

**Next generation cardiac safety testing through the Comprehensive *in vitro*  
Proarrhythmia Assay (CiPA) paradigm**

Bernard Fermini<sup>1</sup>, Jules C. Hancox<sup>2</sup>, Najah Abi-Gerges<sup>3,4</sup>, Mark Bridgland-Taylor<sup>3</sup>,  
Khuram Chaudray<sup>5</sup>, Thomas Colatsky<sup>6</sup>, Krystle Correll<sup>7</sup>, William Crumb<sup>8</sup>, Bruce  
Damiano<sup>9</sup>, Gul Erdemli<sup>10</sup>, Gary Gintant<sup>11</sup>, John Imredy<sup>12</sup>, John Koerner<sup>6</sup>, James  
Kramer<sup>13</sup>, Derek Leishman<sup>14</sup>, Paul Levesque<sup>15</sup>, Zhihua Li<sup>6</sup>, Andrew Lindqvist<sup>16</sup>,  
Carlos Obejero-Paz<sup>13</sup>, David Rampe<sup>17</sup>, Kowei Sawada<sup>18</sup>, David Strauss<sup>6</sup>, Jamie  
Vandenberg<sup>19</sup>, and Hugo Vargas<sup>20</sup>

**Corresponding Author:**

Bernard Fermini, Pfizer Inc, MS8274-1347, Eastern Point Road, Groton, CT 06340

Email: [Bernard.fermini@pfizer.com](mailto:Bernard.fermini@pfizer.com)

## **Abstract**

For the last decade, cardiac safety screening to evaluate the propensity of drugs to produce QT interval prolongation and Torsades de Pointes (TdP) arrhythmia has been conducted according to the ICH-S7B and ICH-E14 guidelines. hERG channel assays and in vivo QT measurements have been central to the existing approach. While effective, the present paradigm carries a risk of unnecessary compound attrition and high cost, especially when “thorough QT” (TQT) studies are initiated. The **Comprehensive *in vitro* Proarrhythmia Assay** (CiPA) initiative is a public-private collaboration with the aim of updating the existing cardiac safety testing paradigm in order to better evaluate arrhythmia risk and remove the need for TQT testing. It is hoped that CiPA will produce a standardized ion channel assay approach, incorporating defined tests against major cardiac ion channels, the results of which then inform evaluation of proarrhythmic actions *in silico*, using human ventricular action potential models. These results are then to be validated using human (stem-cell derived) cardiomyocytes. This article reviews the rationale, progress of and challenges for the CiPA initiative, if this new paradigm to replace existing practice and, in time, lead to updated and widely accepted cardiac safety testing guidelines.

(Abstract 194 words; limit 200 words)

**Key words:** CiPA; Comprehensive *in vitro* Proarrhythmic Assay; hERG; ICH-E14; ICH-S7B; QT interval; safety

## Introduction

On July 23, 2013, a think-tank meeting sponsored by the Cardiac Safety Research Consortium (CSRC), Health and Environmental Sciences Institute (HESI), and the US Food and Drug Administration (FDA) was held at the FDA headquarters in Silver Spring, MD, to discuss a novel approach to assess the pro-arrhythmic potential of drugs that prolong the QT interval. Prolongation of the QT interval is used as a surrogate marker for predicting the risk of a compound to induce a potentially fatal ventricular cardiac arrhythmia called *Torsade de Pointes* (TdP). However, the link between QT interval prolongation and TdP appears multifaceted, and is influenced by a number of underlying factors including age, gender, underlying disease state, electrolyte imbalance, concomitant medication and more <sup>1,2</sup>. While the majority of compounds that can induce TdP are known to inhibit cardiac potassium channels encoded by *human-ether-à-go-go Related Gene (hERG)* <sup>3-5</sup>, block of ionic current ( $I_{hERG}$ ) carried by recombinant hERG channels alone is not always predictive of delayed repolarization or proarrhythmic risks. For example, the L-type calcium channel blocker, verapamil, is a potent blocker of hERG current <sup>6</sup> but does not prolong the QT interval or pose a risk of TdP <sup>7</sup>. The current preclinical (ICH S7B) and clinical (ICH E14) safety guidelines require first a preclinical electrophysiology test against hERG (or the native cardiac equivalent, the rapid delayed rectifier current  $I_{Kr}$ ; usually hERG) and an *in vivo* QT measurement followed, for drugs that pass preclinical testing, by a thorough QT (TQT) study <sup>8,9</sup>. All these measures are surrogates for rather than automatic predictors of TdP and following a decade of testing using this approach, it has become clear that the strong initial focus on a single ion channel (hERG) favours a conservative approach that may have led to the attrition of potentially useful drugs, mainly on the grounds of their activity on hERG

rather than due to arrhythmia induction *per se*. There is good reason, therefore, to reconsider approaches to the evaluation of drug-induced arrhythmia.

This article will focus on the pre-clinical aspects of the new paradigm and will not address in detail its impact on the E-14 guidance document or the dedicated TQT study. To touch on those briefly, collaboration between the Consortium for Innovation and Quality in Pharmaceutical Development and the CSRC was established to design a clinical study in healthy subjects demonstrating that the TQT study can be replaced by robust ECG monitoring and exposure-response analysis of data generated from First-in-Man single ascending dose studies. Results from that study were presented at a meeting held in Silver Spring, MD, in December 2014, and readers should see Darpo et al <sup>10</sup> for more information.

