The Comprehensive *in Vitro* Proarrhythmia Assay

The focus of the Comprehensive *in Vitro* Proarrhythmia Assay (CiPA) is on delayed repolarisation and *Torsades de Pointes* (TdP) proarrhythmia, a rare but potentially lethal drug-induced arrhythmia that can lead to ventricular fibrillation and death. To address limitations with the present approaches (refer to other papers in series), the goal of CiPA is to provide a new *in vitro*-based paradigm that provides a more accurate and comprehensive mechanistic-based assessment of proarrhythmic liability of evolving drugs.[1,2] Fortunately, the electrophysiologic mechanisms responsible for TdP are now well understood.[3] This allows CiPA to move beyond the traditional, presently accepted paradigm for evaluating preclinical proarrhythmic liabilities (drug block of the influential repolarising current in human ventricles (hERG/iKr)), as discussed in the ICH S7B Guidance,[4] as well as the evaluation of clinical proarrhythmic liability based on QT prolongation assessed in thorough QT (TQT) studies as discussed in the ICH E14 Guidance.[5] Indeed, CiPA is intended to replace the cumbersome (and expensive) TQT study typically conducted late in drug development with earlier, mechanistic-based studies that will include robust preclinical and clinical assessments. This paradigm will also guide a more rational drug candidate selection process, coupling early clinical studies (using exposure response modelling derived from first in human studies: refer to other papers in series) to confirm non-clinical findings of minimal electrophysiologic effects.

The CiPA paradigm relies on four components to define an overall integrated proarrhythmic risk assessment, as illustrated in Figure 1.

First, drug effects on multiple cardiac ionic currents are defined using human ionic channels in simplified heterologous expression systems. The net electrophysiologic effect of a drug on ventricular electrical activity is then characterised based on the integrated electrophysiologic response provided by well-defined *in silico* reconstructions of human ventricular myocytes. Results from the *in silico* reconstructions are then compared to known clinic risks characterised for 28 drugs divided into three categories of proarrhythmic risk (high, intermediate, and low/no proarrhythmic risk), providing a clinically-based "gold standards" metric by which to calibrate the CiPA paradigm. The *in silico* findings are also verified empirically using human stem cell-derived ventricular myocytes, ensuring that any novel mechanisms are considered. Finally, drug effects on ECGs from early, well-controlled, first in human clinical studies are evaluated for unanticipated electrophysiologic effects. More recent clinical studies have demonstrated that specific morphological characteristics of human ECGs could serve as additional reliable biomarkers of proarrhythmic risk.[6]

This overall strategy for CiPA is founded on deep mechanistic insights of the underlying cellular ionic factors responsible for interfering with cardiac repolarisation that are directly amenable to study. Drugs that hinder repolarisation may affect the strength or synchrony of repolarisation or generate premature electrical activation. This cellular effect may be manifest as irregular activity in one area that propagates away and subsequently returns, thus creating an electrical circuit supporting TdP. It should be noted that all facets of CiPA are based on human-derived data, thus avoiding potential species differences that may be present when translating non-clinical data from animals to human studies.

CiPA evolved based on remarkable progress made

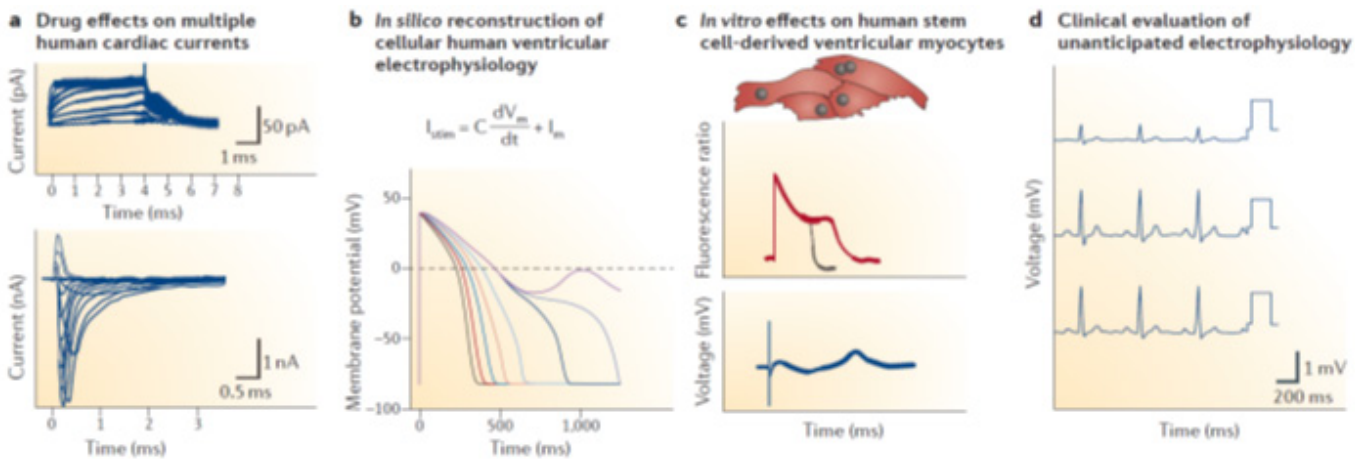

Figure 1. Elements of the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) (Reproduced with permission from Gintant *et al*., Nature Reviews Drug Discovery, 2016).

