO) cell lines with stable and heterologous hERG channel expression are widely used owing to their commercial availability [20,21]. Currently used *in vitro* assays based on these heterologous expression systems include 1) binding assays based on the displacement of radio labeled I_{Kr} blocker, such as [3H] dofetilide, 2) ion flux assays based on fluorescent measurement of thallium flux through hERG channels, 3) measurement of membrane potential by membrane potential sensitive fluorescent dyes, and 4) measurement of hERG current by manual or automated patch clamping [21,22]. Currently used *ex vivo* cardiac preparations include Purkinje-fibers, rabbit ventricular wedge preparations and Langendorff-perfused rabbit hearts [21,22].

The "hERG-centric" approach, while effective in reducing drug induced TdP, is too conservative, resulting in a high percentage of false positives abandoned early in development [23]. False negatives have also been reported [20]. The misrepresentation of the clinical outcome (TdP) by the surrogate biomarker (I_{Kr} blockade) can be found on two levels. On the first level, hERG inhibition does not always lead to QT prolongation. Cardiac repolarization is a result of complex interplay between inward and outward currents conducted by multiple ion channels and drugs' effects on cardiac cells are more promiscuous than expected [20]. Changes of I_{Kr} alone do not represent a drug's integrated effect on the net outward current which determines the QT interval. For example, Verapamil, despite being a potent hERG inhibitor, does not cause significant QT prolongation because of its concurrent blockade of the inward calcium current which counteracts the effects of reduced I_{Kr} outward current [24]. On the second level, QT prolongation lacks a clear and direct correlation with TdP [25,26]. For example, Ranolazine, a novel anti-anginal agent and multi-ion channel blocker, prolongs the QT interval clinically but is not typically linked with TdP [27]. More detailed studies of the mechanisms responsible for TdP suggest that early after depolarization (EAD)-induced triggered activity and increased Transmural Dispersion of Repolarization (TDR) are more direct causes of TdP [28,29]. Since prolongation of action potential duration (APD) and QT by Ranolazine is rate independent, it is not associated with EAD or increased TDR, thus not linked to TdP [30]. Mechanistic understanding of these limitations provides the opportunity to develop more comprehensive approaches for predicting proarrhythmic risk.

New Testing Paradigm

The new paradigm for cardiac safety evaluations cannot be discussed without the mentioning of an important meeting held on July 23, 2013 at the US Food and Drug Administration's White Oak facilities, Silver Spring, MD. The white paper of the meeting provides a summary of a proposal to replace the "hERG-centric" approach with a Comprehensive *In vitro* Proarrhythmia Assay (CiPA) [31]. A more in-depth discussion of the components and challenges of the CiPA initiative can be found in an excellent article published recently [20]. Briefly, the CiPA approach includes three steps [31,32]. First, drug effects on multiple individual cardiac ionic channels (which mediate inward and outward currents) in heterologous expression systems are evaluated [31]. Second, the data obtained from the first step are integrated using *in silico* modeling to reconstruct the ventricular action potential and evaluate the propensity for EADs and repolarization instability [31]. Third, Human Stem Cell-Derived Cardiomyocytes (hSC-CMs) are used to confirm the drug's integrated effects on an intact human-based physiologic system [31]. A non-clinical *in vivo* study and a clinical element that includes the assessment of ECGs from Phase I studies are also included [31].

Since this proposal was put forward, an international network of consortia and experts have been working together to develop and standardize these assays for general use with a target date of December 2017 set for completing validation [32]. For the first step, 7 ionic currents have been chosen including I_{NaFast}, I_{NaLate} and I_{CaL} for inward currents and I_{Kr}, I_{Ks}, $I_{K1}$and I_{to} for outward currents [32]. Automated patch-clamping is proposed as the suitable platform to characterize the effects of drugs on these currents using HEK or CHO cells expressing individual ion channels [32]. 28 compounds with established high, intermediate and low risk of Torsade de Pointes (TdP) have been selected and divided into a set of 12 drugs to be used for CiPA training and calibration, and the remaining 16 used for CiPA validation [32]. A standardized protocol for hERG channel has been established by manual patch-clamp technique [32]. Non-hERG channels are being tested and the protocol is being adapted for automated platform [32].