### **What is CiPA?**

The new paradigm, termed **Comprehensive *in vitro* Proarrhythmia Assay (CiPA)**, has the objective to engineer, early in the drug discovery and development process, assays allowing the evaluation of the proarrhythmic risk of compounds, instead of concentrating on their ability to inhibit the hERG current and to prolong the QT interval <sup>11</sup>. This new paradigm is based on the fundamental mechanistic understanding of the role of ion channels in delayed ventricular repolarization, alterations to which lead to repolarization instability and arrhythmias. It is composed of two distinct series of tests: 1) the *in vitro* study of drug effects on multiple ion channels (not just hERG), and incorporation of these effects in an *in silico* model of a human ventricular action potential (AP) in an effort to reconstruct the effects on

ventricular repolarization and reveal potential markers of proarrhythmia (such as early afterdepolarizations (EAD); delayed afterdepolarization (DAD) and AP triangulation<sup>3,5,12</sup>), and 2) confirmation of the *in silico* results using human ventricular myocytes, likely derived from human induced pluripotent stem cell (iPSC) cardiomyocytes. The CiPA initiative is being taken forwards by a consortium composed of a number of collaborators including, FDA, HESI, CSRC, Japan Nation Institute of Health Sciences (NIHS), Health Canada, European Medicines Agency (MEA), Pharmaceutical and Medical Devices Agency (PMDA, Japan), Japan iPS Cardiac Safety Assessment (JiSCA), academics, *in silico* modellers, and partners from contract research organizations, the pharmaceutical industry and device companies. Because of the complexity of the task proposed, several work streams have been established in support of CiPA. As such, the Safety Pharmacology Society (SPS) has positioned itself as a foremost contributor to this effort by establishing the Ion Channel Working Group (ICWG). This group builds on considerable experience and expertise of its members in the field of ion channels biophysics, pharmacology and the understanding of translation from *in vitro* to *in vivo* models. The ICWG is tasked of bringing together expertise and resources required to deliver best practice recommendations for generating ion channel data needed for *in silico* human cardiac AP simulations of proarrhythmic liabilities. The SPS also supports the CiPA effort in the form of staged funding of the experimental work to be performed. Other committees critical to the success of CiPA include the *In silico* Working group (ISWG), under the direction of the FDA, that is responsible for the selection and development of the best *in silico* model of human ventricular electrophysiology for the AP reconstruction of drug effects on the individual ion channel as determined by the work of the ICWG, and the Cardiac Stem Cell Working

group (SCWG), sponsored by the HESI who will define best practice for performing experiments using human stem cell-derived cardiomyocytes in an effort to validate the effects observed on ion channels, and/or *in silico* modelling, and to unmask effects that, for various reasons, were not revealed in either the ion channel or *in silico* work. Finally, a sub-committee from the joint HESI/CSRC Clinical Translation Working Group (CTWG) selected a series of 29 compounds to be tested in the CiPA paradigm and categorized in 3 different risk groups based on their torsadogenic potential (high, intermediate and very low, or none) according to published and publically available data and expert opinion (<sup>7</sup>; <sup>13,14</sup>, Credible Meds; FDA AERS Database, FDA labelling). The intent in the selection of the compounds is to cover a wide spectrum of electrophysiological endpoints including not only the degree of TdP risk, but also their effects on ion channels, as well as inclusion of some compounds with non-hERG TdP risk. Compounds in the high risk category include: azimilide (antiarrhythmic), bepridil (angina), dofetilide (antiarrhythmic), ibutilide (antiarrhythmic), quinidine (antiarrhythmic), vandetanib (anti-cancer), methadone (opioid addiction), and D, l sotalol (antiarrhythmic). Those in the intermediate risk include astemizole (antihistamine), chlorpromazine (antipsychotic), cisapride (gastrokinetic), clarithromycin (antibacterial), clozapine (antipsychotic), domperidone (anti-mimetic), droperidol (anti-mimetic), terfenadine (antihistamine), pimozide (antipsychotic), risperidone (antipsychotic), and odansatron (anti-mimetic). Finally, the very low risk compounds include diltiazem (hypertension/angina), loratadine (antihistamine), metoprolol (hypertension/angina), mexilitine (antiarrhythmic), nifedipine (hypertension/angina), nitrendipine (hypertension/angina), ranolazine (angina), tamoxifen (anti-cancer), verapamil (hypertension/angina), and flecainide (antiarrhythmic).

## The need for a new paradigm

In order to appreciate the necessity to modify the current strategy, one must revisit the evolution of the regulatory guidelines in order to appreciate our current position. Indeed, beginning in the late 1980's, spontaneous case reports of cardiotoxicity related to the use of the non-sedating H1-antihistamine, terfenadine, began to appear in the literature. Eventually, this drug was shown to cause TdP and sudden death by prolonging cardiac repolarization following inhibition of the delayed rectifier potassium channel,  $I_{Kr}$ <sup>15</sup>. As similar reports with other non-cardiovascular agents began to appear more frequently, in 1997 the Committee for Proprietary Medicinal Products (CPMP) took action and issued a "Points to Consider" document that advocated a series of *in vitro* and *in vivo* experiments to assess the risk of QT prolongation (CPMP/986/96). More specifically, the guidance recommended the undertaking of *in vitro* electrophysiological studies (effects on AP duration) using cardiac tissue that included ion channels corresponding to those contributing to repolarization in human cardiac tissue. Following increasing regulatory scrutiny by various regulatory agencies and expert working groups, in 2005 the International Conference on Harmonization (ICH) issued the two imperative guidance documents that are still the standard today as safety pharmacology guidelines for the development of new chemical entities: the ICH-S7B, entitled "Non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval Prolongation) by human pharmaceuticals" describes a non-clinical testing strategy for assessing the potential of a test compound to delay ventricular repolarization, and the ICH-E14 entitled "Clinical evaluation of the QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs" requesting that all sponsors submitting new drug applications conduct a thorough TQT study to determine if a drug prolongs the

