

# hERG safety screening assay using IonWorks™ HT

IONWORKS HT APPLICATION NOTE #2



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## INTRODUCTION

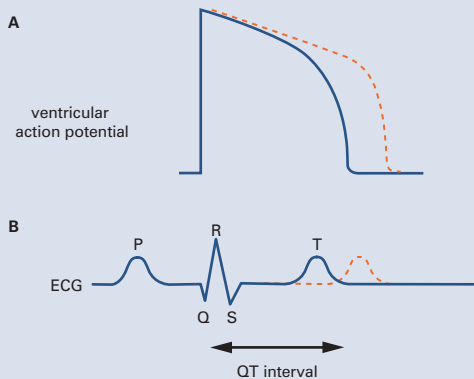
Over the past decade a number of non-cardiovascular drugs have either been withdrawn from the market, or have had severe restrictions placed on their clinical use, due to their propensity to prolong the QT interval of the cardiac electrocardiogram (ECG) and subsequently cause a potentially fatal ventricular tachyarrhythmia called *Torsade de Pointes* (TdP).<sup>1</sup> As a result, the International Committee on Harmonization (ICH), comprised of representatives from the pharmaceutical industry and regulatory authorities, have provided guidelines for suitable pre-clinical methods for

determining the potential of drugs to prolong the QT interval (ICHS7B). The suggested methods should enable pharmaceutical companies to improve their assessment of cardiac risk and minimize the chances of drugs entering the marketplace with these undesirable side effects. This application note describes the use of the IonWorks HT system to test the effects of compounds on the human *ether-à-go-go-related gene* (hERG) potassium channel.

## Long QT syndrome

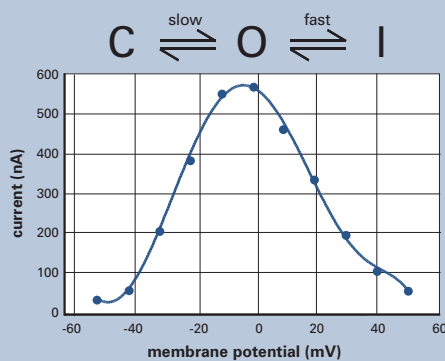
The QT interval of the ECG is measured from the start of the QRS complex to the end of the T wave and represents the time taken for full depolarization and repolarization of the ventricles.<sup>1-3</sup> More specifically, this interval has been shown to be directly correlated to the duration of the cardiac ventricular action potential. (See Figure 1.) Under normal circumstances in healthy individuals, the QT

cardiac action potential (figure 1)



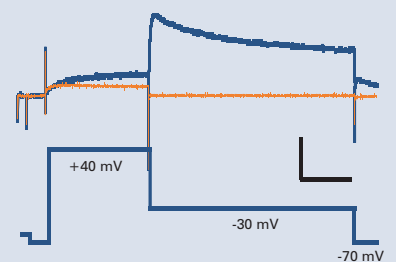
A: Schematic showing action potential waveform in ventricular muscle cells and relationship to surface electrocardiogram (ECG). B: Prolongation of the QT interval is due to an increased plateau phase of cardiac action potential (A, orange dotted line), the underlying electrophysiological basis of the clinical condition known as Long QT Syndrome. (Adapted from reference 3.)

hERG I/V relation (figure 2a)



hERG channels have unusual gating properties. Simplified hERG gating scheme consisting of closed (C), open (O) and inactivated (I) states. Kinetics of channel opening is slow whereas the kinetics of inactivation is fast. Idealized current-voltage curve showing I/V relation of hERG channels. (Adapted from reference 2.)

hERG currents on IonWorks HT (figure 2b)



hERG potassium channel currents measured with the IonWorks HT system. Pre-compound trace shown in blue, blockade with 10  $\mu$ M dofetilide shown in orange. Note the large "tail currents" at a simulated repolarization holding potential of -30 mV. Vertical bar is 500 pA, horizontal bar is 500 ms. Command voltage protocol shown below traces.

