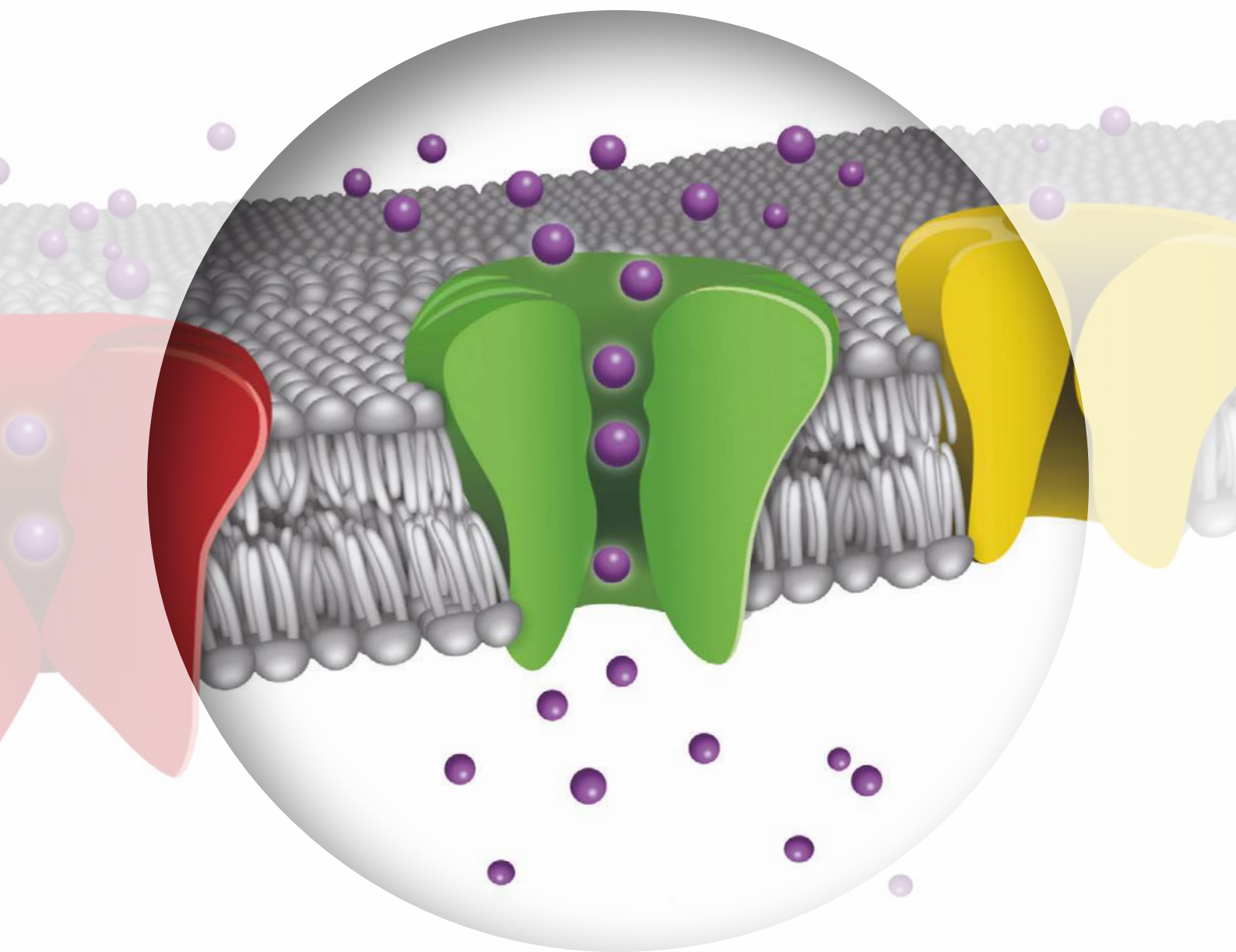


CARDIAC SAFETY SCREENING WHITE PAPER

# The changing landscape of cardiac safety testing



## The changing landscape of cardiac safety testing

### Rationale for more predictive cardiac safety assays in drug discovery

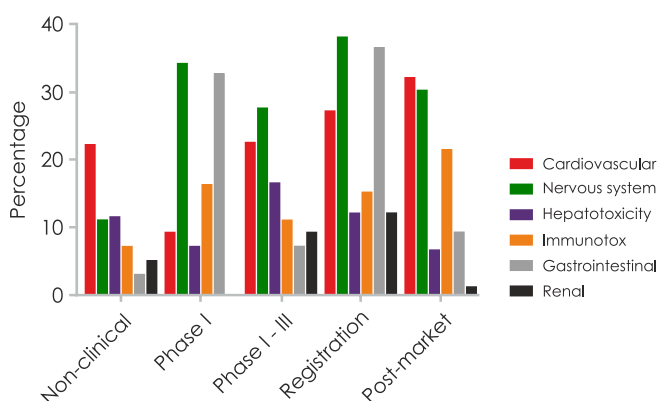
Drug discovery is an expensive and complicated process, with recent estimates of the cost of bringing each new drug successfully to market of \$1-\$2 billion. Implicit in these calculations are the cost of failed drug candidates, which, in industry surveys over the last two decades (Arrowsmith & Miller 2013; Hay *et al.*, 2014; Cook *et al.*, 2014), is attributed firstly to lack of efficacy (~50% of failed compounds) followed by safety issues (~30% of failed compounds). Significantly, cardiac toxicity remains the leading cause of new drug safety side effects (Valentin & Redfern, 2017). Drug attrition due to cardiotoxicity occurs in all phases of drug development (Fig. 1), and the later it occurs the greater the out-of-pocket cost.

What is apparent from these industry surveys is that existing pre-clinical cardiac safety assays that rely on *in vitro*, *ex vivo* and *in vivo* animal models are not sufficiently reliable in predicting the human cardiac risk of new compounds being tested in clinical trials

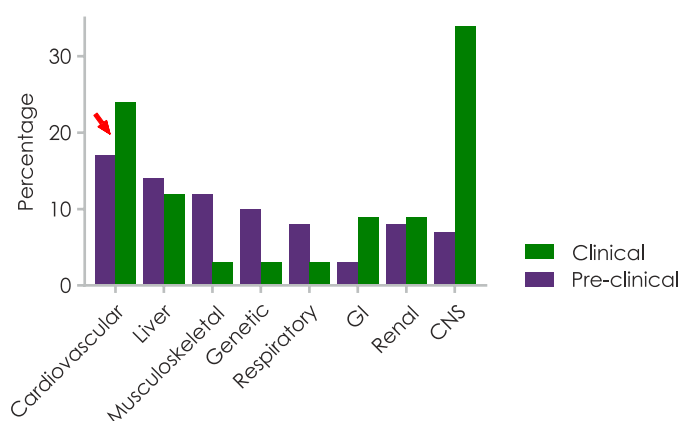
or approved for marketing (red arrow, Fig. 1). Given the ongoing poor predictability of existing cardiac safety testing regimes, industry and regulatory groups across the globe have initiated efforts to improve the translational potential of cardiac safety assays. One key aspect of this is to reduce reliance on animal tissues and models derived from different species. This is because there are key species differences in cardiac physiology, as well as a growing commitment in industry to meet recommended guidelines for reducing animal testing. Three general approaches have been proposed to improve the predictability of future cardiac safety assays, all of which have been successfully implemented at Metrion Biosciences:

- i Include additional human cardiac ion channels for *in vitro* screening panels to capture the full cardiac risk profile of test compounds;
- ii Use high quality *in vitro* data in sophisticated *in silico* models of the human cardiac action potential (AP) to predict arrhythmias, and;
- iii Test compounds empirically, and confirm the arrhythmia predictions of *in silico* models, using translational models employing human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).

