CiPA update: Refining *in vitro* cardiac ion channel assays, *in silico* models and iPSC cardiomyocyte reagents for improved proarrhythmia risk prediction

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Introduction

Metrion is working towards the requirements of the FDA's Comprehensive *in vitro* Proarrhythmia (**CiPA**) initiative (*cipaproject.org*) which comprises 3 parts: 1) High quality *in vitro* cardiac ion channel assays, 2) Comprehensive *in silico* action potential (AP) models, and 3) Predictive assays using induced pluripotent stem cell derived cardiomyocytes (iPSC-CM).

We are building upon our panel of *in vitro* human cardiac ion channel assays and applying the data to various *in silico* cardiac models, and more recently assessing commercially available iPSC-CM for use in phenotypic assays to assess the pharmacological and risk predictions from our *in vitro* and *in silico* cardiac safety data.

Here we outline our progress in validating and implementing all 3 pillars of the CiPA regime by building upon work presented previously at the 2015 SPS meeting in Prague.

1. Validation of automated patch clamp cardiac assays using CiPA-approved protocols and compounds on the gigaseal QPatch platform (Sophion)
2. Comparing web-based *in silico* models of cardiac risk based on action potential prolongation (APD or QT) using our QPatch CiPA ion channel dataset
3. Characterisation of three commercially available iPSC-CM
   • Profiling spontaneous and evoked action potentials
   • Determining the mix of atrial *vs.* ventricular phenotype
   • Creating a voltage clamp "snapshot" of the core cardiac ionic currents (Nav, Cav and hERG) to better understand the underlying cardiac pharmacology
   • Pharmacological sensitivity of core channels to 'in-class' positive controls

Materials and Methods

Automated Patch Clamp: CHO-K1 or HEK-293 stably expressing exogenous human a-subunits of each cardiac ion channel were grown using standard cell culture conditions. The hKv4.3 cell line also expressed KChiP2 accessory subunits and KCNQ1 cell line co-expressed minK subunit. Cells were prepared for assays using proprietary protocols.

All cell lines were validated biophysically and pharmacologically 'in house' on QPatch48 platform (Sophion, Denmark). All recordings were in conventional whole cell configuration using standard single hole chips. Standard recording solutions specific for each ion channel were used and classical voltage protocols in line with CiPA guidelines were used.

Manual patch clamp: Human iPSC-CM were obtained from three commercially available vendors and seeded according to manufacturers instructions. APs were recorded 7-10 days after cell seeding at RT in current clamp mode using perforated patch (100 µg/ml gramicidin). For evoked AP cells were paced at 1 Hz with a field stimulator. Voltage clamp recordings were obtained from single cells using the conventional whole-cell patch clamp configuration with protocols and solutions designed to isolate the ionic current of interest.

Data were acquired with EPC10 amplifiers and PatchMaster software (HEKA Elektronik, Germany). Analog signals were low-pass filtered at 10 kHz before digitization at 20 kHz. Spontaneous AP were analysed with CAPA software (SSCE UG, Germany) and evoked AP data in FitMaster. The analysed AP parameters are shown in Figure 1. Data are reported as mean ± SEM.

Analysed Parameters
MDP Minimum Diastolic (mV)
APA Action Potential Amplitude (mV)
MDR Maximum Diastolic Rate (V/s)
TTP Time To Peak (ms)
APD20 AP Duration at 20 % Repolarisation (ms)
APD50 AP Duration at 50 % Repolarisation (ms)
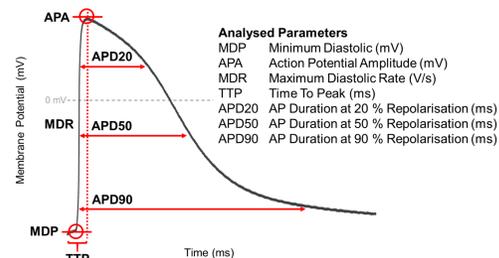APD90 AP Duration at 90 % Repolarisation (ms)

Figure 1: Action potential parameters
Example action potential indicating the parameters which are quantified using HEKA FitMaster (evoked AP) and CAPA software

In silico modelling: We previously used the web portal tool of Williams and Mirams[1] to evaluate the QT prolongation and torsadogenic risk of test compounds by incorporating our *in vitro* IC$_{50}$ data into a simulation of all 6 CiPA cardiac channels in the O'Hara-Rudy model of the human ventricular myocyte action potential. We also evaluate another online AP simulator (EasyAP)[2] and a machine learning cardiotoxicity risk assessment algorithm (CardioTox)[3], both employ the core panel of Nav1.5, Cav1.2 and hERG channels. Where known pacing frequency was 1 Hz and drug effects modelled for 5 min.