interval varies with heart rate; the faster the rate, the shorter the interval. Therefore correction factors are applied to allow for this phenomenon giving a corrected QT interval (QTc).<sup>3</sup> However, even when the QT interval has been corrected for heart rate, a number of individuals have QTc intervals that are prolonged, typically > 450 ms in males and > 470 ms in females.<sup>3</sup> These individuals are defined as having Long QT Syndrome (LQTS) and may be at risk of subsequently developing TdP.<sup>1-5</sup> One of the principle causes of LQTS involves reduced outward potassium flux during the repolarizing phase of the ventricular action potential, a process principally controlled by hERG.<sup>6</sup> Thus, it may come as no surprise to find that many patients with inherited LQTS have defects in the expression/functionality of hERG and that drug-induced LQTS invariably results from hERG channel blockade.

**hERG channel properties**

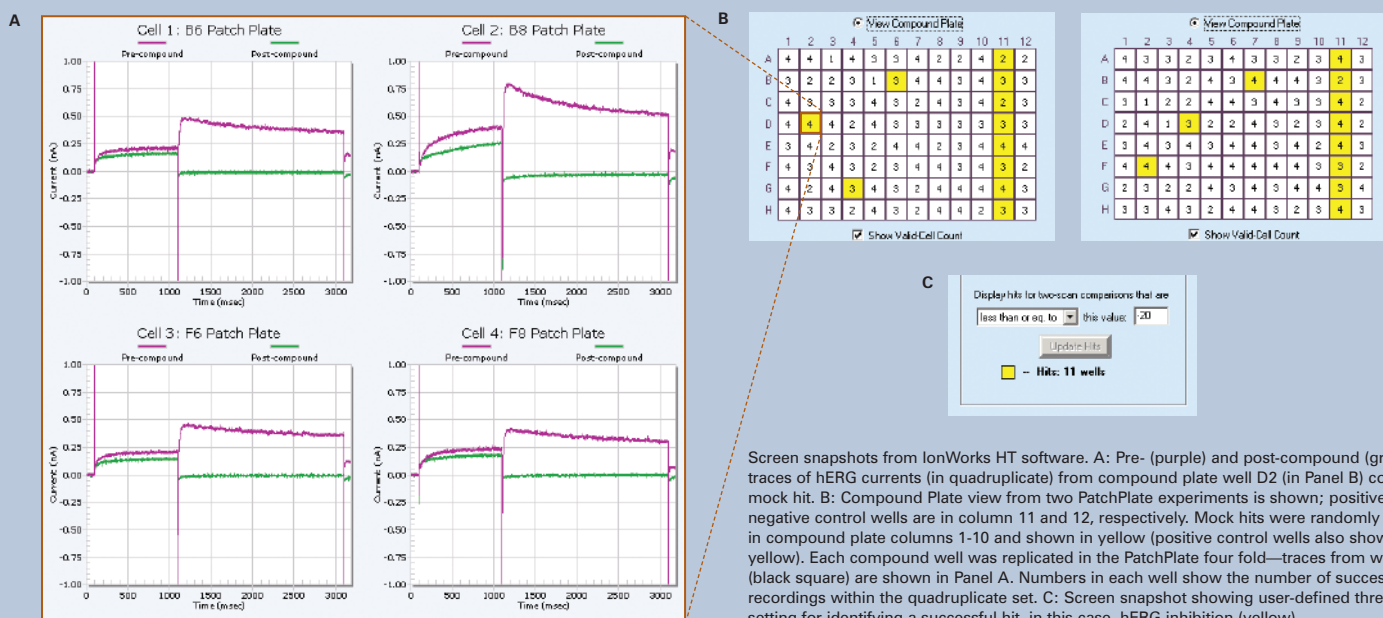
The hERG potassium channel has voltage-dependent gating properties that make it ideally suited to its principal role in controlling the repolarization phase, and therefore the duration, of the cardiac action potential. These include significant, fast inactivation coupled with slower activation when the voltage is rapidly stepped from a negative potential (-80 mV) to more depolarized potentials (+40 mV); e.g. during the upstroke of the action potential. The consequence is that little hERG current flows during the plateau (depolarizing) phase of the action potential. However as the membrane begins to hyperpolarize, hERG channels recover from inactivation, going through the open state prior to closure. This results in a transient increase in the outward flow of potassium ions, causing repolarization of the membrane and termination of the cardiac action potential.