**A: Safety side-effects by organ system**



**B: Drug safety failures by organ system**



**Figure 1: Cardiac toxicity is a major cause of modern drug attrition.**

Surveys of regulatory filings, pharmaceutical company publications and public domain drug testing datasets reveal occurrence of drug safety side-effects by development stage and organ system, with cardiac safety being the leading cause of project closures and drug withdrawals. Data replotted and adapted from:

**A:** Safety Pharmacology Society industry survey, 2000-2016 (Valentin & Redfern 2017).

**B:** AstraZeneca data, 2005-2010 (Cook *et al.* 2014).

## Current status – hERG and Thorough QT clinical evaluation

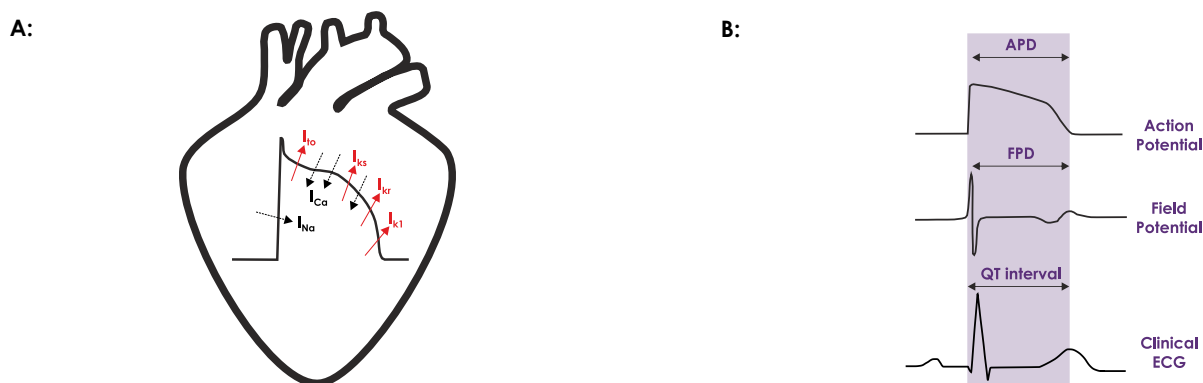
Current cardiac safety testing guidelines were established in 2005 and are based on evaluating *in vitro* and *in vivo* assay surrogates of human cardiac dysrhythmia. International Council on Harmonization (ICH) regulatory guideline S7B focuses on the potential for drugs to inhibit the human ether-à-go-go related gene channel (hERG; the molecular correlate for the rapid delayed rectifier potassium channel,  $I_{kr}$ ), whilst ICH E14 assesses the propensity of a drug to affect the QT duration in human patients (a prolongation of > 10 ms in the rate corrected QT (QTc) interval raises safety concerns).

The simple rationale for this two pronged approach is that many drugs which produce unwanted prolongation of APD in ventricular cardiomyocytes and QT interval in the intact heart also inhibit the hERG potassium channel (e.g. Redfern *et al.*, 2003), referred to as drug-induced long QT syndrome. The linkage between hERG modulation and QT prolongation became apparent during the 1990's and early 2000's when a number of marketed drugs elicited a rare polymorphic ventricular tachycardia called Torsades de Point (TdP). While TdP is not necessarily deadly, it can degenerate into potentially fatal ventricular fibrillation. As a consequence, a number of approved drugs have been removed from the market, or had their use severely restricted, because of a propensity to prolong the QT interval and/or induce cardiac arrhythmias. Examples include antihistamines, such as terfenadine and astemizole, GI drugs, such as terolidine and cisapride, and antibiotics such as

sparfloxacin and grepafloxacin. Unfortunately, hERG is a pharmacologically promiscuous ion channel that interacts with a wide range of structurally diverse compounds (Sanguinetti & Tristani-Firouzi, 2006), which has resulted in a considerable effort being expended to measure and reduce interactions with this cardiac liability target.

The scientific rationale for ICH S7B and E14 was outlined in Redfern *et al* (2003). A comprehensive analysis of academic and regulatory data, clinical trial filings and post-market adverse event reports for 52 clinical drugs indicated that a 30-fold margin between hERG  $IC_{50}$  and unbound plasma  $C_{max}$  should minimise the risk of APD and QT prolongation effects ( $[(hERG\ IC_{50})/[EFTPC_{max}]]$ ). Compounds could be assigned to five different categories based on their proarrhythmic liability, primarily the incidence of TdP. A follow-up study in 2011 of 39 clinical drugs confirmed the reliability of the new guidelines, showing that predicting human cardiac risk (defined as APD or QT prolongation > 5 ms) achieved 64% sensitivity and 88% specificity if a safety margin of 45-fold of hERG  $IC_{50}$  over free plasma concentration was used (Gintant 2011).

The 'hERG-centric' approach to cardiac safety screening has proven effective at preventing dangerous hERG blockers from entering the market, but is associated with substantial costs and drawbacks. From a clinical perspective, although the ICH guidelines employ cardiac safety assays that are highly sensitive and can detect small changes to QT duration, they are not very specific for predicting proarrhythmic liability in human patients (e.g. Li *et al.*, 2018).



**Figure 2: Cardiac electrophysiology and underlying ion channels.**

- A:** The major depolarising inward (black) and repolarising outward (red) currents involved in generating the human ventricular AP.
- B:** Schematic overlay of electrophysiological recordings of single cell AP duration (APD), cardiomyocyte monolayer field potential (FP), and whole heart ECG to indicate correlation between their repolarisation parameters (APD, FPD, QT interval).

From a drug discovery perspective, the current focus on hERG inhibition and QT prolongation has increased costs and timelines of developing and marketing safe new drugs. An over-reliance on hERG selectivity can lead to extensive modification, de-prioritization or even removal of many potentially promising chemical scaffolds from the development pipeline, even though some of these compounds may not manifest a proarrhythmic liability. In addition, extensive screening against the hERG channel and use of a variety of pre-clinical animal tissues and models has increased the effort and timelines required to design safe new drugs. We would argue that a medicinal chemistry strategy that attempts simply to reduce hERG liability in order to minimise proarrhythmic risk is not supported under current guidelines, and is likely to be even less successful under the proposed expansion of *in vitro* screening guidelines where data from additional ventricular cardiac ion channels will be required for FDA-compliant drug discovery.

Analysis of all these factors has raised questions about the traditional reliance on hERG-centric cardiac safety testing regime. It is now clear that there is an imperfect correlation between hERG activity, QT prolongation and, most importantly, TdP and other harmful cardiac arrhythmias; significant exceptions to the ICH rationale are challenging the underpinnings of current cardiac safety testing practices. In addition, the sometimes poor translational ability of *in vitro* hERG and pre-clinical animal assays to model or predict human clinical cardiac risk is driving a re-assessment of ICH cardiac safety guidelines (Cavero & Holzgrefe, 2014; Sager *et al.*, 2014; Gintant *et al.*, 2016).

## Moving beyond hERG – core panel of human cardiac ion channels

The disconnect between hERG inhibition and cardiac APD prolongation has been long known, and more recently has been used as an incentive to determine what missing factors should be assessed to improve cardiac risk prediction. For example, a key industry figure concluded that “*the overall limitations of hERG safety margins shown using quantitative, evidence-based approaches highlight the need for additional pre-clinical assays and adaptive strategies throughout drug discovery to reliably mitigate QTc prolongation risk*” (Gintant 2011). Empirically, observations that drugs with strong hERG activity do not excessively prolong cardiac APD or lead to clinically significant increases in ECG QT duration or arrhythmia (e.g. propafenone, verapamil, ranolazine, amiodarone), suggest that hERG activity alone does not translate to proarrhythmic risk. Conversely, some drugs with

little hERG activity have reached the market after completing ICH S7B and standard industry pre-clinical animal model testing only to cause serious cardiac side-effects in humans (e.g. tedisamil). These disconnects question a simplistic link between hERG potency and human cardiac risk (the “hERG-centric hypothesis”), which has led many academic and industry groups and regulatory agencies to seek a new understanding of this complex issue.

