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# Comprehensive *in vitro* Proarrhythmia Assay (CiPA): Pending issues for successful validation and implementation

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ABSTRACT

*Introduction:* The Comprehensive *in vitro* Proarrhythmia Assay (CiPA) is a nonclinical Safety Pharmacology paradigm for discovering electrophysiological mechanisms that are likely to confer proarrhythmic liability to drug candidates intended for human use.
*Topics covered:* Key talks delivered at the 'CiPA on my mind' session, held during the 2015 Annual Meeting of the Safety Pharmacology Society (SPS), are summarized. Issues and potential solutions relating to crucial constituents [*e.g.*, biological materials (ion channels and pluripotent stem cell-derived cardiomyocytes), study platforms, drug solutions, and data analysis] of CiPA core assays are critically examined.
*Discussion:* In order to advance the CiPA paradigm from the current testing and validation stages to a research and regulatory drug development strategy, systematic guidance by CiPA stakeholders is necessary to expedite solutions to pending and newly arising issues. Once a study protocol is proved to yield robust and reproducible results within and across laboratories, it can be implemented as qualified regulatory procedure.


## 1. Introduction

CiPA is a novel Safety Pharmacology paradigm undergoing systematic evaluation for fitness to discover candidate drugs with the potential to trigger ventricular arrhythmic events in humans. If these evaluation efforts succeed, CiPA will become a Safety Pharmacology screening tool for drug research and development purposes (Cavero & Holzgrefe, 2015).

The CiPA paradigm has been designed to provide an accurate and comprehensive assessment of the cardiac ventricular electrophysiological properties of candidate drugs for identifying mechanisms that may mediate life-threatening ventricular proarrhythmic events. This preclinical approach can be considered as an extension of the currently applied ICH S7B guideline strategy (ICH, 2005a) which is designed to detect whether a candidate drug adversely affects the physiological function

*Abbreviations:* AP, action potential; $ADP_{90}$, action potential duration at 90% repolarization; $Ca_V$, voltage-dependent calcium channel; CDI, Cellular Dynamics International; CiPA, Comprehensive *in vitro* Proarrhythmia Assay; CRISP, Clustered Regularly Interspaced Short Palindromic Repeats; CROs, contract research organizations; CSA, consortium for safety assessment; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; FP, field potential; FPD, field potential duration; FPDcB, FPD corrected according to the Bazett formula; FPDcF, FPD corrected according to the Fridericia formula; GEVI, genetically-encoded voltage indicators; GLP, good laboratory practices; HESI, Health and Environmental Sciences Institute; HPF, high pass filter; *hi*PSC-CMs, human induced pluripotent stem cell cardiomyocytes; HTS, high throughput screening; ICH, International Conference on Harmonization; $I_{CaL}$, L-type (long-lasting voltage-gated) depolarizing calcium current; $I_{Kr}$, rapidly activating delayed rectifier potassium current; $I_{hERG}$, human ether-a-go-go-related gene potassium current; $I_{Ks}$, slowly activating delayed rectifier potassium current; $I_{K1}$, inward rectifier potassium current; $I_{to}$, transient outward potassium current; $I_{NaFast}$, fast depolarizing sodium current; $I_{NaLate}$, late depolarizing sodium current; ICWG, ion channel working group; ILSI, International Life Sciences Institute; *hi*PSC-CMs, human induced pluripotent stem cell derived cardiomyocytes; ISI, interspike interval; ISWG, *in silico* working group; LQTS, long QT syndrome; JiCSA, Japan iPS Cardiac Safety Assessment consortium; JSPS, Japan Safety Pharmacology Society; LQT, long QT interval syndrome; LPF, low pass filter; MEA, multi-electrode arrays; MWG, myocyte working group; $Na_V$, voltage-dependent sodium channel; QMS, Quality Management System; QTc, QT interval corrected according to the Fridericia algorithm; SD, standard deviation; SEM, standard error of the mean; SPS, Safety Pharmacology Society; TdP, Torsade de Pointes arrhythmia; TQT, thorough QT study; $V_{max}$, maximal velocity of depolarization; VSD, voltage-sensitive dyes; VSO, voltage-sensitive optical devices; WG, working group.

