Rechanneling the Cardiac Proarrhythmia Safety Paradigm: An Update
Synopsis of the December 11th, 2014 FDA/CSRC/HESI/SPS Think Tank Meeting

Introduction and Background

The year 2015 marks the 10th anniversary of the release of two ICH Harmonised Tripartite Guidelines, S7B and E14, whose principles have governed the (ventricular) proarrhythmic cardiac safety landscape since around 2003. While this landscape has undoubtedly been effective in preventing the approval of drugs with unanticipated proarrhythmic liability, it has increasingly acknowledged limitations. First, it is predicated on a surrogate marker, QT interval prolongation, that is an imperfect predictor of Torsades de Pointes (TdP), the polymorphic ventricular arrhythmia of primary interest. Second, QT prolongation is sensitive but not highly specific for predicting which compounds can cause TdP. Third, overemphasis on this marker and drug effects on the hERG-encoded current has adversely impacted the development of potentially valuable therapeutics by resulting in their premature discontinuation from development. Some of these drugs would likely have had an acceptable benefit-risk balance, been of significant therapeutic benefit, and hence contributed positively to public health.

Over the last several years, therefore, there has been considerable interest in modifying the current landscape. An important driver in this initiative was a scientific proposal presented at the July 23rd, 2013, Think Tank co-sponsored by the Cardiac Safety Research Consortium (CSRC), the Health and Environmental Sciences Institute (HESI), and the US Food and Drug Administration (FDA), held at the FDA's White Oak facilities, Silver Spring, MD. This proposal's intent was to move towards consensus on defining a new paradigm within the domain of cardiac proarrhythmic safety in which risk would be primarily assessed using nonclinical *in vitro* human models. At that Think Tank, interested stakeholders discussed the public health benefits of adopting such an integrated nonclinical *in vitro/in silico* paradigm, the Comprehensive In Vitro Proarrhythmia Assay (CiPA), for the assessment of a candidate drug's proarrhythmic liability. Those discussions were captured in a publication in the *American Heart Journal*.[1]

CiPA is a proposal to evaluate the proarrhythmic risk of compounds based on a mechanistic, electrophysiological understanding of this risk. It is based on the study of multiple human cardiac ionic currents expressed in heterologous systems, and the use of a standardized *in*

silico model of human ventricular action potential (AP) to predict the proarrhythmic risk of a drug by integrating the ion channel data in this model, as confirmed based on responses using human stem cell-derived cardiomyocytes.

Following the July 23rd, 2013, Think Tank, various workstreams were created to pursue further research within various components of the overall CiPA model. Members of these workstreams came together at a December 11th, 2014, Think Tank co-sponsored by FDA, HESI, FDA, and the Safety Pharmacology Society (SPS) to present progress reports. This synopsis summarizes those reports.

A Summary of CiPA Activities

To set the scene for information from the December 2014 Thank Tank provided in the rest of this document, CiPA is based on the significant advances over the last 15 years in the mechanistic understanding of ventricular arrhythmias via investigations on over-expressed genes influencing human cardiac ion channels and ionic currents, computer models of ventricular myocyte electrophysiology, and isolated human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs). Under CiPA, the bulk of proarrhythmia assessment is being directed towards measuring the effect of a new chemical entity on multiple ionic currents and the employment of *in silico* reconstruction to estimate the compound's relative TdP liability. Additional data will be derived from: a) iPSC-CM studies; b) nonclinical ECGs obtained from robust studies such as those typically used to support the ICH S7B guidance; and c) ECGs derived from First-in-Human (FIH) clinical trials to confirm in humans that there are no unexpected electrophysiologic effects based on the nonclinical findings.

Synopsis of the December 2014 Think Tank

The Think Tank included updates from each respective workstream: the Ion Channel Working Group; the Myocyte Working Group; the In Silico Reconstruction Working Group; and the Clinical Translational Working Group. Each of these updates is summarized in turn.

The Ion Channel Working Group (ICWG)

The purpose of CiPA is to address gaps in the current nonclinical component of the cardiac proarrhythmic safety paradigm. A decade of testing has confirmed that block of hERG alone is not always predictive of delayed repolarization or proarrhythmic risk.