For *in silico* modeling, the O'Hara-Rudy model has been adopted as the starting point for further development [33,34]. A dynamic model of the hERG channel that enables quantitative predictions of AP modulation was developed and shown to be a significant improvement over traditional conductance reduction methods [35]. Different metrics are being evaluated to develop a more sophisticated model that could distinguish different levels of TdP risk rather than identifying a drug as torsadogenic or non-torsadogenic [32].

Due to the difficulties in obtaining human cardiac tissues and issues related to poor viability and proliferation capacity, the use of adult human ventricular tissues or myocytes for routine drug screening is not feasible [36]. Recent advance in stem cell technology provides a viable alternative. Both Human Embryonic Stem Cells (hESCs) and Human Induced Pluripotent Stem Cells (hiPSCs) are being used to derive Cardiomyocytes (CMs) for drug development, each with its pros and cons. Developed almost a decade earlier than hiPSCs, hESC-CMs are better characterized [37-40]. The use of somatic cells generated hiPSCs, however, can avoid many ethical issues associated with hESCs and make the study of patient specific cells (with different genetic backgrounds) possible [36,40,41]. A major concern for both CMs is the heterogeneity in maturity and cell types. Even fully differentiated, these CMs are usually a combination of cells at different developmental stages and of different subtypes (nodal, atrial, or ventricular) [39,41]. Both also face several technical challenges of the same magnitude including low efficacy in establishing cell lines, poor scalability and difficulties in standardization [38].

Despite these limitations, Human Stem Cell Derived Cardiomyocytes (hSC-CMs) emerge as a novel platform that could bridge the gaps found in *in vitro* heterologous expression systems and *in vivo* animal models during preclinical development. A more comprehensive risk assessment can be made by using hSC-CMs to identify electrophysiological effects not detected from isolated

current measures. The use of human CMs instead of animal tissues also partly addresses the species related differences in ventricular repolarization and responses to drugs [20,22]. Techniques that are currently used to assess electrophysiological changes in hSC-CMs include Microelectrode Array (MEA) field potential measurements [42], Voltage-Sensing Optical (VSO) approaches [20] and impedance measurements [43]. The biggest challenge of evolving the hSC-CMs approach in the CiPA paradigm lies in myocytes validation and calibration. In 2014, a pilot study was conducted by the CiPA Myocyte Working Group to evaluate the reproducibility and variability of the electrophysiologic response to 8 compounds across 4 hSC-CMs types and 12 volunteer sites [32]. An extended phase II validation study of 28 compounds across 6 technology platforms (4 MEA and 2 VSO) using two commercial hSC-CM preparations is undergoing to establish benchmarks [32]. As stated in the recent CiPA report, the hSC-CMs approach in the CiPA will move away from confirming the ionic current studies and *in silico* reconstructions and "towards a platform that more closely resembles mature electrophysiologic phenotypes from normal (and diseased) states" [32].

Future directions

As mentioned above, a major challenge faced by the use of hSC-CMs is the immaturity of the cell preparations. To achieve greater maturation of hSC-CMs, ongoing efforts include mechanical and electrical stimulation [44], prolonged culture time [45], 3D culture and co-culture systems [46,47]. Although these new systems still await validation and standardization, it is anticipated that hSC-CMs will be able to faithfully recapitulate a mature electrophysiological and contractile phenotype.

While the pharmaceutical industry and regulatory agencies have largely focused on drugs' proarrhythmic effect, detecting other types of cardiotoxicity during early stage of drug development is no less important. In an internal study conducted by Astra Zeneca on ~1000 compounds with cardiac liability, as high as 75% of the compounds were found to affect aspects of cells other than electrophysiology [48]. With the availability of more physiologically relevant hSC-CMs, new methods are being developed to examine structural and contractile cardiotoxicity. High-content screening which combines automated, high-throughput platform with image-based, multi-probe, multi-parametric analysis techniques is a promising method that allows comprehensive phenotypic profiling of test compounds [49,50]. Mitochondrial functions, calcium handling, ROS production, and apoptosis are some of the important parameters being monitored. Similar to analyzing proarrhythmic effect, mechanistic understanding of these other types of cardiotoxicity is needed for more accurate risk assessment. How to process and interpret the large amount of data generated by high-content screening remains a challenge. Development of methods to more comprehensively assess the multi-parametric cellular responses should be the focus of future studies.