References

[1]Williams & Mirams (2015) JPET; [2]EasyAP (Physiomics, easyap.co.uk); [3]CardioTox Predictor (ardiotox-predictor.com)

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1. CiPA compliant panel cardiac assays on QPatch

A. Potency determination of 'in-class' and CiPA tool compounds with QPatch
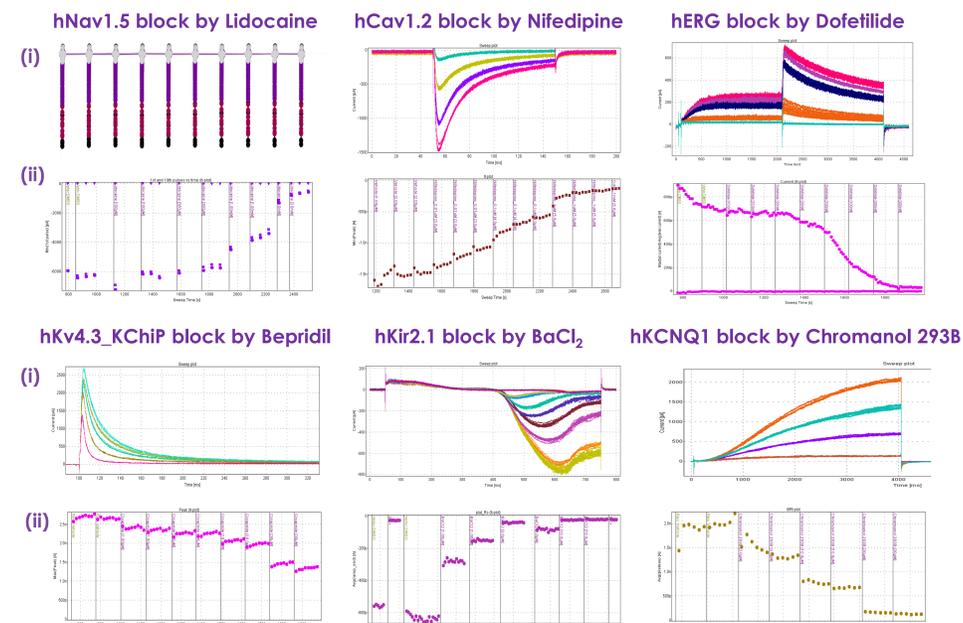*Reliable potency data obtained from Metrion QPatch assays can be applied to in silico models*

hNav1.5 block by Lidocaine   hCav1.2 block by Nifedipine   hERG block by Dofetilide

hKv4.3_KChiP block by Bepridil   hKir2.1 block by BaCl$_2$ hKCNQ1 block by Chromanol 293B

Figure 2: Exemplar GΩ seal quality recordings obtained on QPatch system with cardiac cell lines
(Ai); Gigaseal quality patch clamp current recordings with optimised QPatch assays for a panel of CiPA cardiac cell lines (Aii); Corresponding current vs. time plots showing stable current recordings for the core cardiac CiPA cell lines in control (0.1% DMSO) conditions followed by concentration-dependent inhibition by compounds applied cumulatively as either mini-3pt or full 4-pt IC$_{50}$ testing paradigm. Shown are example compounds from the CiPA working group test set that represent different Torsade de Point risk categories and/or 'in-class' controls.

2. Comparison of *in silico* models that predict human clinical cardiac arrhythmia risk

A. The accuracy of different models in predicting arrhythmogenesis
Potency data from multiple ion channels are required to elucidate full proarrhythmic risk

(i) Comparison of three *in silico* models on the market

Parameter	ApPredict	EasyAP	CardioTox Predictor
# channels	6 (no late Nav)	3 (Nav, Cav, hERG)	3 (Nav, Cav, hERG)
Pacing?	0.05 - 5 Hz	1 Hz	no details
# models	5, incl O'Hara-Rudy	5, not O'Hara-Rudy	1, proprietary
Output/display	AP waveform, EAD	AP waveform, EAD	qualitative risk
	APD (90% only)	APD, upstroke	ΔQTc, TdP +/-
Expertise level	Medium/Expert	Medium/Expert	Novice
Compound class	inhibitors	inhibitors & activators	inhibitors
Compound data	IC$_{50}$	IC$_{50}$ or EC$_{50}$	IC$_{50}$ and EFTPC
Power (time/run)	15 min	30 min	instantaneous
Cost	Free (opensource)	paywall	paywall