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of the cardiac channel encoded by the ether-à-go-go related gene (*h*ERG) which conducts the delayed rectifier $K^+$ current ($I_{Kr}$). This concern arises primarily from clinical findings that drug-induced *h*ERG inhibition can provoke a particular type of polymorphic ventricular arrhythmia called torsade de pointes (TdP). This tachyarrhythmia, at times, culminates in irreversible ventricular fibrillation. However, within the large number of drugs demonstrating potent $I_{Kr}$ channel blocking activity in the *in vitro* patch clamp assay, some are free of proarrhythmic effects in integrated nonclinical assays, as well as in man (Kannankeril, Roden, & Darbar, 2010; Vandenberg et al., 2012).

*Ci*PA is an initiative sponsored by a multi-partner international consortium which includes the FDA, HESI, CSRC, SPS, Japan National Institute of Health Sciences, Health Canada, European Medicines Agency, Pharmaceutical and Japan Medical Devices Agency, Japan iPS Cardiac Safety Assessment Group, academic electrophysiologists, *in silico* modelers, pharmaceutical industry associations, contract research organizations (CROs), stem cell manufacturers, and companies producing hardware and software for Safety Pharmacology research.

The C*i*PA core components are:

1) *In vitro* patch clamp assays utilizing stably expressed recombinant human ion channels. The aim of these studies is to evaluate the effects of candidate drugs on key depolarizing and repolarizing ion currents participating in the formation of human ventricular action potential (AP).
2) An *in silico* AP assay which is performed to verify whether the results obtained in the *in vitro* patch clamp investigations engender either phenotypic indicators signaling proarrhythmic liability on the human ventricular AP [*e.g.*, prolongation of AP duration (*e.g.*, $ADP_{90}$), EADs (Gintant, 2008)] evidenced on the AP profile generated by a mathematical model of the human ventricular myocyte AP (O'Hara, Virag, Varro, & Rudy, 2011)] or mechanism-related proarrhythmic metrics (Section 2.3) allowing the *in silico* model to classify each drug candidate as no/low, intermediate or high proarrhythmic risk agent.
3) An *in vitro* assay designed to investigate the electrophysiological effects of candidate drugs in ventricular cardiomyocytes derived from human induced pluripotent stem cells (*h*iPSC-CMs). The aim is to confirm or cast doubt on the *in silico* predictions and to broaden the cardiac safety assessment of the candidate drug to include additional proarrhythmic mechanisms not discoverable by the ion channel assay investigation or *in silico* analysis.

The CiPA initiative encompasses also the E14 guideline (ICH, 2005b) since it has the potential to de-emphasize the clinical thorough QT/QTc study (TQT) and replace it with an intensive Phase ECG investigation (Cavero, Holzgrefe, & Clements, 2016).

The first part of this article provides extended summaries of key presentations on the C*i*PA paradigm given at the 'C*i*PA on my mind session' which was part of the scientific program of the 15th Annual Meeting of the Safety Pharmacology Society (SPS) held in Prague in October 2015.

The second part of this report threads through issues concerning biological material, experimental platforms, measured parameters, and data analysis approaches that need to be resolved to qualify each C*i*PA core assay for Safety Pharmacology research and regulatory purposes. While available solutions for these issues are mentioned in this report, it will be the role of the C*i*PA Steering Committee and the various C*i*PA Working Groups and Teams to recommend the best possible solutions for bench stakeholders.