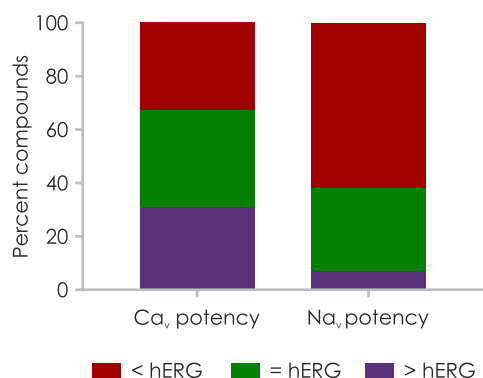
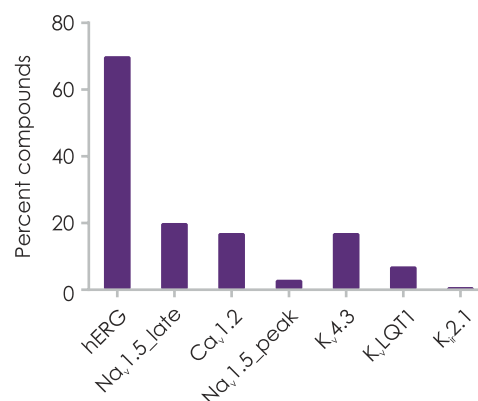
## Core cardiac panel

Several studies (see below) have shown that clinically approved drugs not only exhibit a range of activity against the hERG channel but also significantly inhibit other human ventricular ion channels (Fig. 3, Table 1). For example, an FDA-sponsored study of 30 clinical compounds spanning all TdP risk categories (at 1x free  $C_{max}$ ) showed that as well as inhibiting hERG, these clinical drugs also affected the activity of other major ventricular channels (Crumb *et al.*, 2016). The consensus from this and other work (e.g. Mirams *et al.*, 2011, Kramer *et al.*, 2013) is that inclusion of cardiac screening data from the major depolarising currents of the human heart (carried by  $Na_v1.5$  and  $Ca_v1.2$  channels), inhibition of which would shorten APD and QT duration, can compensate for inhibition of  $I_{Kr}$ , and thereby occlude or offset the hERG liability of drug candidates. This mechanism has been labelled the Multiple Ion Channel Effect (MICE) hypothesis (Kramer *et al.*, 2013), and forms the scientific rationale for including hERG,  $Na_v1.5$  and  $Ca_v1.2$  as part of the so-called “core” cardiac ion channel panel.

The combination of hERG plus  $Na_v1.5$  and  $Ca_v1.2$  channels into the “core” panel of cardiac safety screening assays is important, as it captures the majority of potential clinical cardiac arrhythmia risk (Johannesen *et al.*, 2014), and does so earlier and more reliably than waiting to test new drug candidates in complex pre-clinical animal and tissue models. Critically, the use of high quality automated patch clamp (APC) screening platforms has greatly improved the throughput, turn-around time, and cost-effectiveness of modern cardiac safety ion channel screening, providing the ability to deliver key data from the core cardiac panel in a cost-effective manner to facilitate the development of safer new medicines.

Key findings that support the inclusion of  $Na_v1.5$  and  $Ca_v1.2$  data alongside hERG potency in cardiac safety screening assessment include the following:

- *In silico* modelling groups identified the importance of including MICE data from

**A: MICE activity in clinical drugs****B: Crumb (2016) CiPA compd MICE activity**

**Figure 3: Multiple cardiac ion channel effects are common in drugs reaching the clinic.**

**A:** Data replotted from Kramer *et al.*, (2013) shows relative potency of 55 clinical drugs for Ca<sub>v</sub>1.2, Na<sub>v</sub>1.5 and hERG channels.

**B:** Data replotted from Crumb *et al.*, (2016) showing distribution of inhibitory effect (> 20% activity) of 30 CiPA compounds against a full panel of human cardiac ion channels at 3x free plasma C<sub>max</sub>.

hERG, Na<sub>v</sub>1.5 and Ca<sub>v</sub>1.2 channels in cardiac risk assessment. By utilising *in vitro* IC<sub>50</sub> values from the core panel and free plasma drug levels of 31 clinical compounds, Mirams *et al.* (2011) were able to show that a range of MICE-based parameters greatly improved *in silico* AP simulations and predictions of arrhythmic events and torsadogenic risk, compared to using hERG potencies alone or the [hERG IC<sub>50</sub>]/[EFTPC<sub>max</sub>] 30-fold safety margin outlined by Redfern *et al.*, (2003).

- Kramer *et al.*, (2013) used logistic analysis to show that hERG activity alone is a poor predictor of proarrhythmic risk for 55 clinical compounds. TdP prediction was markedly improved by including *in vitro* APC screening data against Na<sub>v</sub>1.5 and Ca<sub>v</sub>1.2 channels. The best model (Figs. 3 and 4) achieved 83% sensitivity and 97% specificity, a large improvement over using hERG data alone and empirically confirming the idea that MICE should be assessed as part of a holistic cardiac screening cascade. Verapamil and ranolazine were identified as key examples of compounds exhibiting a MICE profile that rendered a hERG blocking drug safe in human patients.
- Similarly, a comprehensive *in vitro* screening and *in silico* modelling study from Eisai in Japan showed that proarrhythmic risk for 12 clinical drugs was poorly predicted for low vs high

Redfern risk categories if only hERG IC<sub>50</sub> values, or hERG and Ca<sub>v</sub>1.2 IC<sub>50</sub> values were used (Okada *et al.*, 2015). Inclusion of hERG, Na<sub>v</sub>1.5 and Ca<sub>v</sub>1.2 data from the gigaseal quality QPatch platform correctly predicted TdP risk for 11/12 drugs.

### Metrion Biosciences Core Cardiac Panel

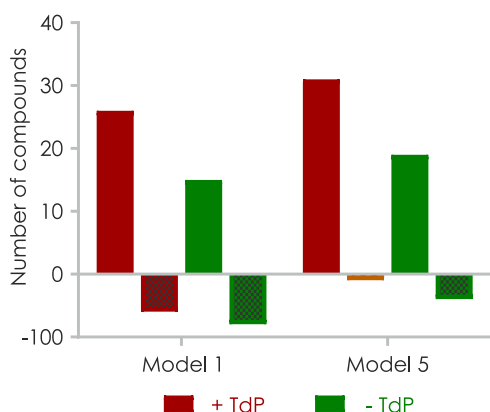
Metrion Biosciences offer a validated 'core' cardiac panel, composed of hERG, Na<sub>v</sub>1.5 and Ca<sub>v</sub>1.2 assays to enable more reliable assessment of proarrhythmic risk and to help clients develop a less hERG-centric approach. We implement these cardiac safety assays on gigaseal quality manual and high throughput screening (HTS) APC platforms, since both data quality and high throughput are essential for reliable and cost-effective AP modelling and cardiac risk prediction.