When translating information acquired at the preclinical level into clinical situation, many more factors at the human physiology and population levels have to be taken into account [51]. These factors may include 1) pharmacokinetics and drug-drug interactions, 2) demographic and genetic factors and 3) environmental factors. Studies have emerged which use *in silico* modeling as a vital tool to achieve *in vitro-in vivo* extrapolations [51]. In one such study, Cardiac Safety Simulator, a software which integrates Physiologically-Based Pharmacokinetic (PBPK) modeling and simulation of a cardiomyocytes model was tested to evaluate drug cardiac safety at the population level [52]. The variation in the population was only partially reconstructed, indicating additional unknown factors. In another study, based on the same platform, a combination of Quantitative Structure - Activity Relationship (QSAR), PBPK, Pharmaco Dynamics (PD), and systems biology was used to predict the effect of drug-drug interaction at the population level, although some disparities were detected [53]. While well-designed clinical trials are still necessary, these *in silico* methods can become valuable tools for cost-effective decision making. Future development may include integration of more preclinical and clinical data, more detailed physiology descriptions of different target populations and additional cardiac safety endpoints.

Conclusion

The field is coming to a consensus on the use of the hSC-CMs combined with *in silico* modeling as the center approach for early cardiac safety screening. Once the cell preparations and calibration protocols are standardized, it will provide a unifying platform for the efficient evaluation of not only proarrhythmic, but also contractile and structural cardiotoxicity. Since the first discussion of CiPA in 2013, significant progress has been made to define, standardize, and validate more comprehensive, mechanistic-based testing system. As these efforts continue, regulatory implementation and eventual benefits to patients are not far away.

Reference

1. Nicola F, Peter S, Alberto C, Joerg H, Amir L, Renee B. Drug attrition during pre-clinical and clinical development: Understanding and managing drug-induced cardiotoxicity. Pharmacol Therapeut. 2013; 138: 470-484.
2. Wilke RA, Debbie WL, Dan MR, Paul BW, David F, Issam Z, et al. Identifying genetic risk factors for serious adverse drug reactions: current progress and challenges. Nat Rev Drug Discov. 2007; 6; 904-916.
3. Onakpoya IJ, Heneghan CJ, Aronson JK. Post-marketing withdrawal of 462 medicinal products because of adverse drug reactions: a systematic review of the world literature. BMC Med. 2016; 14: 10.
4. Brana I, Tabernero J. Cardiotoxicity. Ann Oncol. 2010; 21: 173-179.
5. Ewer MS, Ewer SM. Cardiotoxicity of anticancer treatments. Nat Rev Cardiol. 2015; 12: 547-558.
6. Varga ZV, Ferdinandy P, Liaudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardiotoxicity. Am J Physiol-Heart. 2015; 309: 1453-1467.
7. Doroshow JH. Effect of Anthracycline Antibiotics on Oxygen Radical Formation in Rat-Heart. Cancer Res. 1983; 43: 460-472.
8. Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nat Med. 2012; 18, 1639-1642.
9. De Keulenaer GW, Doggen K, Lemmens K. The Vulnerability of the Heart as a Pluricellular Paracrine Organ Lessons From Unexpected Triggers of Heart Failure in Targeted ErbB2 Anticancer Therapy. Circ Res. 2010; 106: 35-46.
10. Grazette LP, Boecker W, Matsui T, Semigran M, Force TL, Hajjar RJ et al. Inhibition of ErbB2 causes mitochondrial dysfunction in cardiomyocytes: Implications for herceptin-induced cardiomyopathy. J Am Coll Cardiol. 2004; 44: 2231-2238.
11. Chen MH, Kerkelae R, Force T. Mechanisms of cardiac dysfunction associated with tyrosine kinase inhibitor cancer therapeutics. Circulation. 2008; 118: 84-95.
12. Kabbinavar F, Hurwitz HI, Fehrenbacher L, Meropol NJ, Novotny WF, Lieberman G, et al. Phase II, randomized trial comparing bevacizumab plus Fluorouracil (FU) Leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol. 2003; 21; 60-65.