in recent years in understanding TdP proarrhythmia. Based on multiple years of research (which started with the description of polymorphic ventricular tachycardia by the French clinician Francois Dessertenne some 50 years ago), we now have a firm understanding of the mechanisms responsible for genetic- and drug-induced TdP. Technological advances now provide commercially available human ionic currents expressed in various "background" cell types for all prominent cardiac ionic currents. Advances in engineering now provide automated patch clamp platforms able to easily interrogate the functional concentration-dependent effects of drugs on ionic currents. Such studies have demonstrated the importance of considering block of multiple cardiac currents (beyond simply iKr/hERG current alone) in defining proarrhythmic risk, emphasising that the potency of block of only one of multiple cardiac currents defining ventricular repolarisation is insufficient.[7] *In vivo* and *in vitro* studies with human ventricular myocardium have provided sufficient data to support the development of robust *in silico* models of human ventricular activity to define drug effects on cardiac repolarisation. Such reconstructions[8] (based on the O'Hara-Rudy model[9]) are already freely available online.[10] Also, advances in stem cell technologies have provided human-derived ventricular myocytes for *in vitro* studies (thus overcoming the lack of human tissues for studies). While not fully recapitulating all aspects of adult ventricular myocytes, stem cell derived cardiomyocytes have repeatedly demonstrated their ability to detect well-recognised proarrhythmic drugs. Higher-throughput techniques are available to evaluate effects on repolarisation using stem cell derived ventricular myocytes (including multi-electrode arrays[11,12] and voltage-sensing dyes[13] [which provide for direct measures of drug-induced changes in extracellular and intracellular electrical activity] as well as measures of extracellular impedance[14] and calcium transients[15] [providing indirect measure of electrophysiologic effects]). Clinical experience, along with multiple TQT study results generated over the last decade, have provided the basis for classifying drugs for proarrhythmic risk. Finally, the standardisation of the clinical evaluation of ECGs (involving clinical protocols as well as waveform analysis and interpretation) based on TQT study experience has proved invaluable in defining the clinical standards for the evaluation of QT effects in early first in human studies. Indeed, such studies support the exposure-response modelling and evaluation of T-wave morphology changes as part of the mechanistic assessment of proarrhythmic risk.

Organised as a public-private collaboration, CiPA represents a consortia effort including the United States Food and Drug Administration (FDA), Health and Environmental Sciences Institute (HESI), Cardiac Safety Research Consortium (CSRC), Japan National Institutes of Health Sciences, Health Canada, European Medicines Agency (EMA), and the Japanese Pharmaceutical and Medical Devices Agency. At present, multiple working groups are further defining the roles of multiple CiPA components.[16] The Ion Channel Working Group is conducting studies defining potency of drug block of seven cardiac ionic currents (four repolarising potassium currents [iKr/hERG, IKs, Ito, iK1], as well as early (INa,Fast) and later (INa,Late, ICa) depolarising currents). In anticipation of a validation study, the *In Silico* Working Group is identifying candidate proarrhythmic metrics that classify TdP risk using a training subset of 12 drugs categorised for proarrhythmic risk by the Clinical Working Group. Recognising the importance of the dynamics of block of iKr/hERG current, the *In Silico* group is also evaluating the utility of incorporating a dynamic model of hERG block into the O'Hara-Rudy *in silico* reconstructions. Following up on an initial pilot study, the Myocyte Working Group is conducting a validation study defining the concentration-dependent electrophysiologic effects of the 28 CiPA compounds on two commercially available stem cell derived cardiomyocyte lines across two technology platforms (microelectrode array (MEA) and voltage-sensing optical (VSO)) at multiple sites. Finally, the Clinical Translation Working Group (under joint direction from the CSRC and HESI) completed the

categorisation of proarrhythmic risk for a set of 28 clinical drugs representing a diverse class of chemical structures and therapeutic indications.

Since the initial public discussion of CiPA in July 2013, significant scientific progress has been made within CiPA. The CiPA Steering Committee is providing the ICH S7B/E14 Discussion Group with ongoing updates on working group progress and seeking input from ICH on the regulatory implementation of CiPA as the effort evolves over the next ~ 1.5 years, and the CiPA effort is on track to complete the necessary work by the end of 2017. A meeting to discuss progress of the CiPA initiative is scheduled for December 2016.

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Given these considerations, other paradigms for investigating proarrhythmic risk are of considerable interest. These are discussed in the next two papers.

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