### New translational initiatives - extended cardiac panel, *in silico* models, and iPSC-CM

The drug discovery industry is now moving away from an over-reliance on the hERG channel assay and QT prolongation readouts to develop new cardiac safety testing approaches. These new screening paradigms are designed to improve the reliability and efficiency



A:



**Figure 4: Including MICE data improves proarrhythmic risk prediction.**

Data replotted from Kramer *et al.*, (2013) illustrating that correct (positive) and incorrect (negative, hashed bars) TdP risk prediction for 55 compounds is more reliable using MICE data (Model 5) compared to hERG-centric Model 1. Compounds are classified according to having (+) or lacking (-) known torsadogenic risk in the clinic.

of pre-clinical cardiac safety assays, and achieve more robust translation of early stage *in vitro* results to the clinic. The new proposals integrate drug effects measured on multiple human cardiac ion channels with assessment of their mechanistic effects using *in silico* AP models, as well as screening for outcomes in phenotypic human iPSC-CM assays. The aim is to predict torsadogenic effects more reliably and provide a more balanced assessment of patient cardiac risk, focusing in particular on human cell assays and clinical arrhythmia (e.g. TdP) liability.

The highest profile and largest international effort to test and implement new translational cardiac safety assays is the FDA's Comprehensive *in vitro* Pro-arrhythmia Assay (CiPA) initiative, which involves academic, industrial and regulatory entities in North America, Europe and Japan ([www.cipaproject.org](http://www.cipaproject.org)). Metrion is an active participant in the CiPA ion channel HTS sub-team and a member of the Health and Environmental Sciences Institute (HESI) cardiac committee. There are also two similar cardiac safety consortia in Japan that both focus on the use of iPSC-CMs, namely JiCSA (Japan iPS Cardiac Safety Assessment, [www.jicsa.org](http://www.jicsa.org)) and CSAHi (Consortium for Safety Assessment using Human iPS cells, [www.csahi.org/en/](http://www.csahi.org/en/)).

### Extended cardiac ion channel panel (1<sup>st</sup> CiPA pillar):

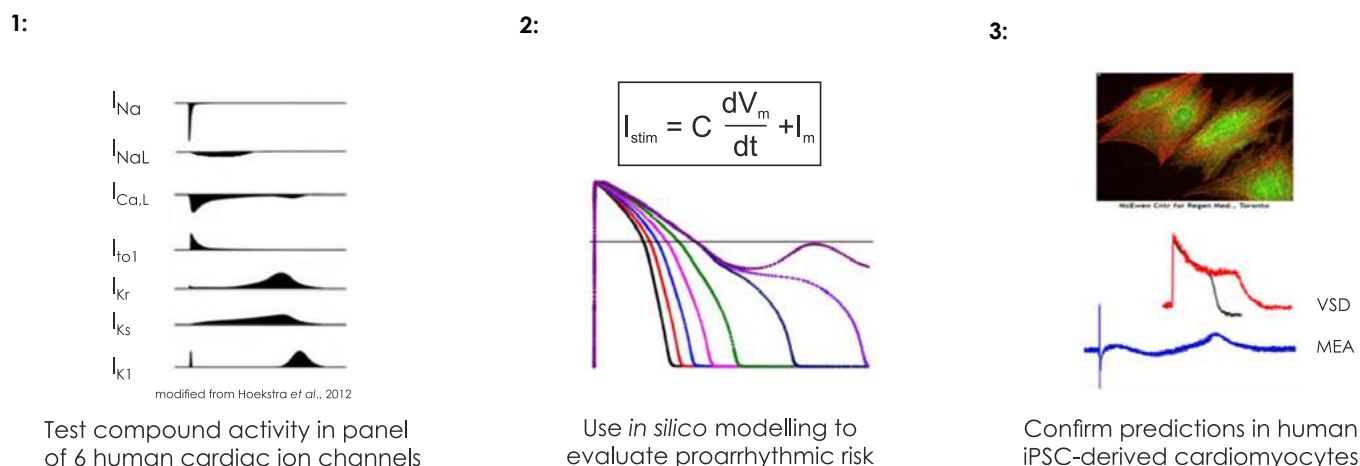
The incorporation of 'core panel' *in vitro* potency data in various *in silico* models and risk prediction analyses demonstrated a marked improvement in cardiac arrhythmia prediction, but there are still some known torsadogenic drugs that cannot be detected.

A major hypothesis of the FDA's CiPA initiative (1<sup>st</sup> pillar, Fig. 5) is that including data from the other major depolarising and repolarising human ventricular cardiac ion channels should provide a more complete picture of underlying cardiac physiology and pharmacology, and thus deliver more accurate data for *in silico* models and iPSC-CM assay validation and screening.

There have been considerable discussions, laboratory tests, and *in silico* comparisons to decide the minimum, or necessary, composition of a human ventricular cardiac ion channel panel that is sufficient to capture the majority of proarrhythmic risk and reliably predict human clinical cardiac liability (Sager *et al.*, 2014; Gintant *et al.*, 2016; Fermi *et al.*, 2016; Li *et al.*, 2017). Although included in early versions of the CiPA ion channel pillar (Sager *et al.*, 2014), the cardiac pacemaker, HCN4, has now been removed. The current CiPA 'big 6' panel is shown in Fig. 5 and Table 1, and the rationale for its composition is given below.

Several observations suggested that addition of  $K_vLQT1$  (the molecular correlate of  $I_{Ks}$ ) biophysics and  $IC_{50}$  potency values could improve the accuracy and sensitivity of existing cardiac risk prediction models, perhaps by accounting for decreases in repolarisation reserve. For example:

- *In silico* modelling revealed large effects of  $I_{Ks}$  blockade on APD (Mirams *et al.*, 2011, 2014).
- Eisai showed that accurate prediction of cardiac arrhythmia risk of 12 clinical drugs can be best achieved using screening data against the core panel (hERG,  $Na_v1.5$  and  $Ca_v1.2$ ) and  $K_vLQT1$  (Okada *et al.*, 2015).



**Figure 5: CiPA *in vitro* and *in silico* pillars for integrative assessment of cardiac arrhythmia risk.**

CiPA cell-based validation and testing efforts are split between the ion channel working group (ICWG) (1), *in silico* modelling (2) and cardiomyocyte working groups (3). The ICWG is comparing manual and APC (HTS sub-team) screening data generated from several protocols and platforms. The *in silico* group is developing cardiac AP models of native and iPSC-CM for use in APD prolongation and arrhythmia predictions. Human iPSC-CM assays validated on imaging and electrophysiology (multi-electrode array, MEA) platforms are being used to test predictions of risk from pillars 1 and 2. Graphic adapted from CiPA publications (Gintant 2015).

- Compounds developed by Johnson & Johnson to treat a variety of non-cardiac diseases were shown to be potent inhibitors of  $I_{Ks}$  and safe in *in vitro* cardiac safety assays, but produced severe cardiac side-effects in *in vivo* dog safety models (Towart *et al.*, 2009).

Groups working with the FDA and HESI CiPA initiative showed that inclusion of the remaining major potassium ion channels present in human ventricular cells, namely  $K_{v4.3}$  and  $K_{ir2.1}$ , is also necessary and desirable to fully capture potential human clinical cardiac risk.

- $K_{v4.3}$  and its accessory subunit KChIP underlie  $I_{to}$ , which contributes to rapid repolarisation after the human ventricular AP peak. Crumb *et al.*, (2016) reported that the most common non-core panel activity of 31 clinical compounds involved  $K_{v4.3}$ , followed by  $K_{vLQT1}$  (seen at 3x free plasma  $C_{max}$ ).
- Mirams *et al.*, (2014) incorporated hERG,  $Na_v1.5$ ,  $Ca_v1.2$ ,  $K_{vLQT1}$  and  $K_{v4.3}$  data from manual and APC platforms into their modified O'Hara-Rudy *in silico* AP model simulation to achieve TdP risk prediction with 79% sensitivity and 95% specificity. This represented a considerable improvement when compared with a model using manual patch clamp hERG data alone (Gintant 2011). Significantly, when using data solely obtained from the Ionworks Quattro, which is a low megaohm

seal quality platform, the model only yielded 14% sensitivity. Overall, their data was equivalent to the performance of a rabbit ventricular wedge *ex vivo* QT assay, but removes the use of animal tissues and non-human data.