heart-rate corrected QT interval (QTc)<sup>8,9</sup>. Clearly the introduction and adoption of these guidance documents has proven successful: there has been no withdrawal of marketed drugs for concerns of TdP since they were adopted in 2005, while 6 non cardiac medications were pulled from the US market by regulatory agencies between 1995 and 2003 at great risks to the patients, and costs to the pharmaceutical industry. Nonetheless, current regulatory concern for any candidate drug to inhibit hERG current, and to prolong the QT interval by a magnitude greater than 5ms leads to extensive evaluation in Phase IIb and III trials at significant costs; since 2005, approximately 450 thorough QT/QTc studies have been performed at an estimated cost of approximately 1 billion dollars<sup>16 17</sup>. Moreover, the major learning from a decade of clinical studies is that increase in QTc is a sensitive, but not specific endpoint to predict proarrhythmia. Therefore, the current preclinical and clinical strategies are suitable to predict QT prolongation, but inadequate to assess the risk of TdP, and need to be revised.

### **Drawbacks of the existing approach and predominant focus on the hERG channel**

Since the launch of the ICH guidelines, pharmaceutical companies have established a number of preclinical assays to identify, early in the discovery process, compounds that have the propensity to block the hERG channel<sup>3,5,12</sup>. Arguably, the ideal preclinical assay for drug-induced  $I_{Kr}$  inhibition would involve electrophysiological recording of native  $I_{Kr}$  from ventricular cardiomyocytes from an appropriate model species. However, the requirement for enzymatic and mechanical myocyte dispersion from intact tissue, together with challenges to effective  $I_{Kr}$  measurement make native  $I_{Kr}$  measurements impractical as a primary safety assay, as inevitably



such measurements would be of low throughput<sup>5</sup>. Additionally, native  $I_{Kr}$  is of small amplitude, necessitating its pharmacological isolation from other overlapping currents, further complicating its use as a primary screen<sup>5</sup>. Consequently, for over ten years, recombinant hERG channel assays have been employed in preclinical drug safety testing: as far back as 2005 hERG assays were used by 93% of surveyed respondents from 119 pharmaceutical for preclinical evaluation of drug modification of repolarization in line with ICH-S7B<sup>18</sup>. Mammalian cell lines over-expressing hERG are attractive, because they permit moderately high throughput automated patch clamp assays, with measurements of a current of large amplitude compared to native  $I_{Kr}$ , in the absence of contamination from overlapping conductances<sup>5,19,20</sup>. Whilst native  $I_{Kr}$  may be composed of hERG1a and hERG1b subunits (Jones et al., 2014) and both KCNE1 and KCNE2 subunits can associate with hERG and, potentially, could influence hERG channel pharmacology<sup>21,22,22-24</sup>, pharmacological potencies derived from hERG 1a alone expressed in mammalian cells approximate well those for native  $I_{Kr}$ <sup>25,26</sup> and co-expression of hERG 1a and 1b or hERG 1a with KCNE1 or KCNE2 subunits is not routinely employed for screening.

The ICH 7SB guidelines stipulate that radioligand competition binding assays are not a viable alternative to an electrophysiological assay, as they provide no information on a compound's ability to modify the current itself<sup>8</sup>. However, beyond indicating that  $I_{Kr}$ / hERG ionic current should be assayed, ICH S7B guidelines are not prescriptive<sup>8</sup>. This has the advantage of permitting flexibility of user approach, both in measurement platform and experimental protocol. However, the potential disadvantage of this flexibility is variability of data due to a lack of standardized approach. The potency of some hERG blockers shows marked sensitivity to stimulus protocol and/or measurement temperature<sup>27-29</sup>. The extent to which measurement

conditions/protocol influence half-maximal inhibitory effect concentration ( $IC_{50}$ ) may vary significantly between drugs: one comparative analysis of cisapride and dofetilide reported variability of  $I_{hERG}$   $IC_{50}$  for dofetilide between 4 and 46 nM and for cisapride between 7 and 240 nM in published literature derived from mammalian cell lines<sup>29</sup>. This further correlated with greater variability for cisapride than for dofetilide  $IC_{50}$  (7-72 nM versus 4-15 nM) in experiments at a single temperature in a single study, between step, step-ramp and action potential voltage commands<sup>29</sup>. A difficulty that this issue poses for the study of novel chemical entities (NCEs) is that protocol dependence of hERG block is not possible to predict in advance. At present, there is no consensus on a single 'best' protocol for studying  $I_{hERG}$  sensitivity to NCEs, though adoption of a standardized approach could reduce variability: a comparative blinded patch-clamp investigation of 12 hERG inhibitors between two contract research organizations employing similar measurement methods and standardized solutions, temperature and protocol showed an  $IC_{50}$  variability between laboratories was in the order of ~3-fold or less for all but one compound<sup>30</sup>.

