

## CARDIAC TOXICITY hERG

One of the major reasons for drug withdrawal or drug label revision is the drug induced sudden cardiac death associated with a prolongation of the QT interval in the electrocardiogram (ECG). When the QT interval is prolonged, there is an increased risk of ventricular tachyarrhythmia, including the life threatening form *torsade de pointes*. The QT interval of the ECG is a measure of the duration of ventricular depolarization and repolarization. Although a direct link between QT interval prolongation and arrhythmogenesis is still unclear, QT prolongation is now the subject of increased regulatory review and is considered a significant risk factor for predicting human safety of new chemical entities. Although prolongation of QT can occur through modulation of several types of ion channels, inhibition of the delayed rectifier K<sup>+</sup> current (I<sub>Kr</sub>), which is conducted by human Ether-a-go-go Related Gene (hERG) potassium channel, is the most common mechanism responsible for drug-induced prolongation of QT interval in humans. Therefore, testing the interaction of a compound with the hERG potassium channel in heterologous expression systems is recommended by the International Conference on Harmonisation (ICH)<sup>1</sup> as one of the non-clinical testing methods for assessing the potential of a test compound to prolong QT interval.

Cerep employs two technologies to evaluate the inhibitory effect of test compounds on hERG potassium channel: patch-clamp assay (automated patch-clamp and conventional patch-clamp) and binding assay. Conventional patch-clamp is offered in both non-GLP and GLP format.

### K<sup>+</sup> CHANNEL (hERG) PATCH-CLAMP ASSAY

Electrophysiological assays using whole cell patch-clamp technique are extensively used to study effects of test compounds on the hERG channel. It is a functional assay and provides the highest quality and the most physiologically relevant data. Cerep offers two platforms of patch-clamp assay: automated patch-clamp and conventional (manual) patch-clamp. Mammalian cell lines (HEK-293 or CHO-K1) stably expressing hERG channel gene are used in our patch-clamp assays.

#### ■ CONVENTIONAL PATCH-CLAMP (NON-GLP)

Conventional patch-clamp remains as the gold standard of studying ion channels. The hERG conventional patch-clamp assay provides the most accurate and detailed information of interaction of test compounds with hERG channel. There are some advantages of conventional patch-clamp over automated patch-clamp. Conventional patch-clamp is manually operated by an assay operator. Any abnormality of the test system or the test compound will be notified. The incubation duration of the test compound can be adjusted by the assay operator during the experiment based on its binding kinetic, which assures that the steady-state inhibition is achieved. Therefore, it produces the most accurate data, especially for test compounds with slow blocking rate on hERG channel. Moreover, the test concentrations of dosing solution used in conventional patch-clamp can be verified by dosing solution analysis. It takes into consideration the reduction of the concentration by possible adsorption to plastic or glass surface during perfusion process. The combination of patch-clamp and dosing solution analysis provides the most accurate assessment. However, conventional patch-clamp is technically difficult and offers low throughput. It is suitable for a thorough study of test compounds to determine the IC<sub>50</sub> value and the mechanism of drug-ion channel interaction.

##### ▶ CEREP'S hERG CONVENTIONAL PATCH-CLAMP ASSAY

Cerep offers hERG assay by conventional patch-clamp. The IC<sub>50</sub> values of some hERG channel blockers determined at Cerep by conventional patch-clamp and their potency ranking are presented in table on next page. HEK-293 cells stably expressing hERG channel are used. The test compound can be evaluated from 1 to 5 concentrations (n=2). Customized protocols are available. The amplitude of hERG potassium channel tail currents are recorded under the control and test compound dosing solution at room temperature (22–24° C). The percentage inhibition of the tail current by test compound is reported. The dose-response curve and IC<sub>50</sub> value are generated if 5 concentrations are tested and the inhibition at the highest test concentration is greater than 50%. The reference compound (positive control), E-4031, is tested to ensure the sensitivity of the test system.

#### ■ CONVENTIONAL PATCH-CLAMP (GLP-COMPLIANT)

Good Laboratory Practice (GLP)<sup>2</sup> is a quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported<sup>3</sup>. Evaluation of inhibitory effect of a test article on hERG potassium channel by conventional patch-clamp in compliance with FDA GLP is recommended by ICH for regulatory submission. According to ICH-S7B, the test article concentrations should cover and exceed the anticipated maximal therapeutic plasma concentration. Ascending concentrations should be tested until a concentration-response curve has been characterized or physicochemical effects, such as solubility limitation and cytotoxicity effect on the testing cell, become concentration-limiting.

<sup>1</sup> ICH-S7B: *The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals* (2005).