- The  $K_{ir2.1}$  inward rectifier channel, which is the molecular correlate of  $I_{k1}$ , is a key determinant of resting membrane potential and AP repolarisation termination. Therefore, any change in its activity will affect the delicate interplay between the other voltage-dependent cardiac ion channels and influence experimental and *in silico* APD and QT parameters (e.g. Mirams *et al.*, 2014, Okada *et al.*, 2015; Dutta *et al.*, 2017). Few clinical drugs show significant inhibition of  $I_{k1}$ , but such activity needs to be ruled out prior to human clinical trials, which is why it remains part of the full CiPA panel and proposed FDA cardiac safety testing guidelines.

Another compelling rationale for including all of the major human ventricular ion channels in an extended cardiac safety panel (and corresponding *in silico* models) is that patients carrying mutations in these channels (so-called channelopathies) manifest various types of cardiac disease, such as long and short QT syndrome (LQT and SQT), Brugada syndrome, Timothy disease, and sick sinus syndrome (Table 1). If genetic mutations leading to functional anomalies in these cardiac ion channels can cause human disease, then it is logical to anticipate

ION CHANNEL	QT SYNDROME	CARDIAC CURRENT & FUNCTION
hERG	LQT2, LQT6, SQT1	$I_{Kr}$ ; Rapid repolarising rectifier current, QT prolongation
$Na_v1.5$	LQT3, Brugada	$I_{Na}$ ; Major inward current underlying AP upstroke
$Ca_v1.2$	LQT8, Timothy, SQT4	$I_{Ca}$ ; Inward current during AP upstroke & early plateau
$K_vLQT1\_minK$	LQT1, LQT5, SQT2	$I_{Ks}$ ; Slow repolarising rectifier current, accessory protein
$K_{ir}2.1$	LQT7, SQT3	$I_{K1}$ ; Inward rectifier that helps set resting potential
$K_v4.3\_KChIP$	-	$I_{to}$ ; Underlies $I_{to}$ transient outward current after AP peak
HCN4	Sick sinus	$I_h$ ; Pacemaker current that modulates AP firing rate

**Table 1: Cardiac ion channel mutations (channelopathies) associated with human cardiac disease.**

The core panel of hERG,  $Na_v1.5$  and  $Ca_v1.2$  ion channels are grouped in dark grey rows, followed by the remaining CiPA 'big 6' extended cardiac panel (light grey). HCN4 is no longer a CiPA channel. Human cardiac diseases associated with rare mutations in each channel are shown, including LQT and SQT.

that drugs that modulate these same channels could also perturb their activity and cause arrhythmia.

More recently, there has been considerable work, and some consensus achieved, around the necessity to also include data from two additional cardiac ion channel assays, which are variants of the existing hERG tail current and  $Na_v1.5$  peak current protocols.

### • Dynamic hERG

Part of the apparent disconnect in hERG-centric cardiac safety screening and TdP prediction has been attributed to differences in the binding kinetics of high vs low risk hERG blockers (Pearlstein *et al.*, 2016; Fermi *et al.*, 2016; Li *et al.*, 2017). Drug trapping in the hERG pore and slower dissociation from the channel appears to be linked to higher proarrhythmic risk. Some good examples of compounds that have distinct binding kinetics which correlate with different levels of proarrhythmic liability, include dofetilide (high risk), cisapride (intermediate risk), and verapamil (low risk).

The influence of drug binding kinetics to the hERG channel is being actively investigated by the CiPA ion channel and *in silico* working groups - hence the use of the dynamic O'Hara-Rudy model (see below). Recent data has shown marked improvements in AP model accuracy and cardiac risk stratification after including hERG binding kinetics alongside MICE data for clinical drugs (Li *et al.*, 2017, 2018).

Metrion Biosciences has replicated these findings by incorporating data collected from its *in vitro* 'big 6' ion channel panel into CiPA's *in silico* model (Fig. 7).

The challenge for the CiPA ICWG and HTS sub-teams has been to devise and validate appropriate hERG kinetic voltage protocols (Milnes *et al.*, 2010) and create reliable and successful 'dynamic hERG' screening assays. This technically demanding assay has been mastered on manual patch, but Metrion Biosciences is the first ion channel CRO to develop an assay on a gigaseal quality APC platform: our QPatch application note can be found [here](#). Metrion's dynamic hERG assay is able to detect differences in drug binding kinetics reliably for compounds with high, intermediate and low proarrhythmic risk in a time- and cost-effective manner; essential features of a cardiac safety screening assay required for post-ICH S7B cardiac safety assessment and regulatory filings.

### • Late $Na_v1.5$

The cardiac sodium channel is also able to contribute late inward currents during the early repolarising phase of the AP plateau, and it has become clear that this small but persistent current can have a major influence on APD, *in silico* AP models, and assessment and prediction of proarrhythmic risk (Wu *et al.*, 2005; Johannesen *et al.*, 2014; Li *et al.*, 2018). Inhibition of late  $I_{Na}$  can shorten APD and QT duration, and more importantly for MICE blockers (such as ranolazine) this effect can attenuate APD prolongation that



results from  $I_{Kr}$  inhibition. Such an effect could prevent the induction of early after depolarizations (EADs) that are considered to underlie the development or arrhythmias *in vivo* (Belardinelli *et al.*, 2013), making it a powerful mechanism to moderate cardiac arrhythmias. Surprisingly, a manual patch study by the FDA (Crumb *et al.*, 2016) showed that there was greater activity of 31 clinical drugs against the late component of  $Na_v1.5$  than the traditional peak current parameter, confirming the importance of including the late component of  $Na_v1.5$  in cardiac ion channel screening panels. Our assay reproduces the activity of known late  $Na_v1.5$  modulators (Application Note [here](#)), and has been used in official CiPA *in silico* AP models to further improve their predictions of arrhythmia and TdP risk for known clinical drugs (Fig. 7). The same has recently been shown to be true for *in silico* AP models (see below).

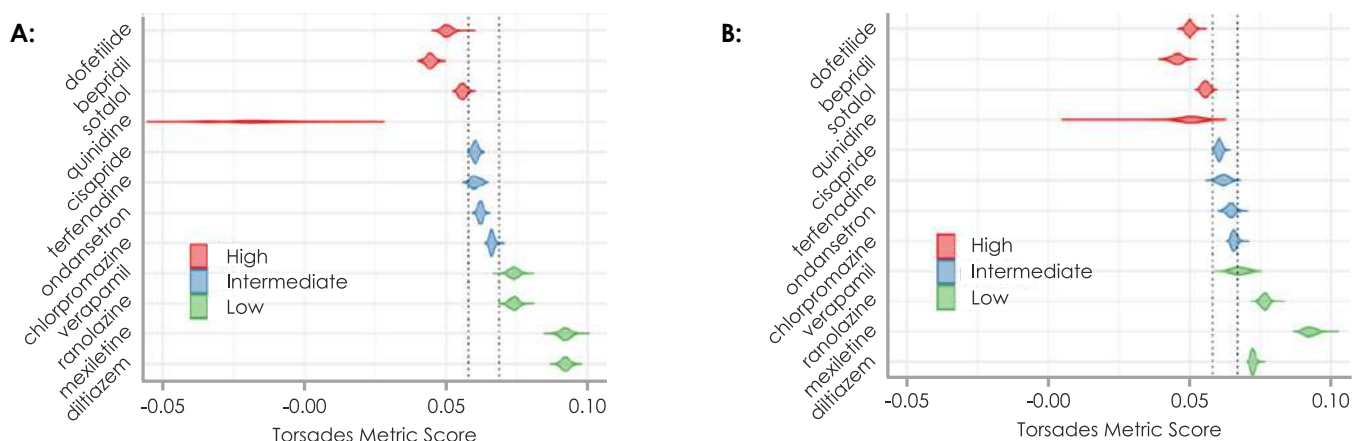
The probability of late  $Na_v1.5$  channel re-openings is low, and most groups use pharmacological tools such as ATX-II toxin, veratridine, or pesticides such as deltamethrin to block channel inactivation artificially and promote late openings. These are fairly blunt and somewhat unreliable ligands; there is considerable variation in the efficacy of commercial sources of ATX-II, all agents are toxic and difficult to use and safely dispose of, and none are selective for the cardiac  $Na_v1.5$  channel (and are thus able to activate endogenous  $Na_v1.x$  channels present in some heterologous cell expression systems). Metrion Biosciences is the first ion channel CRO to build and