The ICH S7B guidelines recommend that where possible full concentration-response relations are obtained for compounds under examination, except where physicochemical properties limit the maxima concentration that can be tested<sup>8</sup>. Given the hERG channel's now well-recognised pharmacological promiscuity<sup>5,12,31</sup>, this poses problems in the assessment of NCEs: up to ~70% of compounds may interact with hERG at some concentration<sup>32</sup>. Automatically eliminating from further development all drugs that inhibit hERG would be costly and may be unjustified in some cases. Consequently, issues arise as to how 'safe' and 'dangerous' hERG blockers may reliably be distinguished from one another. Considerable effort has been expended to define a 'safety index' or 'safety margin', that takes into account

both potency of an agent against hERG and its effective therapeutic concentration against its intended target (reviewed in <sup>5,7,12</sup>). However, hERG safety margins alone may not suffice for decisions whether or not to proceed with development or approval of an NCE. First, a sole focus on hERG may miss rare instances in which QT prolongation/TdP could arise from compound effects on other ionic currents than hERG. Second, drug discovery projects may not have available the measured  $C_{max}$  values necessary for accurate safety margin assessment and may have to rely on  $C_{max}$  estimates <sup>12</sup>. Third, relating hERG safety margin values to  $QT_c$  prolongation and risk can be complex. A comparison of hERG block potency with thorough QT (TQT) data for 39 drugs found that a hERG safety margin of 45 optimally linked safety margin to  $QT_c$  interval prolongation, but that  $QT_c$  prolonging drugs were only 5-7 fold more likely to exhibit safety margins between 1-30 than drugs that do not prolong the  $QT_c$  interval <sup>33</sup>.

Additional preclinical *in vitro* repolarization screens are not currently prescribed, with multiple different approaches used, ranging from single Purkinje fibres, to ventricular wedge and perfused heart preparations – each of which has its own strengths and potential disadvantages <sup>3,5</sup>. Although such additional tests are not mandatory according to ICH S7B <sup>8</sup>, when used they are likely to result in variability in type and comprehensiveness of these complementary data.

### **Functional effects on multiple human cardiac ionic channels**

As a first measure, CiPA proposes to evaluate the proarrhythmic risk of compounds by studying the effects on multiple human ventricular ionic channels. Because the study of native currents from primary human cardiac tissue is not a viable approach

(due to the same limitations as discussed above in respect of  $I_{Kr}$ ), the core strategy will consist of measuring key ionic currents from recombinant human channels expressed in various heterologous systems. The initial voltage clamp work will be performed manually both at physiologic (~35-37°C) and room temperature in an effort to establish biophysical and pharmacological characterization of the effects of selected compounds on each current using the “gold standard” approach. These results will then be used as reference as the work is transitioned to automated high throughput methods in order to adapt CiPA to the current screening environment of most pharmaceutical companies.

But one of the first questions to ask is which ionic currents play a significant role in conferring proarrhythmic properties to compounds, which are the most *important*? The duration of the QT interval on the electrocardiogram is defined as the period between the beginning of the QRS complex (depolarization of the ventricles) and the end of the T wave (repolarization of the ventricles)<sup>34</sup>. It is a reflection of ventricular action potential duration (APD) and several distinct ionic channels contribute to defining the morphology and duration of action potentials (AP) including: the rapid inward sodium current ( $I_{Na}$ ) responsible for the rising phase of the AP, the late sodium ( $I_{Na-Late}$ ) and L-Type calcium channel ( $I_{Ca-L}$ ) in control for the plateau phase, and multiple overlapping outward repolarizing potassium currents comprising of the transient outward current ( $I_{to}$ ), the slow ( $I_{Ks}$ ) and rapid ( $I_{Kr}$ ) components of the delayed rectifier potassium channels, and the inward rectifier channel ( $I_{K1}$ ), the latter responsible for setting the resting potential in cardiac myocytes<sup>35</sup>. Based on their fundamental role in defining human APD, and together with the information obtained from a Safety Pharmacology Society survey, the ICWG selected these 7 different human ionic channels as initial working material for the CiPA assays. More

specifically, as it will be recombinant rather than native channels that are viable for safety testing assays, experiments will focus on the following human recombinant channels expressed in mammalian cell lines: Nav1.5 (rapid  $I_{Na}$ ), toxin-modified Nav1.5 ( $I_{Na,Late}$ ), Cav1.2 ( $I_{Ca,L}$ ), Kv4.3 ( $I_{to}$ ) hERG ( $I_{Kr}$ ); KCNQ1+KCNE1 ( $I_{Ks}$ ), Kir2.1 ( $I_{K1}$ ). Currents will be studied individually using protocols that will not only assess potency of block ( $IC_{50}$  determination) but also evaluate kinetics of block that might prove critical in understanding the potential proarrhythmic potential of compounds (*cf*<sup>36</sup>). Ultimately, only the most informative protocols will be retained as final and it is likely that the list of 7 targeted channels will narrow once their role as proarrhythmic markers are confirmed, or not, based on experimental outcomes. A significant benefit to be expected from this effort will be the establishment of best practices for ion channel studies used to characterize drug effects, such that standardized protocols and methods are developed and adopted in an effort to minimize intra- and inter-laboratory variability, and allow a more level playing field across the pharmaceutical industry in sanctioning more accurate predictions of proarrhythmic risk.

### ***In silico* cellular simulations**

The integration of *in silico* modelling into the CiPA effort makes perfect sense since this approach offers the potential to provide integrative, cost effective and high throughput solutions to predict drug-induced changes in action potential duration by combining the ICWG's results on individual ion channels. The O'Hara-Rudy (OHR) model of the human ventricle was selected following a consensus meeting attended by many of the leading *in silico* modelers in the field at the think-thank meeting of July 2013. The OHR model offers several advantages, including the fact that it is

free of intellectual property (it can be accessed on the Rudy Laboratory research section of the website: <http://rudylab.wustl.edu>), and that all constants (extracellular ionic concentrations, cell geometry, ionic conductance), and all the initial conditions for state variable and scaling factors have been determined and, finally, that it is fully validated with ionic data described for a human ventricular action potential model<sup>37</sup>.

