hERG Safety

Background Information

- The human ether-a-go-go related gene (hERG) encodes the inward rectifying voltage gated potassium channel in the heart ($I_{Kr}$) which is involved in cardiac repolarisation.

- Inhibition of the hERG current causes QT interval prolongation resulting in potentially fatal ventricular tachyarrhythmia called Torsade de Pointes.

- A number of drugs have been withdrawn from late stage clinical trials due to these cardiotoxic effects, therefore it is important to identify inhibitors early in drug discovery.

- The hERG Safety service is performed by our parent company, Evotec, and is a cell-based assay which employs the QPatch HTX System (Sophion Bioscience A/S) or the SyncroPatch 384PE (Nanion Technologies) as automated patch clamp electrophysiology measurements.

- The QPatch HTX and the SyncroPatch 384PE systems deliver high quality, accurate and sensitive data which are comparable with the traditional single cell patch clamp method.

Protocol

- Instrument
  QPatch HTX (Sophion Bioscience A/S) or SyncroPatch 384PE (Nanion Technologies)

- Analysis Method
  Electrophysiology

- Cell Line
  hERG stably transfected HEK293 cells

- Patch Clamp Technique
  Whole cell patch clamp (QPatch)
  Perforated patch clamp (SyncroPatch)

- Test Article Concentration
  Typically, 0.1, 1, 10 µM, applied sequentially to the same cell (different range and number of concentrations available)

- Number of Replicates
  Each test article is usually tested in at least 2 cells

- Quality Controls (QPatch)
  0.1-0.5% DMSO (negative control)
  E-4031 (positive control)
  $R_m > 100$ MOhms
  Pre-compound tail current $> 0.2$ nA

- Quality Controls (SyncroPatch)
  0.25-0.3% DMSO (negative control)
  E-4031 (positive control)
  $R_{seal}$ $\geq 50$ MOhms before compound addition (4-hole chips)
  Peak current $\geq 0.2$ nA before compound addition (4-hole chips)

- Test Article Requirements
  100 µL of 10 mM solution

- Data Delivery
  Mean % inhibition and $IC_{50}$ determination (if appropriate)
To date, electrophysiology remains the 'gold standard' method with which to characterise ion channel properties, as binding, flux and fluorescence assays only indirectly measure ion channel properties.  

The SyncroPatch 384 Patch Engine (PE) automated patch clamp instrument simultaneously performs electrophysiology measurements for multiple single cells in specialised 384 well plates. After initiating the experiment, cell catching, sealing, perforated-cell formation, liquid application, recording, and data acquisition are performed sequentially. Initially, the chip is primed with appropriate extracellular and intracellular solutions. The suspended single cells, stored in a cell hotel reservoir with orbital shaking speed at 200 rpm, are aspirated from the reservoir, pipetted into the planar 384-well patch-clamp chip, and entrapped in the holes of the wells by an automatically applied vacuum. Seal generation, the establishment of the perforated cell mode and also the electrophysiological recordings are controlled by PatchControl 384. Once a stable patch has been achieved, recording commences in voltage-clamp mode.

<table>
<thead>
<tr>
<th></th>
<th>QPatch IC₅₀</th>
<th>SyncroPatch IC₅₀</th>
<th>Literature</th>
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<tbody>
<tr>
<td>E-4031</td>
<td>16-50 nM</td>
<td>10-30 nM</td>
<td>26.3 nM (QPatch)(^5) 17 nM (SyncroPatch)(^6)</td>
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<tr>
<td>Verapamil</td>
<td>0.28 μM</td>
<td>0.63 μM</td>
<td>0.2 μM (QPatch)(^5) 0.59 μM (SyncroPatch)(^6)</td>
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<tr>
<td>Quinidine</td>
<td>1.3-1.7 μM</td>
<td>0.73 μM</td>
<td>0.64 μM (QPatch)(^5) 0.5 μM (SyncroPatch)(^6)</td>
</tr>
<tr>
<td>Astemizole</td>
<td>5-14 nM</td>
<td>41 nM</td>
<td>28.1 nM (QPatch)(^5) 19.8 nM (SyncroPatch)(^6)</td>
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<tr>
<td>Bepridil</td>
<td>0.07-0.36 μM</td>
<td>0.16 μM</td>
<td>0.13 μM (QPatch)(^5) 0.2 μM (Manual Patch Clamp)(^6)</td>
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Data from the Evotec QPatch and SyncroPatch hERG inhibition assay are consistent with literature data as shown in Table 1. This includes the positive control compound E-4031, a known hERG inhibitor.

The hERG inhibition assay forms part of the GPR cardiac ion channel panel for determining cardiac safety. Further cardiac ion channel assays are available as a non-GLP, CiPA-compliant panel from our parent company, Evotec.

References