validate a successful human LQT3 'channelopathy' assay for late  $Na_v1.5$ , using one of the original genetic mutants,  $\Delta KPQ$ , to promote late openings (Wang *et al.*, 1996). Our assay reproduces the activity of known late  $Na_v1.5$  modulators, and has been used in official CiPA *in silico* AP models to further improve their predictions of arrhythmia and TdP risk for known clinical drugs (Fig. 7).

Thus, the extended panel of cardiac ion channel assays are not only genetically validated targets, but are also the substrate for most clinical drug-induced arrhythmia, making them a key component of the CiPA initiative. The latest information from the FDA's CiPA and cardiac safety committee working groups indicates that the optimal screening panel required for future predictive cardiac safety screening applications may comprise the core panel of hERG (standard and dynamic protocols),  $Ca_v1.2$  and late  $Na_v1.5$  assay.

### Manual vs automated patch clamp - HTS sub-team

The key to reliable prediction of cardiac risk is the use of high quality *in vitro* data, be it for a simple calculation of hERG  $IC_{50}$  safety margin over plasma  $C_{max}$ , or use of ion channel panel screening data in sophisticated *in silico* AP models (in contrast to "rubbish in, rubbish out"). The CiPA ion channel working group began generating data and validating



**Figure 6: Comparison of manual vs APC ion channel data for *in silico* cardiac risk modelling.**

Training set of 12 CiPA drugs categorised by clinical cardiac risk, plotted according to predicted torsadogenic risk score using *in vitro* cardiac ion channel data obtained using manual patch clamp (A) or APC screening platforms (B). *In silico* qNET modelling also included dynamic hERG binding kinetic data generated on manual patch in both datasets. Data taken from Li *et al.* (2018): PMID 30151907 (used under open access license).

*in silico* models using 'gold' standard' manual patch clamp assays, but a separate HTS sub-team (of which Metrion is a leading member) was tasked with setting up and validating similar cardiac ion channel assays on higher throughput APC platforms. These APC devices have become an integral part of drug discovery industry screening cascades, as they can deliver the necessary high quality data in a time-efficient and cost-effective manner required for large scale compound screening and safety assessment. Not all APC platforms or assays offer gigaseal recordings, and part of the HTS sub-team efforts are directed at determining the reliability and consistency of data from different APC machines and sites (Fig. 6). Although there still remains resistance in some quarters to the use of APC screening platforms for cardiac safety screening, recent data comparisons from the FDA, CiPA and industry groups indicate that it can effectively substitute lower throughput and more costly manual patch data without affecting the predictive power of *in silico* models (Kramer *et al.*, 2013; Okada *et al.*, 2015; Crumb *et al.*, 2016; Li *et al.*, 2017, 2018).

Metrion Biosciences has made great efforts to ensure that its APC cardiac safety assays are robust and reliable. As part of the CiPA HTS sub-team, we have validated all our cardiac ion channel assays on the QPatch gigaseal platform, using the official (and blinded) 12 compound test set and the 16 compound validation test set. Metrion was the second site to complete this HTS sub-team validation work, and our data was recently published by the FDA (Kramer *et al.*, 2020; Ridder *et al.*, 2020), as well as being used to train and test the FDA's *in silico* AP and cardiac risk prediction models. In addition, Metrion is the first ion channel CRO to offer a validated APC 'dynamic' hERG assay, as well as a pathophysiological LQT3 mutant late Na<sub>v</sub>1.5 channel assay that does not require the use of variable and non-selective pharmacological activators. Taken together with its core and extended CiPA cardiac panels, this makes Metrion Biosciences one of the leading cardiac safety ion channel service providers, with assays fully aligned with future drug discovery and regulatory requirements.

### **In silico models of the human cardiac AP (2<sup>nd</sup> CiPA pillar):**

The second CiPA pillar (Fig. 5) integrates empirical screening data into *in silico* models of human cardiac APs. Thanks to modern computing power, these models are now very fast and sophisticated, and are able to include fine details of multiple underlying ionic conductances (Mirams *et al.*, 2014), temperature

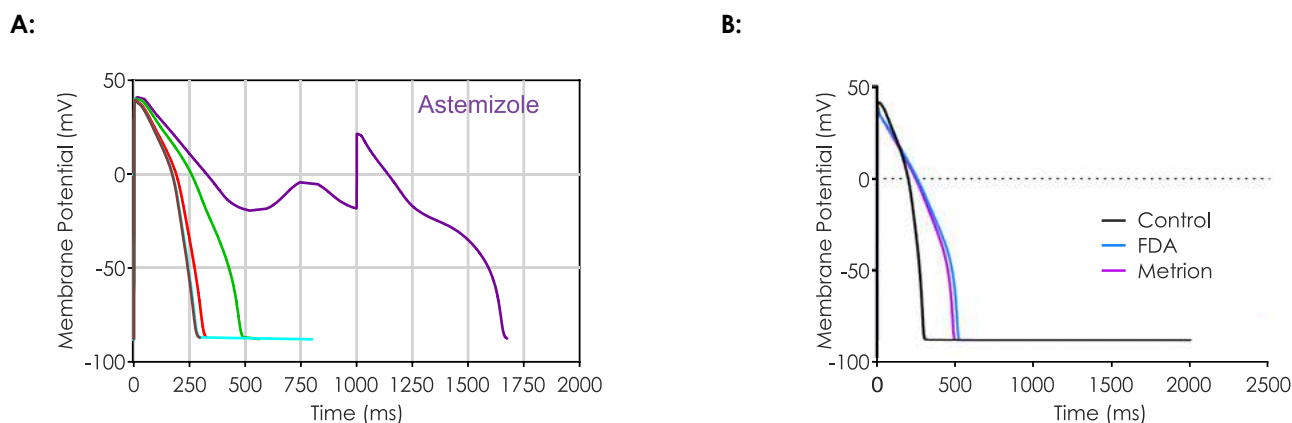
dependence (Li *et al.*, 2016), and drug binding kinetics (Li *et al.*, 2017) that are essential for accurate emulation of the complex electrical activity of native and iPSC-CMs under physiological conditions. The effects of test compounds are assessed by integrating their effects on various cardiac ion channels, based on their patch clamp IC<sub>50</sub> values and virtual screening concentration, and comparing their effects on APD and arrhythmia events to the plasma exposures (e.g. C<sub>max</sub>).