(ii) *in vitro* QPatch data for Astemizole

Ion channel	IC$_{50}$ (µM)
hNav1.5	8.0 ± 0.60
*hCav1.2	8.77 ± 1.10
hERG	0.045 ± 0.01
hKv4.3_KChiP	21.3 ± 0.56
hKir2.1	> 30
hKCNQ1_minK	19.9 ± 3.51

(iii) Simulated AP profiles and APD prolongation for Astemizole using ApPredict and EasyAP

ApPredict   EasyAP

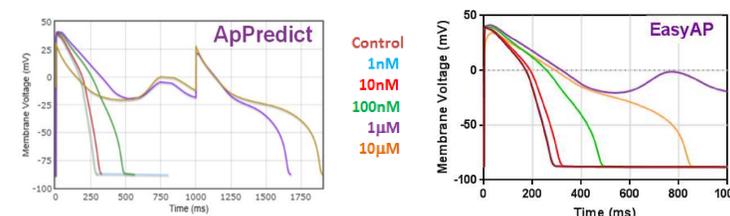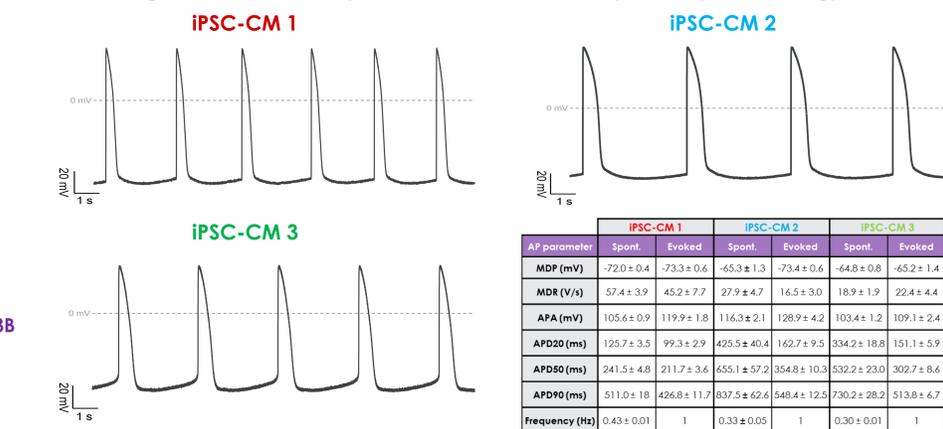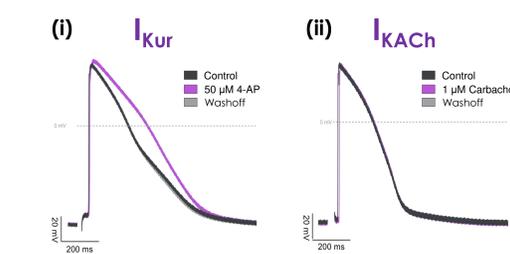Control
1nM
10nM
100nM
1µM
10µM

Figure 3: Performance of APD and torsade de point (TdP) risk simulators using *in vitro* cardiac data
(Ai) Comparison of three *in silico* modelling techniques available on the market, the AP predict using data from 6 channels based on the O'Hara-Rudy method and two models that only use the core panel of Nav1.5, Cav1.2 and hERG, the EasyAP online simulator and the CardioTox predictor a machine learning cardiotoxicity assessment algorithm .
(Aii) Potency data for the CiPA test set compound Astemizole in CiPA QPatch assays (*data from fluorescence assay)
(Aiii) Comparison of AP profiles and simulated APD prolongation by Astemizole showing EAD in ApPredict model (6 channels) but only partial repolarisation with EasyAP model (3 channels)

