Validating Cardiac Ion Channel assays on the IonFlux™ System for the CiPA Paradigm

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Abstract
Drug-induced QT interval prolongation and Torsades de Pointes (TdP) arrhythmia are the leading causes for drug withdrawal from the market. For the past decade, in vitro hERG channel assays and in vitro QT-macro assays have been used as surrogates for proarrhythmic risk propensity according to ICH S7B and ICH E14 guidelines. This paradigm, although effective, has suffered from lack of specificity and led to unnecessary compound attrition during drug development. The Comprehensive in vitro Proarrhythmia Assay (CiPA) is a new paradigm paradigm under development with the goal of addressing this limitation and improving the ability to predict a drug’s proarrhythmic liability. This new paradigm includes a panel of in vitro assays that integrates the effects of test compounds on several cardiac ion channels. In this study, HEK-hERG, HEK-hNav1.5 peak and HEK-hNav1.5 late cardiac ionic currents were validated on the microfluidic-based automated IonFlux™ HT patch clamp system, using HESI-sponsored High Throughput Screening (HTS) protocols and assay solutions with a set of 12 compounds from Phase 1 to assess the variability and reproducibility of HTS platforms/sites and for the calibration and validation of the in silico action potential (AP) model. The results demonstrate suitability of the IonFlux™ HT system for high throughput screening of drug effects on cardiac ionic currents, and provide data for in silico reconstructions in the CiPA paradigm for defining proarrhythmic risk.

1. HEK-hERG assay on the IonFlux™ HT

![Figure 1a](image1.png)

**Figure 1a.** CIPA hERG protocol and elicited current

Onset and steady state block of hERG current was measured using a pulse pattern (shown on the left panel), repeated every 5 sec, consisting of a depolarization to 40 mV amplitude for a 500ms duration, followed by a ramp (1.2 V/s) to -80mV for 100ms. The holding potential was -80mV. Peak tail current was measured during the ramp (shown on the right panel). Peak current was measured after applying a saturating concentration of a blocker such as Cavatride (1µM) at the end of each experiment to completely block hERG current.

2. hNav1.5 peak current assay on the IonFlux™ HT

![Figure 2a](image2.png)

**Figure 2a.** CIPA hNav1.5 peak protocol and elicited current

Onset and steady state block of peak Nav1.5 current was measured using a pulse pattern, repeated every 5 sec, consisting of a hyperpolarizing pulse to -120mV for a 200ms duration, depolarization to -15mV amplitude for a 500ms duration, followed by step to -15mV for 200ms and finally a 100ms ramp (1.2 V/s) to a holding potential of -80mV. Peak tail current was measured during the step to -15mV. Peak current was measured after applying a saturating concentration of a blocker such as lidocaine (20µM) at the end of each experiment to completely block hNav1.5 current.

3. hNav1.5 late current assay on the IonFlux™ HT

![Figure 3a](image3.png)

**Figure 3a.** CIPA hNav1.5 late protocol and elicited current

Late protocol was measured using the same voltage pattern as mentioned above for hNav1.5 peak current. All external solutions contained 20 mM ATP to activate late currents. Late currents were measured at their maxima during the ramp.

4. Comparison of IonFlux™ HT IC50 values to manual patch clamp IC50 values

<table>
<thead>
<tr>
<th>Drug</th>
<th>HEK-hERG</th>
<th>CIPA hNav1.5 peak</th>
<th>CIPA hNav1.5 late</th>
<th>Manual patch clamp</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dofetilide</strong></td>
<td>1.25 µM</td>
<td>2.5 µM</td>
<td>0.5 µM</td>
<td>1.25 µM</td>
</tr>
<tr>
<td><strong>Terfenadine</strong></td>
<td>1.25 µM</td>
<td>2.5 µM</td>
<td>0.5 µM</td>
<td>1.25 µM</td>
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<tr>
<td><strong>Méxiletine</strong></td>
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<td>0.5 µM</td>
<td>1.25 µM</td>
</tr>
<tr>
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</tr>
</tbody>
</table>

Conclusion
1. The twelve (12) CiPA Phase 1 compounds were screened against HEK-hERG, hNav1.5 peak and hNav1.5 late current in the IonFlux™ HT platform using HESI-sponsored High Throughput Screening (HTS) protocols and assay solutions. The IC50 values were generated from HEK-hERG, hNav1.5 peak and hNav1.5 late current inhibition and compared to the IC50 values generated for these compounds using manual patch clamp.
2. The IC50 values were generated from HEK-hERG, hNav1.5 peak and hNav1.5 late current inhibition and compared to the IC50 values generated for these compounds using manual patch clamp.
3. IonFlux HT hERG IC50 values for all compounds, other than Dofetilide, correlated well with the hERG IC50 values generated using manual patch clamp.
4. IonFlux HT hNav1.5 peak and late current IC50 values for all compounds, correlated well with the IC50 values generated using manual patch clamp.

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