# Developing a Package of In Vitro Human Cardiac Ion Channel Assays using Automated Patch Clamp to Predict Clinical Arrhythmia Risk

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## Introduction

Current cardiac safety testing regimes have successfully prevented new drugs coming to market with unknown proarrhythmic risk. However, they are expensive and time-consuming, and an over-reliance on hERG liability as a marker for proarrhythmia has led to exclusion of useful chemical scaffolds from further drug development. In addition, the focus on hERG ignores the risk posed by potential drug interactions with multiple cardiac ion channels (MICE) that can alter cardiac action potentials[1,2].

Conversely, numerous marketed drugs that are potent hERG inhibitors are not associated with arrhythmia, which has prompted the FDA to implement the Comprehensive *in vitro* Pro Arrhythmia initiative (CiPA)[1,2]. This program aims to increase *in vitro* screening against an expanded panel of human cardiac ion channels using automated electrophysiology (APC) and introduce *in silico* computer modelling of human ventricular action potentials and phenotypic stem cell-derived cardiomyocyte assays to assess the overall proarrhythmic risk of new chemical entities.

CiPA working groups have established a testset of 28 compounds with varying Torsades de point (TdP) risk based on a balance of Redfern, Kramer (Chantest), Mirams, and FDA AERS labels[2]. This testset is designed to validate *in vitro* APC cardiac ion channel assays, as well as stem cell-derived cardiomyocyte phenotypic assays, and *in vitro* screening data from these compounds used to train and validate *in silico* action potential models.

## Summary

We developed a package of high quality human cardiac ion channel automated patch-clamp electrophysiology assays and validated it against a panel of compounds with known high (Bepridil), intermediate (Astemizole, Cisapride and Pimozide) and low (Ranolazine) proarrhythmic risk, as identified by CiPA working groups and literature publications.

Assays developed included **hERG ($I_{Kr}$), hNav1.5 ($I_{Na}$), hCav1.2 ($I_{CaL}$), hKCNQ1-minK ($I_{Ks}$), hKir2.1 ($I_{K1}$), Kv4.3-KChIP2 ($I_{to}$) and hHCN4 ($I_f$).** The *in vitro* potency data for these CiPA compounds was modelled using the web portal tool developed by Williams and Mirams employing the O'Hara-Rudy model of the human ventricular myocyte action potential[3].

The combination of high quality screening data and *in silico* modelling was able to separate compounds according to their known pro-arrhythmic risk.

## Methods & Materials

### Tissue culture

CHO-K1 or HEK-293 stably expressing exogenous human α1 subunits of each cardiac ion channel were grown using standard cell culture conditions. Kv4.3 cell line also expressed KChiP2 accessory subunits and KCNQ1 cell line co-expressed minK subunit. Cells were prepared for assays using proprietary protocols.

### Automated patch-clamp electrophysiology using Sophion QPatch

All cell lines, except hCav1.2, were validated biophysically and pharmacologically 'in house' on QPatch platform (Sophion, Denmark). All recordings were in conventional whole cell configuration using standard single hole chips. For Cav1.2, a fluorescence plate based assay was utilised to asses the potency of compounds against calcium mediated fluorescence signals elicited by a depolarising high K+ stimulus.

*Solutions:* Internal solution contained for potassium channels and HCN4 (in mM): either 20/100, 60/60 or 120/0 ratio of KF to KCl, 10 NaCl, 10 HEPES, 5 or 10 Na₂-ATP, 10 EGTA, 1 MgCl₂; pH 7.2, ~290mOsm. External solution contained (in mM): 140 NaCl, 5, 10 or 30 KCl, 10 Glucose, 10 HEPES, 2 MgCl₂ and 1 CaCl₂; pH 7.4, ~310mOsm. For Nav1.5 KF/KCl was replaced with CsF or CsCl and NaCl concentrations were adjusted accordingly.

*Voltage protocols:* The following voltage protocols were used; **hNav1.5** currents were elicited using a 1Hz, 10-pulse chain voltage protocol from a $V_h$ -100mV to activating step at -20mV. **hERG;** a standard +40/-40mV (2s/2s) voltage protocol from $V_h$ -80mV. **hKir2.1;** a ramp-step protocol from +40 to -120mV from $V_h$ -40mV. **hKv4.3-KChIP2.2;** a 500ms step to +30mV from $V_h$ -80mV. **hKCNQ1-minK;** a 4s activating test pulse to +40mV from a $V_h$ -80mV. **hHCN4;** a step-ramp was used, from $V_h$ -40mV a 2s step to -120mV followed by a 1s ramp to +20mV. Series resistance (4-15MΩ) was compensated by 65-85% and leak subtraction calculated using a P/n protocol.