Numerous *in silico* AP models have been developed over the years but many of these are designed to emulate the cardiac electrophysiology of popular pre-clinical animal species such as rabbit, guinea pig and dog. Some groups have developed AP models of human iPSC-CMs to more readily translate data between the CiPA pillars (Paci *et al.*, 2015), but the ultimate goal of CiPA is to develop *in silico* models of native adult human cardiomyocytes to allow prediction of cardiac risk prior to drugs being submitted to human clinical trials.

The O'Hara-Rudy AP formulation has proven to be the most predictive model of human cardiac APs, and is also uniquely able to recapitulate early after depolarisations (EADs), which are crucial to accurately predict human clinical cardiac risk (Mirams *et al.*, 2011). A fully featured version of the O'Hara-Rudy AP simulation is being developed and validated by the FDA as the official CiPA *in silico* model. This consensus O'Hara-Rudy dynamic (ORd) cardiac AP model is based on the 'big 6' ion channel panel but also includes a late Na<sub>v</sub>1.5 component as well as hERG drug binding kinetics. The ORd model was calibrated using *in vitro* ion channel IC<sub>50</sub> data from the same CiPA toolbox of 12 training drugs used in the HTS and cardiac myocyte assays (Li *et al.*, 2017). The resulting model and metrics were then fixed and used to virtually screen the 16 CiPA validation drugs to test the accuracy of proarrhythmic risk predictions against the known clinical risk of the test compounds (Li *et al.*, 2018).

There are several important features of the FDA's *in silico* cardiac AP model that appear to contribute to more accurate predictions of APD changes and proarrhythmic risk.

- First, a measure of net change in depolarising (inward) and repolarising (outward) currents, called qNet, has been shown to be a powerful prediction metric (Dutta *et al.*, 2017). The qNet parameter is heavily weighted by MICE effects against the core the core cardiac panel, and extensive model validation shows that drug inhibition of hERG, Ca<sub>v</sub>1.2 and the



**Figure 7: *In silico* AP models can reliably predict human cardiac arrhythmic risk.**

**A:** O'Hara-Rudy MICE model reports AP prolongation and EADs for increasing concentrations (1 nM – 10 mM) of astemizole (Mirams *et al.*, 2014).

**B:** Concordance between FDA and Metrion ORd model output for verapamil, with FDA using manual patch dynamic hERG and APC MICE data, and Metrion using manual patch MICE data (Crumb *et al.*, 2016) and QPatch dynamic hERG assay data.

late  $\text{Na}_v1.5$  current components that have the most significant impact on proarrhythmic risk prediction (Li *et al.*, 2018). In addition, MICE block of peak  $\text{Na}_v1.5$  is also important due to its potential to cause depolarisation failure.

- Late  $\text{Na}_v1.5$  conductance is now a critical feature in most *in silico* cardiac AP models. More selective inhibitors of hERG channels will prolong the APD and promote additional openings of  $\text{Ca}_v1.2$  and late  $\text{Na}_v1.5$  channels, which are strong factors in eliciting EADs and arrhythmias that make this type of drug high TdP risk. Conversely, a MICE profile, which includes inhibition of late  $\text{Na}_v1.5$  current, is able to offset the effects of hERG blockade, and this may be especially important to reduce the proarrhythmic liability potential of drugs that are trapped in the hERG channel (Li *et al.*, 2017; Fig. 7).
- Finally, incorporation of Markovian hERG drug binding kinetics (as outlined above) can help to further differentiate and predict low from high proarrhythmic risk drugs. Several important kinetic parameters can be extracted from the dynamic hERG patch clamp assay to indicate the channel trapping blocking profile of test compounds.

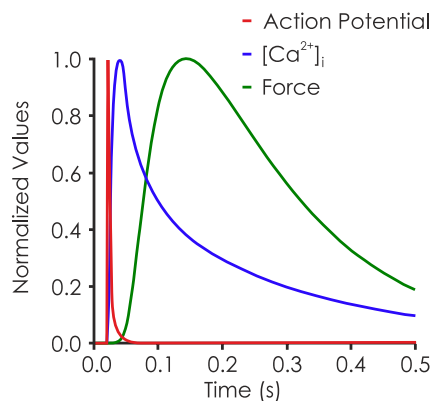
Code for the official FDA *in silico* AP model was recently released and has been successfully

implemented at Metrion Biosciences. This emulation includes dynamic hERG and late  $\text{Na}_v1.5$  components. Generating data for these latter assays remains technically challenging, and only data from reliable manual or APC assays should be used in proarrhythmic risk modelling. Metrion has successfully validated both a dynamic hERG and late  $\text{Na}_v1.5$  assay, the latter using a LQT3 channelopathy gain-of-function mutation, rather than non-selective pharmacological activators. Therefore, we are able to offer an industry-leading and fully validated *in vitro* CiPA cardiac ion channel panel, alongside the latest generation of *in silico* cardiac electrophysiology models, necessary to meet FDA requirements for integrated, accurate and predictive cardiac safety risk assessment.

### Human stem cell cardiomyocyte assays (3<sup>rd</sup> CiPA pillar)

The third *in vitro* pillar of the CiPA initiative is designed to create and validate iPSC-CM and assays that can serve as an accessible experimental model of the human heart. This has proven to be the most challenging of the CiPA pillars, and the work done by the global collection of academic, industry and regulatory groups in North America, Japan and Europe continues to face some resistance from those more closely aligned with *ex vivo* and *in vivo* pre-clinical animal models and native, adult human cardiac tissue.

A:



**Figure 8: Schematic showing different time course of cardiomyocyte functional readouts.**

Excitation-contraction coupling is initiated by electrical activation of ionic currents during the AP (also manifested in field potential and QT electrical recordings), followed by changes in  $\text{Ca}^{2+}$  flux across the cell membrane and from intracellular stores, and finally the activation of contractile proteins. Image taken from doi:10.1371/journal.pone.0063141.g011 and used under open access license CC BY 4.0.

To be clear, human iPSC-CMs have proven to be a useful model of cardiac arrhythmia, but should not be claimed or viewed as a replacement or substitute for native human tissue (Himmel *et al.*, 2013; Sala *et al.*, 2016). Most importantly, iPSC-CMs must replicate the essential biophysical and pharmacological features required to test predictions of cardiac arrhythmia produced from *in vitro* screening data and *in silico* AP models. Indeed, great strides have been made by iPSC providers and screening platform developers to create cardiac safety assays which have been shown to be more cost-effective and predictive than existing toxicology assays (Guo *et al.*, 2013; Navarrete *et al.*, 2013; Nakamura *et al.*, 2014; Yamamoto *et al.*, 2016).

The main advantage (and criticism) of iPSC-CM is that they exhibit an immature phenotype that is most obviously manifest by spontaneous AP firing and cell beating. It is this ongoing activity that makes iPSC-CM amenable to various plate-based screening technologies, such as voltage and  $\text{Ca}^{2+}$  imaging platforms (dye and/or optogenetic reporters) or electrophysiology readouts employing multi-electrode arrays (MEA) or impedance electrodes. It has been claimed that loading cells with voltage and  $\text{Ca}^{2+}$  indicators can affect normal physiological processes and even induce toxicity (e.g. Broyles *et al.*, 2018); this can be avoided by using genetically encoded reporters (e.g. Klimas *et al.*, 2016; Dempsey *et al.*, 2016). The CiPA consortium recognised these issues and chose to compare iPSC-CM data generated using voltage-sensitive dye (VSD) and MEA techniques, although other readouts such as  $\text{Ca}^{2+}$  transients are part of the Japanese JiCSA and CSAHi initiatives, and many groups are also using impedance to study contraction amplitude and inotropic drugs.