<sup>2</sup> FDA: *GLP for Nonclinical Laboratory Studies*, Source: 21 CFR Part 58.

<sup>3</sup> OECD: *Principles on Good Laboratory Practice* (revised in 1997).

► CEREP'S GLP-COMPLIANT hERG PATCH-CLAMP ASSAY

This assay is conducted in compliance with the applicable FDA GLP regulations for nonclinical laboratory studies, 21 CFR Part 58. The study is designed based on ICH-S7B recommendation. The test article is evaluated at 5 concentrations in triplicate to determine the IC<sub>50</sub> value. The HEK-293 cell line stably expressing hERG channel is used. Reference compound, E-4031, is tested on the same batch of cells to verify the sensitivity of the cell line.

An optional non-GLP hERG patch-clamp pilot study is recommended for defining the appropriate test concentrations. The certificate of analysis, MSDS (Material Safety Data Sheet) and stability report of the test article are required. The stability study of the test article in dosing solution (hERG extracellular solution<sup>4</sup>) and the dosing solution analysis study, which determines the concentrations used in the hERG patch-clamp study, are conducted by a sponsor specified GLP-complaint analytical laboratory. Cerep can refer such laboratory upon request. The IC<sub>50</sub> value of the test article is calculated if the inhibition at the highest test concentration is greater than 50%. Otherwise, the IC<sub>50</sub> value will be reported as greater than the highest test concentration.

■ AUTOMATED PATCH-CLAMP

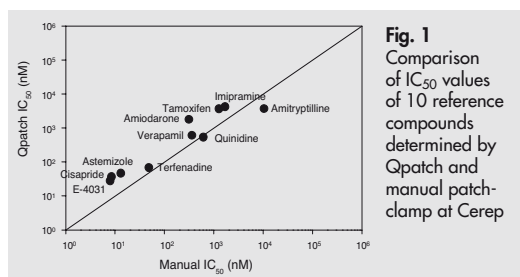
The automated patch-clamp system is based on planar glass-coated silicon chips with micro-etched patch-clamp holes. It forms a true giga-ohm seal patch-clamp, which is the same principle used in conventional patch-clamp. The automated patch-clamp performs multiple independent patch-clamp experiments in parallel in a disposable electrode array. It has been adopted and validated by pharmaceutical industry to screen a variety of ion channels at a higher throughput and lower cost than conventional patch-clamp assays. It provides results comparable with conventional patch-clamp.

► CEREP'S hERG AUTOMATED PATCH-CLAMP ASSAY

Cerep offers the hERG automated patch-clamp assay by using Qpatch 16, an automated patch-clamp system made by Sophion Biosciences (Denmark). CHO-K1 cells stably expressing hERG channel are used. The validation data obtained by Cerep from ten known hERG channel blockers by automated and conventional patch-clamp shows that the automated patch-clamp provides comparable result to the conventional patch-clamp (fig. 1 and table below).

The test compounds can be evaluated at 3 concentrations (0.1, 1 and 10 μM, n=2 by default) or at 5 concentrations for IC<sub>50</sub> determination (0.01, 0.1, 1, 10 and 100 μM, n=2 by default). Customized conditions are available. A reference compound (positive control), E-4031, is tested concurrently with the test compounds to ensure the sensitivity of the test system. The percentage inhibition of hERG tail current by test compound is reported. The dose-response curve and IC<sub>50</sub> value are generated if 5 concentrations are tested and the inhibition at the highest test concentration is greater than 50%. The following criteria were used to qualify the data.

1. Peak tail current >100 pA in control.
2. Initial run-down < 30 % of the initial peak tail current and the run-down stops before first application of the test compound.
3. Leak currents < 50% of the control peak tail current at any time.
4. Rs < 20 MΩ throughout the experiment.



**Fig. 1**  
Comparison of IC<sub>50</sub> values of 10 reference compounds determined by Qpatch and manual patch-clamp at Cerep

Comparison of IC<sub>50</sub> values by Qpatch, manual patch-clamp and potency ▼

Compounds	Qpatch IC <sub>50</sub> (±SE)	Cerep manual IC <sub>50</sub>	Qpatch/manual ratio	Potency
E-4031	25.6 ± 4.3 nM (n=8)	8.4 nM	3.0	high
astemizole	42.7 ± 5.2 nM (n=6)	13.8 nM	3.1	high
cisapride	33.8 ± 7.4 nM (n=9)	9.0 nM	3.8	high
terfenadine	63.4 ± 9.8 nM (n=15)	51 nM	1.2	high
verapamil	0.57 ± 0.05 μM (n=6)	0.38 μM	1.5	high
quinidine	0.49 ± 0.03 μM (n=5)	0.65 μM	0.7	high
amiodarone	1.6 ± 0.4 μM (n=9)	0.33 μM	4.8	high
tamoxifen	3.4 ± 0.2 μM (n=7)	1.3 μM	2.6	moderate
imipramine	3.9 ± 0.3 μM (n=9)	1.8 μM	2.2	moderate
amitriptyline	3.4 ± 0.4 μM (n=8)	11.2 μM	0.3	moderate

■ HOW TO INTERPRET PATCH-CLAMP DATA?