### Compound screening

Vehicle (0.1% DMSO) was applied to the cells to achieve a stable control recording (4min) and then compound potency was determined from cumulative applications of test compound (5-10µL) typically applied as two bolus additions per concentration to obtain 3pt mini-IC₅₀ or 4pt IC₅₀ (at 0.5 log unit intervals) estimates of compound potency.

*Data analysis:* Peak current amplitude during the activating test step was measured for each sweep by QPatch assay software. The % inhibition was calculated from mean peak current measured for the last three sweeps at the end of each concentration application period relative to that measured at the end of the control period after current stabilisation. Concentration response curves (four parameter logistic curve) were fitted to % inhibition data using Prism (GraphPad) from which IC₅₀ (50% inhibitory concentration) and Hill coefficients were determined (Hll slope constrained to $0.5 > n_h < 2.0$). IC₅₀ data is N ≥ 3.

### In silico modelling

Using the web portal tool developed by Williams and Mirams[3] we employed the O'Hara-Rudy model of the human ventricular myocyte action potential as recommended by ISWG working group of CiPA. Pacing frequency was 1Hz and drug effects were modelled for 5 min.
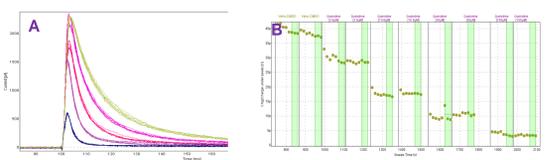
## Results

### 1. Metrion's optimised panel of CiPA-compliant QPatch cardiac ion channel assays
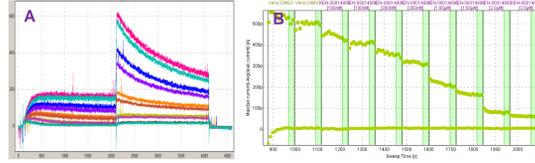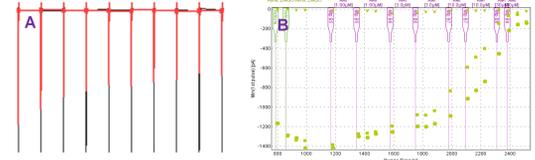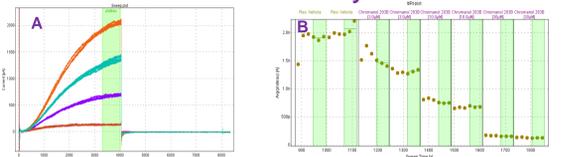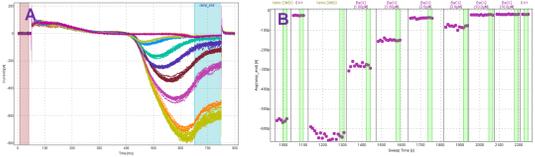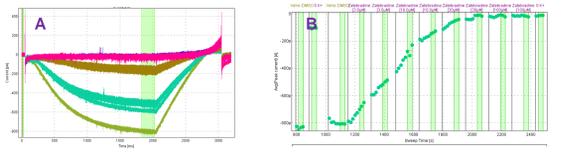


**Figure 1: Example GΩ seal quality electrophysiology recordings obtained from QPatch system for CiPA cardiac cell lines**
**A:** Gigaseal quality patch clamp current recordings obtained with optimised QPatch assays for CiPA cardiac cell lines.
**B:** Stable current recordings over time for each CiPA cell line in control (0.1% DMSO) conditions followed by dose-dependent inhibition by 'in-class' positive control for each channel. Compounds were applied using either a cumulative mini-3pt or full 4-pt IC₅₀ testing paradigm.

### 2. CiPA cardiac ion channel panel – compound test set

We selected 6 compounds from the CiPA working group test set that represent each TdP risk category, including high (Bepridil), intermediate (Astemizole, Cisapride and Pimozide) and low (Verapamil, Ranolazine) proarrhythmic risk. Our potency values showed good agreement with a similar QPatch study by Eisai[4]; there were some discrepancies between Nav1.5 data that are likely due to voltage protocol differences.

| TdP risk category | ETPC_un [uM] | | Metrion hERG | Eisai hERG | Metrion Nav1.5 | Eisai Nav1.5 | Metrion Cav1.2 | Eisai Cav1.2 | Metrion KCNQ1 | Eisai KCNQ1 | Metrion Kir2.1 | Eisai Kir2.1 | Metrion Kv4.3 | Eisai Kv4.3 | Metrion HCN4 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.0003 - 0.003 | Astemizole | 0.045 | 0.028 | 8.0 | 1.9 | 8.77 | 0.99 | 19.9 | > 30 | >30 | >30 | 21.3 | >30 | >30 |
| 2 | 0.007 - 0.05 | Bepridil | 0.370 | 0.13 | 9.7 | 0.64 | 4.75 | 1.46 | 18.5 | 6.03 | 24 | >30 | 14.3 | 4.5 | >30 |
| 1 | 0.003 | Cisapride | 0.044 | 0.015 | 12.2 | 2.07 | >30 | 4.3 | >30 | > 30 | >30 | >30 | 10.3 | >30 | >30 |
| 1 | 0.0003-0.0004 | Pimozide | 0.040 | - | 11.7 | - | 4.13 | - | >30 | - | >30 | >30 | >30 | - | >30 |
| 0 | 0.01 - 0.09 | Verapamil | 0.387 | 0.2 | 64.7 | 4.3 | 1.62 | 0.33 | 27.9 | 29.9 | >30 | >30 | >30 | 3.5 | >30 |
| 0 | 0.4 - 5.3 | Ranolazine | 23.7 | 3.93 | 46.8 | 41.1 | >30 | 118 | >30 | > 30 | >30 | >30 | >30 | 434 | >30 |