13. Kannankeril P, Roden DM, Darbar D. Drug-Induced Long QT Syndrome. Pharmacol Rev. 2010; 62; 760-781.

14. Roden DM. Cellular basis of drug-induced torsades de pointes. Brit J Pharmacol. 2008; 154, 1502-1507.

15. Sanguinetti MC, Jiang CG, Curran ME, Keating MT. A Mechanistic Link between an Inherited and an Acquired Cardiac-Arrhythmia - Herg Encodes the I-Kr Potassium Channel. Cell. 1995; 81: 299-307.

16. Rampe D, Brown AM. A history of the role of the hERG channel in cardiac risk assessment. J Pharmacol Tox Met. 2013; 68: 13-22.

17. Stockbridge N, Morganroth J, Shah RR, Garnett C. Dealing with Global Safety Issues Was the Response to QT-Liability of Non-Cardiac Drugs Well Coordinated? Drug Safety. 2013; 36: 167-182.

18. Guideline, ICH Harmonised Tripartite. The non-clinical evaluation of the potential for delayed ventricular repolarization (QT Interval Prolongation) by human pharmaceuticals. 2005; S7B.

19. Guideline, ICH Harmonised Tripartite. The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. 2005; E14.

20. Gintant G, Sager PT, Stockbridge N. Evolution of strategies to improve preclinical cardiac safety testing. Nat Rev Drug Discov. 2016; 15; 457-471.

21. Shinde V, Chaudhari U, Sotiriadou I, Hescheler J, Sachinidis A. In vitro Methods for Cardiotoxicity Testing. Method Pharmacol Tox. 2014; 45-77.

22. Heijman J, Voigt N, Carlsson LG, Dobrev D. Cardiac safety assays. Curr Opin Pharmacol. 2014; 15: 16-21.

23. Polak S, Pugsley MK, Stockbridge N, Garnett C, Wisniowska B. Early Drug Discovery Prediction of Proarrhythmia Potential and Its Covariates. AAPS J. 2015; 17: 1025-1032.

24. Aiba T, Wataru S, Masashi I, Takashi N, Shunichiro M, Wei-Guang Ding, et al. Cellular and ionic mechanism for drug-induced long QT syndrome and effectiveness of verapamil. J Am Coll Cardio. 2005; l45: 300-307.

25. Schwartz PJ, Woosley RL. Predicting the Unpredictable Drug-Induced QT Prolongation and Torsades de Pointes. J Am Coll Cardiol. 2016; 67: 1639-1650.

26. Ahmad K, Dorian P. Drug-induced QT prolongation and proarrhythmia: an inevitable link? Europace. 2007; 9: 16-22.

27. Cobbe S. Electrophysiological perspectives - what has ranolazine taught us? Eur Heart J Suppl. 2004; 6: 9-11.

28. Antzelevitch C. Role of transmural dispersion of repolarization in the genesis of drug-induced torsades de pointes. Heart Rhythm. 2005; 2: 9-15.

29. Wu J, Zipes DP. Early after depolarizations, U waves, and torsades de pointes. Circulation. 2002; 105: 675-676.

30. Antzelevitch C, Luiz B, Andrew CZ, Alexander B, Di Diego JM, Jeffrey MF, et al. Electrophysiological effects of ranolazine, a novel antianginal agent with antiarrhythmic properties. Circulation. 2004; 110: 904-910.

31. Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N. Rechanneling the cardiac proarrhythmia safety paradigm: A meeting report from the Cardiac Safety Research Consortium. Am Heart J. 2014; 167: 292-300.

32. Colatsky T, Fermini B, Gintant G, Pierson JB, Sager P, Sekino Y, et al. The Comprehensive In vitro Proarrhythmia Assay (CiPA) initiative - Update on progress. J Pharmacol Toxicol Methods. 2016.

33. O'Hara T, Virag L, Varro A, Rudy Y. Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. PLoS computational biology. 2011; 7; e1002061.

34. O'Hara T, Rudy Y. Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species. Am J Physiol Heart Circ Physiol. 2012; 302: 1023-1030.