The ISWG is focused on evaluating and extending the model so that it may provide assessment of ion pharmacology and clinical risk of TdP. The philosophy behind the use of the model is to use the data generated by the ICWG iteratively to parametrize and validate the performance of the model at each step in the development process, whilst keeping the process as simple as possible by using patch clamp protocols that are not overly complicated or challenging to apply experimentally. Pilot patch clamp protocols to parametrize the endogenous  $I_{Kr}$  current (hERG) have been developed and, at the time of writing, manual voltage clamp data have been obtained for 3 drugs (dofetilide, cisapride and verapamil) generated both at room and physiological temperature.

### **Human myocyte studies**

The primary goal of using human iPSC derived cardiomyocytes is to identify repolarization effects not anticipated from the ion channel work or *in silico* reconstruction efforts. Such effects may provide some insight on the modulation of cardiac action potentials by mechanisms that do not directly affect ion channels, or may not manifest themselves in the current version of the *in silico* model. While iPSC-derived cardiomyocytes offer clear advantages over isolated primary human cardiac cells or cardiac tissue preparations, both in terms of availability and ease of

use, numerous aspects of the biology and pharmacology of these cells remain to be determined with certainty (see below).

In an effort to reach their goal, the SCWG has formed a subgroup under the auspices of HESI. Two technological study platforms have been selected: the multi-electrode array (MEA) and the voltage-sensing optical (VSO) action potential platform. A pilot study was initiated in 2014 and comprised 16 work sites, 4 stem cell providers, and 3 VSO/MEA providers. The pilot study evaluated the effects of four blinded compounds selected to test the assays sensitivity to specific ion channel blockers (mexilitine for  $I_{Na}$ , nifedipine for  $I_{CaL}$ , E-4031 for  $I_{Kr}/hERG$ , and JNJ303 for  $I_{Ks}$ ). A preliminary comparison of three drugs across two sites for one stem cell source suggests a good concordance when results were presented as % change vs. baseline values. Additional experiments and validation work will be needed in order to reach definitive conclusions on the functional usefulness of these cells for determining the proarrhythmic risk of established and future drugs.

## **Impact**

If successful, CiPA will have a significant impact on the manner in which cardiac safety assessment is performed in the pharmaceutical industry. Although this is a consortium effort, some general rules around data sharing and use have been developed and compiled under the CiPA Guiding Principles<sup>17</sup>. By moving the evaluation of proarrhythmic risks earlier in the development process, it will allow for the removal of compounds with undesirable cardiac repolarizing effects, alleviating the risk and the costs of unmasking a QT signal of concern in clinical trials.

From a preclinical perspective, the CiPA paradigm will allow standardization of all *in vitro* ion channel assays to characterize the drug effects on cardiac repolarization. It is also envisioned that it will make available a single, common, and fully validated *in silico* model to assess the risk of arrhythmia based on the ion channel data. Finally, it will also establish best practices for the use of stem cell derived cardiomyocytes, in an effort to confirm the translation between ion channels, *in silico* modelling and human cardiac tissue. To a certain extent, it will accomplish what the S7B guidance document has failed to achieve since its inception: standardization of the way preclinical assays are conducted across the pharmaceutical industry in order to remove bias and provide a reliable and reproducible dataset allowing complete assessment of the potential effects of drugs on human cardiac electrophysiology.

Moreover, an early and more complete assessment of proarrhythmic risks, rather than the determination of QT prolongation alone, will reduce the number of “false positive” (high sensitivity) results based on activity at the hERG channel and allow a more accurate determination of true negative (high specificity) compounds. As a consequence, it is expected that CiPA will lead to a reduction in the need to conduct thorough QT studies, as well as improve the accuracy of current or future labelling, and lead to an increase in the number of products available on the market to treat unmet medical needs. In addition, unlike the approach adopted for the S7B guidance document, CiPA is a consortium effort allowing sharing of knowledge and expertise to support the project. By doing so, it lends itself to the sharing of resources with the common goal of defining best practice in developing safer drugs and in re-evaluating the true cardiac risk of drugs that have been discarded because of their activity on the hERG channel, or that are currently marketed but carry a warning for QT prolongation in their label. As a consequence, the CiPA effort may



lead to significant cost savings in the development process, savings that will benefit all organizations. Given an individual cost of approximately US \$2M per TQT study, CiPA has the potential to make a significant positive impact on the cost of developing new medicines.

### **Limitations and challenges**

The CiPA initiative is the next logical step following the review and analysis of a decade of data obtained using the ICH-S7B and ICH E14 guidance documents<sup>8,9</sup>. It has been touted as “revolutionary” and to some extent it is, at the least, a visionary initiative. That said, while it is an attractive proposal, it will require a significant amount of work and likely a few years of testing before it comes to fruition to the point where regulatory guidelines can definitively be updated. The technology is currently available to perform the ion channel work in a high throughput environment, albeit most likely at ambient rather than physiological temperature. However, while recording of ionic currents from recombinant channels expressed in various cell lines offers significant advantages over recording endogenous currents in human cardiac myocytes, the underlying properties of these channels may not fully recapitulate that of endogenous channels. For example, when expressed in cell lines, the cardiac calcium channel Cav1.2 displays very slow rates of recovery from inactivation at room temperature and, therefore, can only be recorded at very slow pacing rates. Given that one important aspect of the CiPA paradigm is to understand not only potency of compounds, but also kinetics of block, such properties will potentially limit the ability to perform experiments at a more physiologic pacing rate. Therefore, full validation of the ion channel assays for the targets chosen will require time,

dedication and funding from sources that may need to go beyond SPS, but have not yet been clearly identified. Additionally, whilst the particular ion channels chosen are those collectively most likely to influence APD and arrhythmia susceptibility, other proteins including the Na-Ca exchanger, Na-K pump in the sarcolemmal membrane and ryanodine receptors in the sarcoplasmic reticulum, can contribute directly or (in the case of the RyR) indirectly to electrogenesis; in principle NCEs might, albeit rarely, influence these processes.

