IN VITRO ELECTROPHYSIOLOGY SAFETY STUDIES (hERG)

ICH S7B guideline regulates the in vitro IKr assay in combination with in vivo QT evaluation prior to first-in-human administration. This guideline has been recommended for adoption by the regulatory bodies of 3 regions (Japan, USA and EU). To support compound development through cardiovascular safety assessment, Charles River Preclinical Services, Edinburgh (PCS-EDI) has developed and operates an in vitro electrophysiology capability in accordance with the ICH S7B guideline so that we can continue to offer and support our clients with a full package of safety pharmacology studies.

ICH S7B Guideline - A Short History

In the 1990s, several drugs were withdrawn from the market due to adverse event reports regarding cardiotoxicity (torsades de pointes (TdP) and sudden cardiac death). The FDA recommended the removal of Seldane™ in 1997, and sales were discontinued (1998).

Since then, sudden cardiac death due to non-cardiac drugs became a major safety issue for the pharmaceutical industry and the regulatory agencies.

Accompanying the ICH S7A non-clinical safety pharmacology guideline, a draft of the S7B guideline focused on cardiotoxicity has been in discussion since 2001. The S7B guideline recommends a general non-clinical testing strategy for determining the propensity of non-cardiovascular drugs to delay ventricular repolarisation, an effect that at times progresses into life-threatening ventricular arrhythmia.
The S7B guideline was recommended for adoption in 2005. The relationship between QT prolongation and TdP is not obvious, and the mechanism linking QT prolongation to TdP is not completely understood. Thus, conduction of S7B non-clinical studies assessing the risk for delayed ventricular repolarisation and QT interval prolongation prior to first-in-human administration should be considered.

The Figure 1 excerpt from the ICH S7B guideline outlines a pair of assays. One is “in vitro IKr assay”, and the other is “in vivo QT assay”. “In vitro IKr assay” represents the evaluation of the effects on the ionic current through a native or expressed potassium (IKr) channel, such as that encoded by the hERG gene. “In vivo QT assay” represents the measurement of indices of ventricular repolarisation such as QT interval. Follow-up studies are intended to provide greater depth of understanding or additional knowledge regarding the potential of the test substance for delayed ventricular repolarisation and QT interval prolongation in humans.

Cardiotoxicity Assessment

1: QT prolongation (in vivo) and ionic current (in vitro)
TdP is linked to a prolongation of the QT interval (LongQT). The prolongation of the QT interval is linked to an increase in the cardiac action potential duration (APD) as a result of delayed repolarisation.

A cardiac action potential is composed of different phases. The first upstroke (depolarisation) phase is the product of Na⁺ influx through the Na⁺ channel. A cell with a well developed outward current shows a transient repolarisation (notch), followed by a plateau phase. This plateau phase is the result of a balance of inward current (mostly Ca²⁺ influx) and outward current (K⁺ efflux). Following the plateau phase, the action potential enters a repolarisation phase towards the resting potential. This repolarisation phase is the result of K⁺ efflux.

Figure 2 demonstrates the link between reduced efflux through the IKr channel and the prolongation of QT interval. It is clear that an inhibition of the efflux (or, a decrease of K⁺ outward movement by a blocking action of drug active on K⁺ channels) can cause a prolongation of APD, resulting in prolonged QT (LQT).
2: Non-clinical testing strategy on cardiotoxicity assessment

Figure 1 (ICH S7B guideline) represents the combination of QT evaluation (ECG) and IKr evaluation (hERG assay).

(a) In vivo QT assay

If QT prolongation always precedes TdP, ECG evaluation is the ideal method to assess cardiotoxicity safety issues. However, in practice:

- Frequency of TdP is usually too low to be detected reliably in preclinical and/or clinical trials
- QT interval varies with heart rate
- Drug induced prolongation of QT interval can be within normal daily variability

Therefore, QT measurement alone is not considered sufficient for cardiotoxicity assessment.

(b) In vitro hERG assay

Research data have shown that the most common mechanism of QT interval prolongation by test compounds is the inhibition of the potassium channel that is responsible for IKr (rapid delayed rectifier). It is suggested that hERG (human ether-a-go-go related gene) encodes for the molecular subunit of the K+ channel for IKr rectifier K+ channel, therefore hERG is important as an established target for non-cardiac drugs carrying the TdP/LQT liability.

However, in some cases, potent block of the hERG channel does not necessarily predict QT prolongation or TdP.

- Some potent hERG channel blockers have no QT prolongation effect probably due to mixed ion channel effects (i.e. verapamil, flunarizine)
- Non-linear relationship between drug concentration and APD prolongation (i.e. cisapride)
- Some weak hERG channel blockers induce QT prolongation (i.e. sotalol)
- Potential for false positive or false negative results

Therefore, for some compounds, the hERG assay in isolation may not be wholly predictive of QT prolongation or TdP liability in vivo.

(c) In vivo and in vitro assays

Considering both the relevance and the limitations in both assays, the complementary package of in vivo QT assay and in vitro IKr (hERG) assay is recommended by S7B guideline for assessment of the safety profile prior to the first-in-human trial.

>In vitro hERG assay technique

The voltage clamp technique, using the whole-cell patch configuration, is most commonly applied for the hERG assay. In general, the assay is carried out with hERG transfected cell lines (normally CHO or HEK cells) and the hERG tail current, activated by a suitable voltage step protocol, is recorded for the evaluation.

As described by Sanguinetti (2006), the hERG channel exists in either a closed, open, or inactivated state, depending on the transmembrane voltage. At a negative potential of -80 mV, the hERG channels are closed. A depolarisation slowly opens the channels, which then inactivate rapidly (this gives the inwardly rectifying property). Repolarisation reverses the transitions between the channel’s states (Sanguinetti et al. (2006) Nature 440:463-469).

*Erwin Neher and Bert Sakmann developed the patch clamp in the late 1970s and early 1980s. They received the Nobel Prize in Physiology or Medicine in 1991 for this work.*
The voltage step protocol commonly applied to the hERG assay is based on this property. At -80 mV (resting potential), hERG channels are closed. A voltage step at -40 mV or less activates the channel and an outward current is recorded. A voltage step positive to 0 mV decreases the outward current due to a rapid inactivation of the channel. Returning the voltage step to -50 mV then rapidly activates the hERG channel, resulting in an evident tail current which slowly inactivates and can be measured.

![Graph showing voltage step protocol](image)

Many different voltage command protocols (VCP) exist for evaluating hERG channel block and can take the form of a square pulse or introduce a ‘ramp’ step during the repolarisation phase (Figure 4). Protocols may vary slightly in the potentials that they use and in the duration that they are applied. All are designed for optimal current flow and measurement of compound effect on the tail current.

![Graph showing examples of voltage command protocols](image)

**Typical experimental procedures**

- **Single concentration assay**: The inhibitory effect of a test compound on the hERG K⁺ current is measured. The appropriate concentration for the study is selected based on IC₅₀ or EC₅₀ for the primary target or a nominal concentration, usually 10 or 100 μM. Additional concentrations can be considered as a follow up to elucidate possible effects.

- **Multi-point concentration response assay**: The inhibitory effect of a test compound on the hERG K⁺ current at multiple concentrations is measured (at least 6 concentrations; observation number ≥ 4). The concentrations are selected based on maximum solubility or data from previous assays or studies. The concentration-response relationship is obtained, and an IC₅₀ value estimated.

For additional information, please visit The Source℠, a secure portal that provides registered users with direct access to the technical, scientific and educational resources available from Charles River. To register, please visit [www.criver.com/thesource](http://www.criver.com/thesource)