The hERG patch-clamp assay is an accurate measurement of interactions between a test compound and the hERG channel. However hERG patch-clamp assay data alone is not sufficient to evaluate a candidate's potential to prolong QT interval. The best evaluation should include the data from hERG patch-clamp assay; result from an *in vivo* assay that measures QT interval; and the assessment from a ventricular repolarization assay that measures action potential parameters.

Cerep adopts a general potency ranking system<sup>5</sup> to indicate the potency of the test compound inhibiting hERG channel: low, IC<sub>50</sub> > 10 μM; moderate, 1 μM ≤ IC<sub>50</sub> ≤ 10 μM; high, IC<sub>50</sub> < 1 μM. Of note here, to assess the potential of QT prolongation, the effective therapeutic plasma concentration of the test compound should be considered. An extensive review<sup>6</sup> of the relationships between hERG IC<sub>50</sub> and clinical QT interval prolongation showed that if a drug's hERG IC<sub>50</sub> is 30-fold higher than the therapeutic plasma concentration, the drug is unlikely to be associated with QT prolongation. Therefore, a 30-fold difference between C<sub>max</sub> and hERG IC<sub>50</sub> might be considered as a desirable safety margin.

<sup>4</sup> hERG extracellular solution composition (in mM): 1.37 NaCl, 4 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 D(+)-Glucose, 10 HEPES [pH adjusted to 7.4 with 1 N NaOH].

<sup>5</sup> Roche, et al. (2002), ChemBioChem, 3: 455-459.

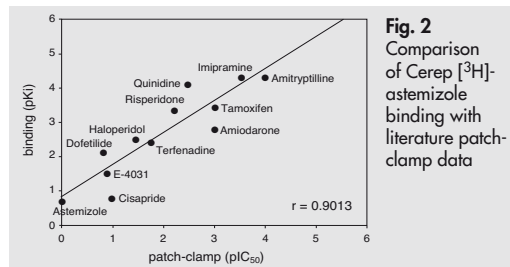
<sup>6</sup> Redfern, et al., (2003), Cardiovascular Research, 58: 32-45.

## K<sup>+</sup> CHANNEL (hERG) BINDING ASSAY

The hERG binding assay uses a radioligand to evaluate affinity of test compound for hERG channel. In this assay test compounds are studied for their ability to displace the radiolabeled ligand from the hERG channel. This assay is high throughput and low cost. It is suitable for primary screening to identify compounds that have a high affinity for the hERG channel. However, the binding assay has inherent limitations. It is not a functional assay. Therefore, it provides no information on agonistic or antagonistic effects of a test compound on the hERG channel. It cannot identify test compounds as positive when they only bind to a certain state (e.g. open or inactivated) of the channel. Moreover, this assay will not identify test compounds that bind to hERG channels at sites other than the radioligand binding sites. Therefore, the hERG binding assay may provide false negative results. But the high throughput and low cost of the binding assay enables it to screen large amount of compounds to identify compounds that have high hERG channel affinity. It is recommended that test compounds that do not show activity in binding assay should undergo the functional testing, either automated or conventional patch-clamp, to confirm the result.

### ► CEREP'S hERG BINDING ASSAY

Cerep's hERG binding assay uses [<sup>3</sup>H]astemizole radioligand. The comparison of [<sup>3</sup>H]astemizole binding at Cerep with literature patch-clamp data is presented in fig 2. The cell membrane homogenate prepared from HEK-293 cells stably transfected with the hERG gene are used. The preparation is incubated with [<sup>3</sup>H]astemizole in the absence or presence of a test compound in a testing buffer. Nonspecific binding is determined in the presence of astemizole. Following incubation, samples are filtered rapidly under vacuum through glass fiber filters presoaked with 0.3% PEI and rinsed several times with ice-cold tris-HCl. The filters are dried and then counted for radioactivity in a scintillation counter using a scintillation cocktail. The reference compound astemizole is tested to validate each experiment. The results are expressed as a percent inhibition of the specific control radioligand.



**Fig. 2**  
Comparison  
of Cerep [<sup>3</sup>H]-  
astemizole  
binding with  
literature patch-  
clamp data

■ **QUESTIONS OR CONCERNS?**  
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