The predictive ability of the ventricular cell model(s) used will depend substantially on the current formulations used and vigilance will be required to limit model-dependency of results. Simulations that allow TdP to manifest (rather than simply cellular precursors such as EADs) will require the use of tissue as well as cell models. Stem cell derived human cardiomyocytes offer numerous advantages over isolated primary cardiac cells (their use circumvents the use of animals; they can be cultured for extended periods of time allowing for chronic exposure to test compounds and, potentially most importantly, derive from humans). However; the currently available cells are composed of a mixed phenotype of cardiac cells that is closer to an immature phenotype and they may not fully recapitulate the (electro)physiological properties of adult ventricular cardiac myocytes (e.g. <sup>38-43</sup>). Also, there have been some reports where the pharmacology of standard compounds has proven inconsistent with cardiac cells, raising the question of the predictability of the assay relative to human cardiac tissue <sup>44,44-46</sup>. Moreover, the study of stem-cell derived myocytes does not recapitulate ECG measurements from the intact heart *in situ*, though it may reveal the propensity of a compound to delay repolarization and favor EAD generation. Additionally, CiPA will not address the issue of compounds that prolong the QT interval via changes in autonomic tone or

blood pressure, or act via mechanisms other than direct cardiac ion channel modulation. However, the intention is to retain *in vivo* preclinical hemodynamic and electrophysiology (ECG) measurements, which should bring to light drug induced changes missed through *in vitro* ion channel and myocyte testing alone <sup>11</sup>.

Although initial timelines proposed to complete the *in vitro* series of tests for this effort were very ambitious (i.e. revision of S7B by June 2016), in reality the CiPA effort is likely to go through multiple iterations before it reaches a point of applicability. The issues addressed are extremely complex and will require the international consensus of protocols and data from all three core assays, as well as the consensus of regulatory bodies across the globe, in order to be accepted internationally. It is an evolving initiative with evolving workflows that will require scientific, intellectual and practical contributions from multiple parties and, in consequence, will require time to be successful. Nonetheless, it is evident that the time has come to consider new ways of testing for drugs that confer proarrhythmic risks. The hope is that this new approach will prevent early inappropriate compound attrition due to hERG liability, provide a complete assessment of proarrhythmic risk, reduce animal and clinical work, rescue drugs labelled with cardiac warnings based on small degrees of QT prolongation, and help bring complete and standardized submission packages to regulatory agencies leading to desperately needed new drugs reaching patients more quickly.

## References

1. Yap, Y. G.; Camm, A. J. Drug induced QT prolongation and torsades de pointes. *HEART* **2003**, (89), 1363-1372.
2. Trinkley, K. E.; Page, R. L.; Lien, H.; Yamanouye, K.; Tisdale, J. E. QT interval prolongation and the risk of torsades de pointes: essentials for clinicians. *Curr. Med. Res. Opin.* **2013**, 29 (12), 1719-1726.
3. Gintant, G. A. Preclinical Torsades-de-Pointes screens: advantages and limitations of surrogate and direct approaches in evaluating proarrhythmic risk. *Pharmacol. Ther.* **2008**, 119 (2), 199-209.
4. Gintant, G. A.; Su, Z.; Martin, R. L.; Cox, B. F. Utility of hERG assays as surrogate markers of delayed cardiac repolarization and QT safety. *Toxicol. Pathol.* **2006**, 34 (1), 81-90.
5. Hancox, J. C.; McPate, M. J.; El Harchi, A.; Zhang, Y. H. The hERG potassium channel and hERG screening for drug-induced torsades de pointes. *Pharmacology and Therapeutics* **2008**, 119, 118-132.
6. Zhang, S.; Zhou, Z.; Gong, Q.; Makielski, J. C.; January, C. T. Mechanism of block and identification of the verapamil binding domain to HERG potassium channels. *Circ. Res.* **1999**, 84 (9), 989-998.
7. Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; MacKenzie, I.; Palethorpe, S.; Siegl, P. K.; Strang, I.; Sullivan, A. T.; Wallis, R.; Camm, A. J.; Hammond, T. G. 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. *Cardiovas. Res.* **2003**, 58, 32-45.
8. Anon . ICH S7B Note for guidance on the nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. 2005. London, 25 May. Reference CHMP/ICH/423/02.  
Ref Type: Generic
9. Anon . ICH E14 Note for guidance on the clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for nonantiarrhythmic drugs. 2005. London, 25 May. Reference CHMP/ICH/2/04.  
Ref Type: Generic
10. Darpo, B.; Garnett, C.; Benson, C. T.; Keirns, J.; Leishman, D.; Malik, M.; Mehrotra, N.; Prasad, K.; Riley, S.; Rodriguez, I.; Sager, P.; Sarapa, N.; Wallis, R. Cardiac Safety Research Consortium: can the thorough

QT/QTc study be replaced by early QT assessment in routine clinical pharmacology studies? Scientific update and a research proposal for a path forward. *Am. Heart J.* **2014**, *168* (3), 262-272.