Although manual voltage clamp remains the industry 'gold standard' for delivering high quality ion channel data, the need to screen an increasing number of compounds for hERG influence has led to the rapid development of automated patch clamp systems. Currently, more than a dozen different automated voltage clamp systems are commercially available. While these systems can generate large amounts of data in a very short time, the quality and reproducibility of these data will depend largely on the ability to establish giga-ohm seals with the cells tested. A recent report showed that repeated measurements of the same positive control compound displayed significant variability when recorded in low seal-resistance conditions. While the ICH S7B guidance allows flexibility in testing paradigms, this freedom has resulted in the development of ion channel strategies that utilize different platforms, solutions, and protocols across the industry. The main disadvantage of this flexibility is variability in data generated.

Given the central role of ion channel data in validating the CiPA paradigm, and in an effort to tap into the significant level of ion channel knowledge, experience, and expertise within the SPS, the SPS Board of Directors endorsed the creation of an "Ion Channel Working Group" (ICWG). This ICWG is tasked with bringing together the expertise and resources required to deliver best practice recommendations for generating ion channel data needed for *in silico* cardiac action potential (AP) simulations of proarrhythmic liabilities. It is composed of members from the pharmaceutical industry, the FDA, contract research organizations (CROs) as well as advisors from academia, and is supported by SPS in the form of staged funding of the experimental portion of this effort.

Since its launch in January 2014, the ICWG has focused on addressing several important questions related to best practices, including the following: Which ion channels should be selected to support *in silico* AP modelling? What properties should be studied (e.g., IC_{50} determinations, kinetics of block and unblock, rate/use/voltage dependence of block)? What requirements are needed to deliver robust, reliable, and reproducible ion channel data in a high throughput screening (HTS) environment in support of *in silico* AP reconstruction?

As a first step, the ICWG distributed a survey to active SPS members to collect frequency/type data on the commonly used ion channels in their laboratories to obtain qualitative information on their relevance to drug-induced cardiac safety concerns, with a specific focus on proarrhythmia. The majority of those who responded (response n=74; see **Figure 1**) were found to work in large pharmaceutical companies (59%), followed by CROs (22%) and various other work places. The survey was critical in identifying 7 ion channels (response n=65; see **Figure 2**) that are routinely studied because of perceived safety concerns (I_{Kr}, I_{Ks}, I_{to}, I_{K1}, I_{Ca} and $I_{Na\ [peak\ and\ late]}$). Consequently, protocols were developed for each of these ionic currents, with the intent of gathering key data to be used for *in silico* AP simulations.

Another important question that was addressed in the survey was the issue of temperature when evaluating drug effects on ionic currents. One critical requisite for the successful implementation of CiPA is the ability to run the proposed ion channel protocols on HTS systems at room temperature, since the majority of these systems do not provide for temperature controlled conditions. As shown in **Figure 3** (response n=54), most of the ionic current testing presently conducted is evaluated at room temperature, highlighting the importance of adapting the protocols that will be developed to room temperature conditions.

The ICWG designed and delivered two sets of protocols for the different ion channels selected. A first set, described as "Dynamic Block" and focused primarily on hERG, provides information on affinity, kinetics, and state-dependency of block. As such, hERG current is elicited by a depolarization pulse of increasing duration (0.05, 0.1, 0.2, 1, 3, 6, 10, 15, and 20 sec) to +20 mV, from a holding potential of -75 mV. A 20 msec hyperpolarizing pulse to -85 mV is introduced prior to the step to +20 mV to allow continuous evaluation of leak current and seal quality. Tail current amplitude is measured upon repolarization to -40 mV for 500 msec. Using this approach, data for hERG have been generated both at physiologic ($\approx 37°C$) and room temperature for three compounds (dofetilide, cisapride, and verapamil) that show very different kinetics of block while presenting similar potency. These data are currently being incorporated by the CiPA In Silico Working Group in a modified O'Hara-Rudy human ventricular AP model. Additional data looking at the other 6 ion channels will be generated at a later time, as current efforts focus on the hERG channel.

A second set of protocols, described as "Physiological," were delivered for all 7 ion channels. These address issues of potency, rate, and voltage and use-dependency, while

remaining practical and amenable to HTS. These protocols are currently being used for a translational cardiac safety study supported by the FDA.

The In Silico Working Group (ISWG)

This past year, the In Silico Working Group (ISWG) continued to focus on evaluating and extending the O'Hara–Rudy computational model of the human ventricular myocyte so that it is able to provide a robust assessment of both ion channel pharmacology and the clinical risk of TdP. Key questions that determined the development strategy employed included the following:

1) How do previously published results and metrics using other computational models of the ventricular myocyte compare with the results obtained using O'Hara-Rudy?
2) Are drug IC_{50}s adequate for characterizing drug effects? If not: (a) how should drug-channel interactions be modeled? and (b) which patch clamp protocols are needed to parameterize the model?
3) What metrics can be used to rank proarrhythmia risk correctly for the initial training set of high, moderate, and low/no risk drugs?
4) What simulation experiments are needed to define the metric(s) and validate the model?