The CiPA cardiomyocyte consortium has recently published its Phase I (test) and Phase II (validation) studies that compared several iPSC-CM cell lines and plate-based readouts (Blinova *et al.*, 2017, 2018), which showed reliable prediction of known cardiac arrhythmia risk (100% specificity, up to 79% sensitivity) and greater variation between cell types than between readout platforms.

Cardiac toxicity is a complex process, and it should be mentioned that the focus of this white paper is on ion channel electrophysiology and acute cardiotoxicology as outlined by the CiPA initiative. Drugs can acutely affect other important cardiac functions, such as  $\text{Ca}^{2+}$  handling and muscle physiology, that will affect contractility and cell health (Fig. 8), which can be assayed with a variety of techniques. There is also growing evidence for chronic cardiotoxicity effects of pre-clinical compounds and clinical drugs such as tyrosine kinase inhibitors, and these may occur independently of acute or chronic ion channel function (Cohen *et al.*, 2011). An important point to note is that much of the iPSC-CM assay development and validation work carried out under current cardiac safety assessment initiatives are also being applied to chronic cardiotoxicity studies, demonstrating the translatability of such new approaches.

It is perhaps not surprising to learn that there is some variation between the biology of different iPSC-CM cell lines. Vendors are striving to reduce batch-to-batch variation in their products (Huo *et al.*, 2016), but Metrion and others have observed that there are reproducible differences in the ion channel repertoire for each provider and cell line, which can underlie variability in their AP biophysics and cardiac pharmacology. For example, Blinova *et al.*, (2017) showed significant differences in hERG,  $\text{Ca}_v1.2$ ,  $\text{Na}_v1.5$  (especially the late component) and

K<sub>v</sub>LQT1 expression between two leading iPSC-CM cell lines. We have observed similar differences in functional cardiac ion channel activity, based on voltage clamp recordings of ionic currents and current clamp recordings of AP pharmacology, across several leading commercial suppliers (poster, Table 2) We take a pragmatic view of the diversity in iPSC-CM cell line ion channel profiles, based on a clear and verified understanding of differences in cardiac electrophysiology and pharmacology. Rather than using, or relying, on a single iPSC-CM reagent for our cardiac safety assessment services, Metrion Biosciences replicated the CiPA cardiac myocyte team efforts (Table 3) and generated an extensive validation test set for several leading commercial iPSC-CM cell lines (e.g. CDI, Axol Biosciences). This proprietary data allows us to choose the best reagent for each assay endpoint or client hypothesis. Moreover, it is now possible to conduct so-called “clinical trial-in-a-dish” (Burrige *et al.*, 2016) and *in vitro* thorough QT studies (Fermini *et al.*, 2018) using a collection of diverse human iPSC-CM cell lines.

Metrion Biosciences and its network of iPSC partners enables clients to access such cardiomyocyte diversity, providing a collection of iPSC reagents and assays that represent the mix of normal epigenetic variation and rare

genetic mutations, which are fundamental to traditional human clinical trials. In this way Metrion Biosciences provides independent access to scientifically verified human iPSC-CM reagents and assays, which complement our FDA-compliant *in vitro* cardiac ion channel panel and *in silico* AP models and contribute to a comprehensive set of translational human cardiac safety assessment services.

## Conclusions

The need for more reliable and predictive cardiac safety testing to reduce clinical arrhythmia risk of new medicines is moving the drug discovery industry away from an over-reliance on hERG testing and pre-clinical animal models to use of sophisticated human *in vitro*, *in silico* and translational iPSC-CM phenotypic assays. Our cardiac expertise and involvement in the FDA's CiPA initiative has enabled Metrion Biosciences to develop and validate a comprehensive array of high quality, industry-leading human cardiac ion channel panels, *in silico* AP models and human iPSC-derived cardiomyocyte assays that are aligned with future cardiac safety testing and drug regulatory needs.

IPSC-CM CELL LINE	AP PROFILE	IONIC CURRENTS	MEA PHASE 1	MEA PHASE 2	IMPEDANCE
CDI (i-cell <sup>2</sup> )	✓	✓	✓	✓	✓
NCardia (Pluricytes)	✓	✓	✓		✓
Axol Biosciences	✓	✓	✓		✓
Takara (Cellartis)	✓	✓	✓		

**Table 2: Metrion has validated all leading commercial iPSC ventricular cardiomyocytes.**

We have profiled the biophysical and pharmacological properties of iPSC-CM cell lines using patch clamp, multi-electrode array (MEA) and impedance techniques to give an independent measure of their underlying cardiac ion channel function. AP profiling utilised current clamp recordings of passive membrane, as well as spontaneous and evoked AP parameters. Voltage clamp was used to measure inward and outward ionic currents. MEA assays assessed baseline and drug effects on FP amplitude and duration, as well as beat rate, whilst impedance measured cell health index, beat rate and contraction amplitude.



Compound	CiPA TdP Risk	ΔFPDc							ARRHYTHMIA						
		Metrion vCor.4U	JiCSA Cor.4U (1)	FDA Cor.4U (3)	Metrion iCell <sup>2</sup>	JiCSA iCell (4,5,6)	FDA iCell (3)	FDA iCell (2)	Metrion vCor.4U	JiCSA Cor.4U (1)	FDA Cor.4U (3)	Metrion iCell <sup>2</sup>	JiCSA iCell (4,5,6)	FDA iCell (3)	FDA iCell (2)
Diltiazem	Low	↓		↓	↓	↓	↓	↓	-		-	-	-	-	-
Mexilitine	Low	↑	↑	=	↑	↑	=	=	-	-	-	-	-	-	-
Ranolazine	Low	↑	↑	↑	↑	↑	↑	↑	-	-	-	+ FP	+ FP	+ FP	+ FP
Verapamil	Low	↓	↓	↓	↓	↓	↓	↓	-	-	-	-	-	-	-
Chlorpromazine	Medium	↑	=	=	↑	=	=	↑	+		- FN	- FN	+	- FN	+
Cisapride	Medium	↑	↑	↑	↑	↑	↑	↑	+	+	- FN	+	+	+	+
Ondansetron	Medium	↑			↑	↑			+			+	+		
Terfenadine	Medium	↑	↑	↑	↑	↑	=	↑	+	- FN	- FN	- FN	- FN	- FN	+
Bepidil	High	↑	↑	↑	↑	↑	=	↑	- FN	- FN	- FN	+	- FN	- FN	- FN
D,L-Sotalol	High	↑			↑	↑			+			+	+		
Dofetilide	High	↑	↑	↑	↑	↑	↑	↑	+	+	+	+	+	+	+
Quinidine	High	↑	↑	↑	↑	↑	↑	↑	+	+	+	+	+	- FN	+

**Table 3: Comparison of Metrion Biosciences MEA iPSC-CM results to published data.**

Compounds from each CiPA risk classification were screened against Axiogenesis vCor.4U and CDI iCell<sup>2</sup> cardiomyocytes on the Maestro MEA platform, measuring change in field potential duration (FPD) and arrhythmic events. Overall, good correlation was observed between our data and published datasets from the FDA and JiCSA. The MEA assay correctly identified all low risk compounds as non-arrhythmic and all high risk compounds showed a significant FPDc prolongation. All high risk compounds produced arrhythmic events in both cell lines, with the exception of bepidil which failed to generate EAD/arrhythmic events in vCor.4U cells.

All high risk compounds produced arrhythmic events in both cell lines, with the exception of bepidil which failed to generate EAD/arrhythmic events in vCor.4U cells.

**KEY:** ↑Increased FPDc; ↓Decreased FPDc; = No change; + Arrhythmia/EAD events; – No arrhythmic events.

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