3. Variable phenotypes from different iPSC-CM vendors
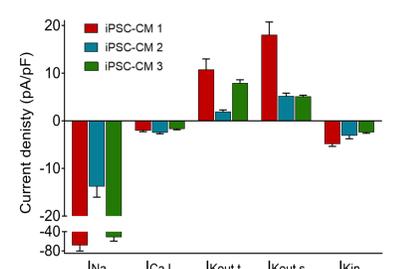
A. Comparison of spontaneous vs evoked AP parameters
Spontaneous APs show varied waveform, firing rate and APD values in different cell-lines
Evoked APs give more consistent parameters suitable for comparative pharmacology

iPSC-CM 1   iPSC-CM 2   iPSC-CM 3

AP parameter	iPSC-CM 1		iPSC-CM 2		iPSC-CM 3	
	Spont.	Evoked	Spont.	Evoked	Spont.	Evoked
MDP (mV)	-72.0 ± 0.4	-73.3 ± 0.6	-65.3 ± 1.3	-73.4 ± 0.6	-64.8 ± 0.8	-65.2 ± 1.4
MDR (V/s)	57.4 ± 3.9	45.2 ± 7.7	27.9 ± 4.7	16.5 ± 3.0	18.9 ± 1.9	22.4 ± 4.4
APA (mV)	105.6 ± 0.9	119.9 ± 1.8	116.3 ± 2.1	128.9 ± 4.2	103.4 ± 1.2	109.1 ± 2.4
APD20 (ms)	125.7 ± 3.5	99.3 ± 2.9	425.5 ± 40.4	142.7 ± 9.5	334.2 ± 18.8	151.1 ± 5.9
APD50 (ms)	241.5 ± 4.8	211.7 ± 3.6	655.1 ± 62.2	354.8 ± 10.3	532.2 ± 23.0	302.7 ± 8.6
APD90 (ms)	511.0 ± 18	426.8 ± 11.7	837.5 ± 62.6	548.4 ± 12.5	730.2 ± 26.2	513.8 ± 6.7
Frequency (Hz)	0.43 ± 0.01	1	0.33 ± 0.05	1	0.30 ± 0.01	1

B. Atrial vs. ventricular phenotype
Evoked AP's are sensitive to 4-AP but not Carbachol

(i) I$_{Kur}$ (ii) I$_{KACh}$

Control
50 µM 4-AP
Washoff

Control
1 µM Carbachol
Washoff

C. Voltage clamp "snapshot"
Variable levels of core cardiac currents

iPSC-CM 1
iPSC-CM 2
iPSC-CM 3

I$_{Na}$ I$_{Ca,L}$ I$_{Kout.t}$ I$_{Kout.s}$ I$_{Kin}$

D. Cardiac ion channel pharmacological sensitivity differs between iPSC-CM
Range of efficacy for 'in-class' reference compounds

I$_{Na}$ Lidocaine (100 µM)   I$_{Ca,L}$ Nifedipine (100 nM)   I$_{Kr}$ Dofetilide (50 nM)
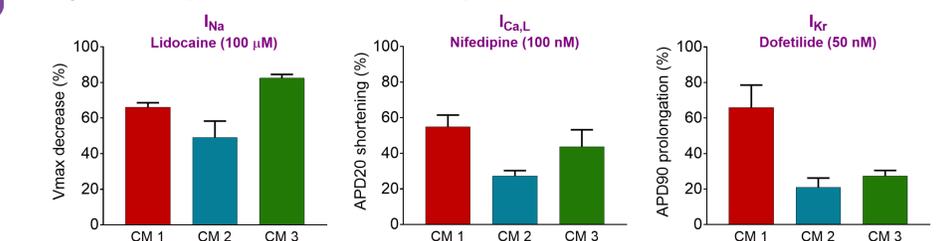
CM 1 CM 2 CM 3

Figure 4: Electrophysiological and pharmacological characterisation of three different commercial iPSC-CM by high quality gigaseal manual patch clamp
(A)Representative spontaneous AP waveforms and a summary table of the AP parameters analysed from both spontaneous and evoked (1Hz) action potentials
(B) Atrial vs. ventricular phenotype was assessed using 4-AP and Carbachol which target atrial-specific I$_{Kur}$ (i) and I$_{KACh}$ (ii) currents, respectively
(C) Comparison of core cardiac current density from conventional whole-cell voltage clamp recordings from single cells
(D) Effect of selective cardiac reference compounds on representative evoked AP parameters (1 Hz, perforated patch clamp)

Conclusions

• **High quality quantitative IC$_{50}$ data from our CiPA panel of human cardiac ion channel assays implemented on the QPatch gigaseal platform enables *in silico* predictions of arrhythmia risk using validated human AP models**

• **Detailed current and voltage clamp profiling reveals varying phenotypes and cardiac pharmacology in commercial iPSC-CM's that are not obvious from simple comparisons of action potential waveform or beat rate**

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