11. Sager, P. T.; Gintant, G.; Turner, J. R.; Pettit, S.; Stockbridge, N. Rechanneling the cardiac proarrhythmia safety paradigm: a meeting report from the Cardiac Safety Research Consortium. *Am. Heart J.* **2014**, *167* (3), 292-300.
12. Pollard, C. E.; Abi-Gerges, N.; Bridgland-Taylor, M. H.; Easter, A.; Hammond, T. G.; Valentin, J. P. An introduction to QT interval prolongation and non-clinical approaches to assessing and reducing risk. *Br. J. Pharmacol.* **2010**, *159* (1), 12-21.
13. Kramer, J.; Obejero-Paz, C. A.; Myatt, G.; Kuryshev, Y. A.; Bruening-Wright, A.; Verducci, J. S.; Brown, A. M. MICE Models: Superior to the HERG Model in Predicting Torsade de Pointes. *Sci. Rep.* **2013**, *3*, 2100.
14. Mirams, G. R.; Cui, Y.; Sher, A.; Fink, M.; Cooper, J.; Heath, B. M.; McMahon, N. C.; Gavaghan, D. J.; Noble, D. Simulation of multiple ion channel block provides improved early prediction of compounds' clinical torsadogenic risk. *Cardiovasc. Res.* **2011**, *91* (1), 53-61.
15. Paakkari, I. Cardiotoxicity of new antihistamines and cisapride. *Toxicol. Lett.* **2002**, *127* (1-3), 279-284.
16. Park, E.; Willard, J.; Bi, D.; Fiszman, M.; Kozeli, D.; Koerner, J. The impact of drug-related QT prolongation on FDA regulatory decisions. *Int. J. Cardiol.* **2013**, *168* (5), 4975-4976.
17. Anon .  
<https://www.ilsixtra.org/hesi/science/cardiac/cipa/Project%20Documents/CIPA%20data%20sharing%20final.pdf>. 2015.  
Ref Type: Generic
18. Friedrichs, G. S.; Patmore, L.; Bass, A. Non-clinical evaluation of ventricular repolarization (ICH S7B): results of an interim survey of international pharmaceutical companies. *J. Pharmacol. Toxicol. Methods* **2005**, *52* (1), 6-11.
19. Bridgland-Taylor, M. H.; Hargreaves, A. C.; Easter, A.; Orme, A.; Henthorn, D. C.; Ding, M.; Davis, A. M.; Small, B. G.; Heapy, C. G.; Abi-Gerges, N.; Persson, F.; Jacobson, I.; Sullivan, M.; Albertson, N.; Hammond, T. G.; Sullivan, E.; Valentin, J. P.; Pollard, C. E. Optimisation and validation of a medium-throughput electrophysiology-based hERG assay using IonWorks HT. *J. Pharmacol. Toxicol. Methods* **2006**, *54* (2), 189-199.
20. Danker, T.; Moller, C. Early identification of hERG liability in drug discovery programs by automated patch clamp. *Front Pharmacol.* **2014**, *5*, 203.

21. McDonald, T. V.; Yu, Z.; Ming, Z.; Palma, E.; Meyers, M. B.; Wang, K. W.; Goldstein, S. A. N.; Fishman, G. I. A minK-HERG complex regulates the cardiac potassium current I<sub>Kr</sub>. *Nature* **1997**, *388*, 289-292.
22. Du, C.; El Harchi, A.; Zhang, H.; Hancox, J. C. Modification by KCNE1 variants of the hERG potassium channel response to premature stimulation and to pharmacological inhibition. *Physiological Reports* **2013**, *1* (6), e00175.
23. Abbott, G. W.; Sesti, F.; Splawski, I.; Buck, M.; Lehmann, M. H.; Timothy, K. W.; Keating, M. T.; Goldstein, S. A. N. MiRP1 forms I<sub>Kr</sub> potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* **1999**, *97*, 175-187.
24. Sesti, F.; Abbott, G. W.; Wei, J.; Murray, K. T.; Saksena, S.; Schwartz, P. J.; Priori, S. G.; Roden, D. M.; George, A. L.; Goldstein, S. A. A common polymorphism associated with antibiotic-induced cardiac arrhythmia. *Proc Natl Acad Sci U S A* **2000**, *97*, 10613-10618.
25. Weerapura, M.; Nattel, S.; Chartier, D.; Caballero, R.; Hebert, T. F. A comparison of currents carried by HERG with and without the coexpression of MiRP1, and the native rapid delayed rectifier current. Is MiRP1 the missing link? *J. Physiol.* **2002**, *540*, 15-27.
26. Yang, T.; Snyders, D. J.; Roden, D. M. Drug block of I<sub>Kr</sub>: model systems and relevance to human arrhythmias. *J. Cardiovasc. Pharmacol.* **2001**, *38*, 737-744.
27. Kirsch, G. E.; Trepakova, E. S.; Brimecombe, J. C.; Sidach, S. S.; Erickson, H. D.; Kochan, M. C.; Shyjka, L. M.; Lacerda, A. E.; Brown, A. M. Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. *J. Pharmacol. Toxicol. Methods* **2004**, *50* (2), 93-101.
28. Yao, J. A.; Du, X.; Lu, D.; Baker, R. L.; Daharsh, E.; Atterson, P. Estimation of potency of HERG channel blockers: impact of voltage protocol and temperature. *J. Pharmacol. Toxicol. Methods* **2005**, *52* (1), 146-153.
29. Milnes, J. T.; Witchel, H. J.; Leaney, J. L.; Leishman, D. J.; Hancox, J. C. Investigating dynamic protocol-dependence of hERG potassium channel inhibition at 37 degrees C: Cisapride versus dofetilide. *J. Pharmacol. Toxicol. Methods* **2010**, *61* (2), 178-191.
30. Hanson, L. A.; Bass, A. S.; Gintant, G.; Mittelstadt, S.; Rampe, D.; Thomas, K. ILSI-HESI cardiovascular safety subcommittee initiative: evaluation of three non-clinical models of QT prolongation. *J. Pharmacol. Toxicol. Methods* **2006**, *54* (2), 116-129.
31. Sanguinetti, M. C.; Chen, J.; Fernandez, D.; Kamiya, K.; Mitcheson, J.; Sanchez-Chapula, J. A. Physicochemical basis for binding and voltage-

dependent block of hERG channels by structurally diverse drugs. *Novartis. Found. Symp.* **2005**, 266, 159-166.