Model development was guided by the following design principles:

1) Preserve an immediate and direct relation to experimentally-derived, verifiable data sets at all times, using available data to assess the performance of the model at each step in the development process;
2) Keep the model as simple as possible, using patch clamp experiments that are not overly challenging, to provide model parameters.

Key achievements include the following:

1) "Virtual" concentration-response curves were generated by reducing the maximal conductance of the candidate repolarization currents of interest to determine the impact of each channel on repolarization. I_{Kr} was the only current likely to cause early EADs when blocked (requiring ~87% block), and I_{CaL} and $I_{Na-late}$ were the currents most likely to modify (attenuate) these effects.

2) In benchmarking studies using literature IC_{50} values, the O'Hara-Rudy model appeared to perform better than published models in assessing TdP risk determination for the CiPA training set of drugs.

3) It was determined that model performance could be improved if state-dependence of block was included. The I_{Kr} model was then extended to include dynamic drug-block, and validated using independent experimental datasets. The modified model was able to reproduce the generation of EADs consistent with experimental findings.

4) It was also determined that the temperature used to measure channel pharmacology would be a factor (most data obtained from a search of the literature were collected at room temperature, whereas the model simulates the action potential at physiological temperatures), and the I_{Kr} model was subsequently modified to include consideration of temperature-dependent effects.

5) In collaboration with the Ion Channel Working Group, patch clamp protocols were developed to parameterize the I_{Kr} model, and data for 3 drugs generated at room and physiological temperatures.

Further testing and development will await delivery of complete set of ion channel pharmacology data using standardized protocols.

The Myocyte Working Group

The primary goal of the Myocyte Working Group is to provide verification of *in silico* reconstructions of ventricular electrophysiologic proarrhythmic responses using integrated physiologic systems provided by well-characterized human stem cell-derived cardiomyocytes (hSC-CMs). In particular, these hSC-CMs are expected to inform knowledge of repolarization effects not anticipated from ion channel or *in silico* reconstruction efforts. Such effects could reflect modulation of cardiac ion channel function by mechanisms other than direct channel block (e.g., metabolic modulation) and emergent behavior, e.g., early afterdepolarizations (EADs) that may not be manifest in some *in silico* models.

Towards this goal, the Myocyte Working Group formed a subgroup in November 2013. Under the auspices of the Health and Environmental Sciences Institute (HESI), the sub-group's founding members held a face-to-face meeting in March 2014, where they discussed

technological platforms to evaluate electrophysiologic effects. As a result of discussions at that meeting, two subgroups were envisioned: the multi-electrode array platforms (MEA) group, focusing on extracellular recordings (e.g., field potential recordings provided MEAs); and the voltage-sensing optical (VSO) group, focused on optical-based techniques (e.g., voltage-sensitive dyes and voltage-sensing integral proteins).

The sub-group completed the pilot study protocol design in the summer of 2014 with recruitment of volunteer sites, vendors, and stem cell providers. A pilot study was initiated in October 2014, with all data to be received by January 2015. The goals of the pilot study included demonstration of the utility of MEA and VSO approaches with hIPSC-CMs to detect cellular proarrhythmia, as well as evaluation of reproducibility, variability across cell types, study sites, and platforms. A standardized protocol was proposed across all platforms and approaches that encompassed 16 work sites, 4 stem cell providers, and 3 voltage/MEA suppliers. Some examples of preliminary results from the pilot study were presented at the December 2014 CiPA meeting, with data compilation set to commence in February 2015. Specific details of the study protocols and final results will be published along with results in the fall of 2015.