## 2. Key presentations from 'C*i*PA on your mind' session held at the Annual Meeting of the Safety Pharmacological Society

### *2.1. CiPA introduction. Ongoing activities (CiPA Steering Team) and updates. Dr. G. Gintant, Abbvie, North Chicago, IL, USA*

The 2015 C*i*PA *h*iPSC-CM pilot study program was designed to test whether a set of reference drugs yielded reproducible effects on field potentials (FP) measured with multi-electrode arrays (MEA) and action potentials (AP) obtained by using voltage-sensitive optical dyes (VSD) (Cavero & Holzgrefe, 2014). The knowledge acquired from these investigations will provide indications for optimizing experimental protocols with regard to the selection of the most informative parameters to measure, sampling times during the execution of a protocol, analytical approaches, and data reporting formats.

In September 2015, the FDA Cardiac Safety Committee (CSC), through an FDA Broad Agency Announcement (BAA) awarded a grant to HESI for 'Validating human stem cell cardiomyocyte technology for better predictive assessment of drug-induced cardiac toxicity' (HESI, 2014). This endowment will be used to finance studies to characterize the electrophysiological properties of *h*iPSC-CMs in studies using MEA and VSD platforms.

HESI funding will also support a pilot Phase I study (to be completed by the end of 2016) of 12 reference drugs selected by the C*i*PA Clinical Subteam which includes compounds with no/low (diltiazem, mexiletine, ranolazine and verapamil), intermediate (chlorpromazine, cisapride, terfenadine, and ondansetron), and high proarrhythmic risk (quinidine, bepridil, dofetilide, and sotalol). These drugs were selected from a larger set of 28 drugs [Table 1 in (Gintant, Sager, & Stockbridge, in press)] which the C*i*PA Clinical Translation Working Group proposed for achieving the validation of the components of the C*i*PA paradigm (Cavero & Holzgrefe, 2015). The drugs within this set have different physico-chemical properties and cover a wide spectrum of electrophysiological endpoints (in particular, the degree of torsadogenic risk, multichannel blockade, and variable degrees of *h*ERG channel blockade).

The results obtained from ongoing studies will be used to test whether the C*i*PA *in vitro* ion channel assays provide appropriate markers of risk (metrics) for training the *in silico* AP model to recognize and assign an appropriate level of proarrhythmic risk to each of the 12 initial reference drugs. Phase II studies will investigate the entire collection of 28 drugs.

The C*i*PA Steering Committee has accepted a proposal from the Japan iPS Cardiac Safety Assessment (JiCSA) group to sponsor and coordinate, within Japan, an experimental study of 60 compounds with varying degrees of proarrhythmic potential for testing and evaluating the C*i*PA *h*iPSC-CM assays.

The C*i*PA Steering Committee and the ICH S7B and E14 Working Groups have initiated discussions to define the possible place of the C*i*PA paradigm within the upcoming revisions of these regulatory documents. For instance, the E14 Q&As (R3), released by the ICH in December 2015 (ICH, 2015) opens the avenue to perform intensive ECG studies, in replacement of the thorough QT/QTc study, conducted within Phase I safety clinical investigations. The latter trials, if are designed in accordance with the rich cardiac electrophysiological knowledge generated by the C*i*PA assays, can be expected to accelerate and improve the human assessment of the proarrhythmic safety profile of candidate drugs (Cavero et al., 2016).

### *2.2. Ion channel project update. Dr. Bernard Fermini Global Safety Pharmacology, Pfizer, Groton, CT, USA and Dr. Najah Abi Gerges, AnaBios Corporation, San Diego, CA, USA*

The Ion Channel Working Group (ICWG), under the auspices of the Safety Pharmacology Society, has been charged with the following tasks:

1) Selection of cardiac ventricular ion channels requiring experimental scrutiny because drug-induced perturbations of their physiological function have been found to mediate proarrhythmic events in patients (Kannankeril et al., 2010; Vandenberg et al., 2012);
2) Establishing best practice protocols for manual and automated patch-clamp studies to determine whether candidate drugs disrupt human ventricular ion channel functions;

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