35. Di Veroli GY, Davies MR, Zhang H, Abi-Gerges N, Boyett MR. High-throughput screening of drug-binding dynamics to HERG improves early drug safety assessment. Am J Physiol Heart Circ Physiol.2013; 304: 104-117.

36. Mordwinkin NM, Burridge PW, Wu JC. A review of human pluripotent stem cell-derived cardiomyocytes for high-throughput drug discovery, cardiotoxicity screening, and publication standards. J Cardiovasc Transl Res. 2013; 6: 22-30.

37. Kehat I, Dorit KK, Mirit S, Hana S, Michal A, Amira G, et al. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. J Clin Invest. 2001; 108: 407-414.

38. Mandenius CF, Steel D, Noor F, Meyer T, Heinzle E, Asp J, et al. Cardiotoxicity testing using pluripotent stem cell-derived human cardiomyocytes and state-of-the-art bioanalytics: A review. Journal of applied toxicology: JAT. 2011; 31: 191-205.

39. Khan JM, Lyon AR, Harding SE. The case for induced pluripotent stem cell-derived cardiomyocytes in pharmacological screening. Brit J Pharmacol. 2013; 169: 304-317.

40. Liang P, Lan F, Lee AS, Gong T, Sanchez-Freire V, Wang Y, 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.

41. Matsa E, Ahrens JH, Wu JC. Human Induced Pluripotent Stem Cells as a Platform for Personalized and Precision Cardiovascular Medicine. Physiol Rev. 2016; 96: 1093-1126.

42. Li X, Zhang R, Zhao B, Lossin C, Cao Z. Cardiotoxicity screening: A review of rapid-throughput In vitro approaches. Arch Toxicol. 2016; 90: 1803-1816.

43. Peters MF, Lamore SD, Guo L, Scott CW, Kolaja KL. Human Stem Cell-Derived Cardiomyocytes in Cellular Impedance Assays: Bringing Cardiotoxicity Screening to the Front Line. Cardiovasc Toxicol. 2015; 15: 127-139.

44. Stoppel WL, Kaplan DL, Black LD 3rd. Electrical and mechanical stimulation of cardiac cells and tissue constructs. Adv Drug Deliv Rev. 2016; 96: 135-155.

45. Lundy SD, Zhu WZ, Regnier M, Laflamme MA. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Stem Cells Dev. 2013; 22: 1991-2002.

46. Ou DB, He Y, Chen R, Teng JW, Wang HT, Zeng D, et al. Three-Dimensional Co-Culture Facilitates the Differentiation of Embryonic Stem Cells Into Mature Cardiomyocytes. J Cell Biochem. 2011; 112: 3555-3562.

47. Mathur A, Loskill P, Shao K, Huebsch N, Hong S, Marcus SG, et al. Human iPSC-based cardiac microphysiological system for drug screening applications. Sci Rep. 2015; 5: 8883.

48. Chi KR. Revolution dawning in cardiotoxicity testing. Nat Rev Drug Discov. 2013; 12: 565-567.

49. Pointon A, Abi-Gerges N, Cross MJ, Sidaway JE. Phenotypic profiling of structural cardiotoxins In vitro reveals dependency on multiple mechanisms of toxicity. Toxicol Sci1. 2013; 32: 317-326.

50. Mioulane M, Foldes G, Ali NN, Schneider MD, Harding SE. Development of high content imaging methods for cell death detection in human pluripotent stem cell-derived cardiomyocytes. J Cardiovasc Transl Res. 2012; 5: 593-604.

51. Polak S, Wisniowska B, Fijorek K, Glinka A, Mendyk A. In vitro - In vivo extrapolation of drug-induced proarrhythmia predictions at the population level. Drug Discov Today. 2014; 19: 275-281.

52. Glinka A, Polak S. The effects of six antipsychotic agents on QTc An attempt to mimic clinical trial through simulation including variability in the population. Comput Biol Med. 2014; 47: 20-26.

53. Mishra H, Polak S, Jamei M, Rostami-Hodjegan A. Interaction between domperidone and ketoconazole: toward prediction of consequent QTc prolongation using purely in vitro information. CPT Pharmacometrics Syst Pharmaco. 2014; l3: 130.