To assess cellular proarrhythmia in hSC-CMs, the pilot study evaluated the effects of four blinded compounds selected to test assay sensitivity to specific ion channel blockers (Mexiletine for INa, Nifedipine for I_{CaL}; E-4031 for I_{Kr}/hERG; and JNJ303 for I_{Ks}) and integrated responses to mixed channel blockers (represented by flecainide, moxifloxacin, ranolazine, and quinidine). Four point concentration-response curves were generated with MEA- and VSO-based approaches using nominal therapeutic and supratherapeutic clinical exposures. Endpoints for MEA recordings included time to peak field potential repolarization, beat frequency, amplitude of depolarization spike, and incidence of arrhythmias. For VSO recordings, measured parameters included spike amplitude, waveform triangulation (differences in APD_{30-90}), APD_{90}, beat frequency (although some sites also applied regular electrical pacing), and incidence of arrhythmia (manifest as early EADs). A preliminary comparison of the effects of three drugs across two sites for one stem cell source demonstrated good concordance when results were plotted as "percent change vs. baseline values." We wish to acknowledge all the volunteers (sites, vendors, platform manufacturers, and academicians) that contributed to the success of the pilot study.

In addition to the myocyte subgroup efforts, the Japan iPS Cardiac Safety Assessment (JiCSA) and FDA discussed ongoing complementary studies to explore proarrhythmia risk prediction using hSC-CMs. The Japanese National Institute of Health Sciences (NIHS) reported on the efforts of JiCSA, a consortium comprised of grantees from universities in Japan, CRO collaborators, pharmaceutical companies, and the Japanese Safety Pharmacology Society. An initial JiCSA study evaluated the effects of three compounds (E-4031, chromanol 293B, and cisapride) using hSC-CMs from one provider and MEA platform at multiple test sites. As for the myocyte MEA subgroup, the JiCSA study measured field potential duration and incidence of EADs with a focus not only on predictability but also on a measure of inter-laboratory variability. A follow-on study will evaluate the effects of an additional 54 compounds using one stem cell provider, three MEA platforms, and a voltage-sensitive dye approach is ongoing. Results of these studies will be included in a database and will contribute to a global standardization of a core protocol for use in the CiPA paradigm.

The FDA also reported on preliminary results obtained in an independent study testing drug effects on two different sources of hSC-CMs using a MEA and a VSO platform. Among 26 drugs and 3 drug combinations tested, 11 compounds were also tested in a FDA-sponsored clinical electrocardiographic study, thus providing for future comparison of myocyte data with clinical effects.

The results presented to date corroborate the concordance seen in the CiPA myocyte subgroup pilot study and the ability of hSC-CMs to predict the incidence of TdP. With supportive data from these efforts, the CiPA myocyte workstream will be designing a validation study to be conducted in 2015 to support the role of hSC-CMs in the CiPA paradigm.

The Clinical Translational Working Group (CTWG)

This working group (under the auspices of the Cardiac Safety Research Consortium) has evaluated compounds associated with QT prolongation and TdP proarrhythmia and selected a cohort of compounds for the development and testing of the *in silico* model and the myocyte experiments. The intent of the compound set was to provide drugs representing a varied spectrum of multiple electrophysiologic parameters, including the following: the degree of torsadogenic clinical risk; actions on ion channels (with primary attention given to multi-channel blockers); varying levels of block at clinical exposures; and inclusion of some compounds with

non-hERG TdP risk. Selection criteria included that compounds should not have major proarrhythmic active metabolites, be insoluble compounds, and have well defined cardiac electrophysiology.

The ranking of compounds with regard to clinically demonstrated torsadogenic risk/occurrence was based partly on published reports, the FDA AERS database, other published data sources and the expert opinion of clinical electrophysiologists. Compounds were grouped into three risk stratification categories: very low (i.e., none); intermediate; and high risk. Drugs classified into these categories are presented in **Table 1.**

This Working Group is currently assessing the key issues and datasets that will be required to achieve clinical and regulatory acceptance of CiPA. In 2015, a Clinical Translation Working Group subgroup will focus on defining an approach to ECG assessment during Phase 1 clinical trials under CiPA. While QTc assessment will certainly continue, potential novel approaches to elucidate if there are electrophysiologic effects in humans that were not anticipated on the basis of nonclinical testing (e.g., from a metabolite) will be defined. Such approaches may include sophisticated T-wave analysis and other ECG variables.

References

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2. Braam SR1, Tertoolen L, van de Stolpe A, Meyer T, Passier R, Mummery CL. Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes. *Stem Cell Res*. 2010;4:107-16.