**Figure 2: Potencies of 6 CiPA testset compounds against a panel of 7 cardiac ion channel Qpatch assays**
Compounds were tested using the same paradigm as described in Figure 1 for in-class positive controls. IC₅₀ of >30µM indicates that it failed to achieve >40% inhibition at the top concentration tested.

### 3. CiPA cardiac safety testing – *in silico* models to predict arrhythmia risk

As proposed by ISWG working group of CiPA[1] and confirmed by our data (Figure 3a), the O'Hara-Rudy model of the human ventricular myocyte action potentials is most suited to predict the effect of compounds on action potential duration (APD). The QPatch potency data for the test set of 6 compounds (Figure 2) from hERG, hNav1.5, hCav1.2, hKCNQ1, hKir2.1 and hKv4.3 assays was modelled using the web portal tool developed and curated by Williams and Mirams[3].
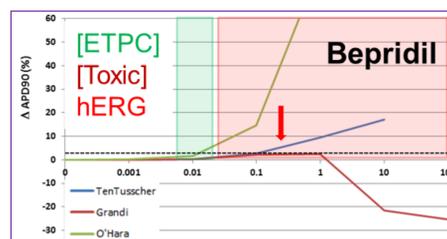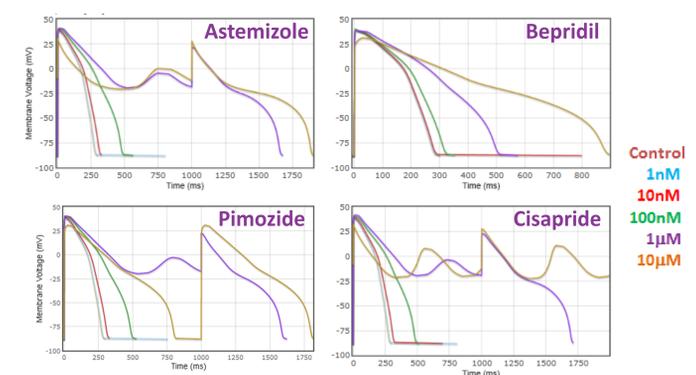


**Figure 3: Results from *in silico* modeling of human ventricular action potential duration using cardiac ion channel potency data from QPatch assays**
**A:** Example of *in silico* prediction for the action of Bepridil on APD₉₀ (%) as determined by three different models, ten-Tusscher, Grandi and O'Hara-Rudy models
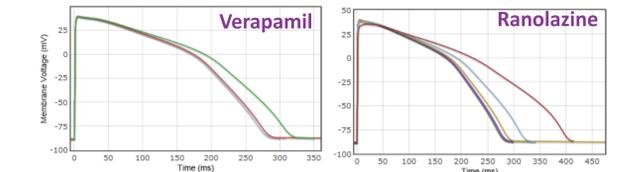**B:** High TdP risk produce arrhythmic AP's with pronounced APD broadening & EAD with O'Hara-Rudy model
**C:** Low TdP risk compounds show less broadening of APD and do not induce EAD in the O'Hara-Rudy model

## Conclusions

• **Automated Patch Clamp assays employing gigaohm seal recordings on the QPatch platform yield high quality data suitable for CiPA human cardiac ion channel panel safety screening**

• **Quantitative cardiac IC₅₀ data from our APC assays enables accurate *in silico* predictions of arrhythmia risk using validated human cardiac action potential models**

• **The remaining challenge is to devise a metric from *in silico* models that can be used to reliably measure and predict TdP and arrhythmia risk from *in vitro* screening data**

## References

[1]Cavero and Holzgrefe (2015) JPET *in press* doi: 10.1016/j.vascn.2015.06.004 [2]Sager P.T (2014) Am Heart J; 167:292-300
[3]Williams and Mirams (2015) JPET *in press* doi:10.1016/j.vascn.2015.05.002 [4]Okada *et al* (2015) Sci. Adv. 1:e1400142i:10.1016/j.vascn.2015.05.002