32. Shah, R. R.; Hondeghem, L. M. Refining detection of drug-induced proarrhythmia: QT interval and TRLaD. *Heart Rhythm* **2005**, 2 (7), 758-772.
33. Gintant, G. An evaluation of hERG current assay performance: Translating preclinical safety studies to clinical QT prolongation. *Pharmacol. Ther.* **2011**, 129 (2), 109-119.
34. Antzelevitch, C. Cellular basis for the repolarization waves of the ECG. *Ann. N. Y. Acad. Sci.* **2006**, 1080, 268-281.
35. Abriel, H.; Schlapfer, J.; Keller, D. I.; Gavillet, B.; Buclin, T.; Biollaz, J.; Stoller, R.; Kappenberger, L. Molecular and clinical determinants of drug-induced long QT syndrome: an iatrogenic channelopathy. *Swiss. Med. Wkly.* **2004**, 134 (47-48), 685-694.
36. Di Veroli, G. Y.; Davies, M. R.; Zhang, H.; bi-Gerges, N.; Boyett, M. R. High-throughput screening of drug-binding dynamics to HERG improves early drug safety assessment. *Am. J. Physiol Heart Circ. Physiol* **2013**, 304 (1), H104-H117.
37. O'Hara, T.; Virag, L.; Varro, A.; Rudy, Y. Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. *PLoS. Comput. Biol.* **2011**, 7 (5), e1002061.
38. Yang, X.; Pabon, L.; Murry, C. E. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. *Circ. Res.* **2014**, 114 (3), 511-523.
39. Robertson, C.; Tran, D. D.; George, S. C. Concise review: maturation phases of human pluripotent stem cell-derived cardiomyocytes. *Stem Cells* **2013**, 31 (5), 829-837.
40. Bett, G. C.; Kaplan, A. D.; Lis, A.; Cimato, T. R.; Tzanakakis, E. S.; Zhou, Q.; Morales, M. J.; Rasmusson, R. L. Electronic "expression" of the inward rectifier in cardiocytes derived from human-induced pluripotent stem cells. *Heart Rhythm.* **2013**, 10 (12), 1903-1910.
41. Ma, J.; Guo, L.; Fiene, S. J.; Anson, B. D.; Thomson, J. A.; Kamp, T. J.; Kolaja, K. L.; Swanson, B. J.; January, C. T. High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents. *Am. J. Physiol Heart Circ. Physiol* **2011**, 301 (5), H2006-H2017.
42. Rao, C.; Prodromakis, T.; Kolker, L.; Chaudhry, U. A.; Trantidou, T.; Sridhar, A.; Weekes, C.; Camelliti, P.; Harding, S. E.; Darzi, A.; Yacoub, M. H.; Athanasiou, T.; Terracciano, C. M. The effect of microgrooved culture

substrates on calcium cycling of cardiac myocytes derived from human induced pluripotent stem cells. *Biomaterials* **2013**, *34* (10), 2399-2411.

43. Lee, Y. K.; Ng, K. M.; Lai, W. H.; Chan, Y. C.; Lau, Y. M.; Lian, Q.; Tse, H. F.; Siu, C. W. Calcium homeostasis in human induced pluripotent stem cell-derived cardiomyocytes. *Stem Cell Rev.* **2011**, *7* (4), 976-986.
44. Scott, C. W.; Zhang, X.; Abi-Gerges, N.; Lamore, S. D.; Abassi, Y. A.; Peters, M. F. An impedance-based cellular assay using human iPSC-derived cardiomyocytes to quantify modulators of cardiac contractility. *Toxicol. Sci.* **2014**, *142* (2), 331-338.
45. Qu, Y.; Gao, B.; Fang, M.; Vargas, H. M. Human embryonic stem cell derived cardiac myocytes detect hERG-mediated repolarization effects, but not Nav1.5 induced depolarization delay. *J. Pharmacol. Toxicol. Methods* **2013**, *68* (1), 74-81.
46. Jonsson, M. K.; Vos, M. A.; Mirams, G. R.; Duker, G.; Sartipy, P.; de Boer, T. P.; van Veen, T. A. Application of human stem cell-derived cardiomyocytes in safety pharmacology requires caution beyond hERG. *J. Mol. Cell Cardiol.* **2012**, *52* (5), 998-1008.



This is the author's version of a work that was accepted for publication. Changes introduced as a result of publishing processes such as copy-editing and formatting may not be reflected in this work. For a definitive version of this work please refer to the published source.

The official published article is available online at <http://dx.doi.org/10.1177/1087057115594589>

**Citation:**

Fermini, Bernard; Hancox, Jules C; Abi-Gerges, Najah; Bridgland-Taylor, Matthew; Chaudhary, Khuram W; Colatsky, Thomas; Correll, Krystle; Crumb, William; Damiano, Bruce; Erdemli, Gul; Gintant, Gary; Imredy, John; Koerner, John; Kramer, James; Levesque, Paul; Li, Zhihua; Lindqvist, Anders; Obejero-Paz, Carlos A; Rampe, David; Sawada, Kohei and Strauss, David G and Vandenberg, Jamie I (2016) A New Perspective in the Field of Cardiac Safety Testing through the Comprehensive In Vitro Proarrhythmia Assay Paradigm. *Journal of Biomolecular Screening*, 21 (1): 1-11. ISSN 1552-454X