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The Clinical Translational Working Group (CTWG): Philip Sager

Table and Table Legend

Table 1: Compounds Classified at Various Risk Stratifications

Very Low (no) Risk	Intermediate Risk	High Risk
Diltiazem	Astemizole	Azimilide
Loratadine	Chlorpromazine	Bepridil
Metoprolol	Cisapride	Dofetilide
Mexiletine	Clarithromycin	Ibutilide
Nifedipine	Clozapine	Quinidine
Nitrendipine	Domperidone	Vandetanib
Ranolazine	Droperidol	Methadone
Tamoxifen	Flecainide	D, l-sotalol
Verapamil	Ondansetron	
	Pimozide	
	Risperidone	
	Terfenadine	

Figures and Figure Legends

Figure 1: Affiliation of the various participants to the SPS survey
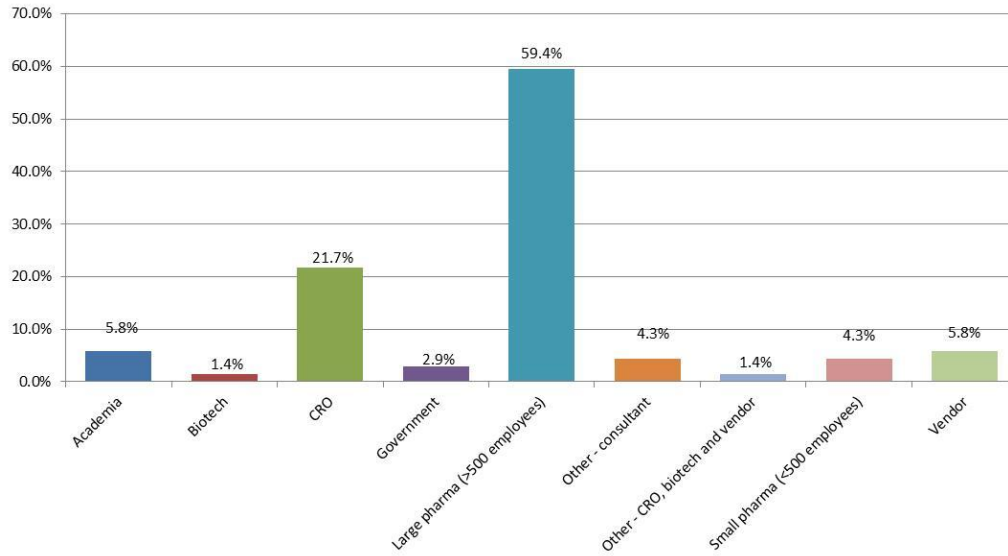

Figure 2: Inventory of ion channels studied for safety purpose
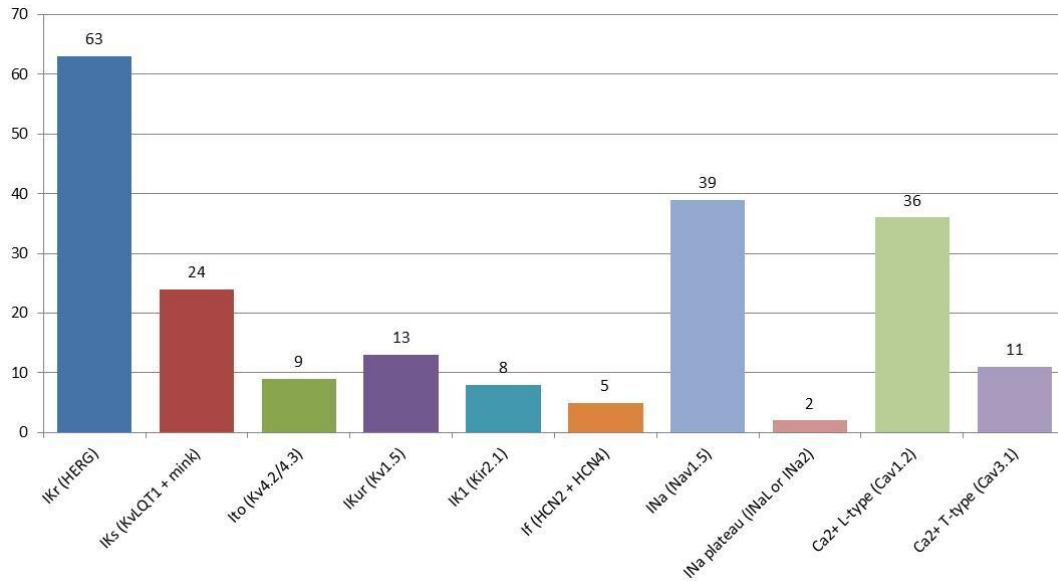
Figure 3: Distribution of temperature for the different ion channels tested