Some of these characteristics are illustrated in Figure 2a, which shows the current-voltage relationship of hERG, recorded using the whole-cell voltage clamp technique. This plot shows that hERG channels are predominantly closed at potentials negative to -50 mV and only begin to open at potentials greater than -40 mV. Further incremental depolarization up to 0 mV shows further increases in macroscopic current since, at these voltages, hERG channels are predominantly in the open state. At depolarizing voltages above 0 mV, the amplitude of the current becomes progressively smaller due to greater number of channels entering the inactivated state. hERG currents recorded on the IonWorks HT system are shown in Figure 2b.

**MATERIALS**

→ Cells: Chinese hamster ovary (CHO) cells expressing hERG

mock hERG safety screening experiment using the IonWorks HT system (figure 3)



- Reagents and buffers: Amphotericin (Sigma Cat. # A-4888), DMSO (Sigma Cat. # D-2650) were used; Internal buffer: High K<sup>+</sup>, Low Cl<sup>-</sup> internal buffer containing (in mM): 100 K-Gluconate (Sigma Cat. # G-4500, 40 KCl (Sigma Cat. # P-9333), 3.2 MgCl<sub>2</sub> (Sigma Cat. # M-2670), 5.0 EGTA (Sigma Cat. E-0396), 5.0 Hepes (Sigma Cat. # H-7523), pH 7.25 with KOH; External buffer: Phosphate Buffered Saline (PBS, Gibco Cat. # 14040)
- Tissue culture flasks: Cells were grown in T-175 flasks (Corning Cat. # 431080)
- Cell culture media: Dulbecco's Modified Eagle Medium containing L-glutamine, glucose, pyroxidine HCl, without Na pyruvate (Gibco Cat. # 11965-092) supplemented with the following: 50 ml Fetal Bovine Serum (FBS, Irvine Cat. # 3000), 5 ml Penicillin/Streptomycin (Irvine Cat. # 9366) and 5 ml Geneticin (G418, Gibco Cat. # 10131) used to grow cells; Versene™ (Gibco Cat. # 15050) used to remove the cells from the flasks
- PatchPlate™ consumables (Molecular Devices Cat. # 9000-0688)
- Compound plates: Costar® 96-well plate (Cat. # 3355)

## METHODS

### *hERG channel inhibitor screen*

A mock hERG screening experiment was performed on the IonWorks HT system by randomly including hERG channel inhibitors (dofetilide, E-4031, terfenadine) among mock “unknowns.” Six compound plates were used, each containing eight positive control wells (10 μM cisapride), eight negative control wells (buffer), three wells per plate containing mock hits (i.e., hERG inhibitors, see Figure 3, Panel B)

and seventy-seven wells containing buffer. For each PatchPlate experiment, every compound was replicated fourfold. A total of six PatchPlate experiments were conducted on a single day (~5-6 hours), representing a 480-compound screen. The results of two compound plates are shown in Figure 3, Panel B, where positive controls and mock hits are highlighted in yellow. The IonWorks HT software was configured to highlight wells that had post-compound hERG currents reduced by 20% or more. (See Figure 3, Panel C.)

### *Statistical analysis*

Z-Factor statistical analysis of the mock hERG inhibitor screen is summarized in Figure 4. Data was expressed as a percent of the pre-compound current at -30 mV test potential used to elicit hERG tail currents.

Z-factor was calculated as:

$$Z = 1 - \frac{[3(\text{SD}_{\text{sample}}) + 3(\text{SD}_{\text{control}})]}{|\text{mean of sample} - \text{mean of control}|}$$

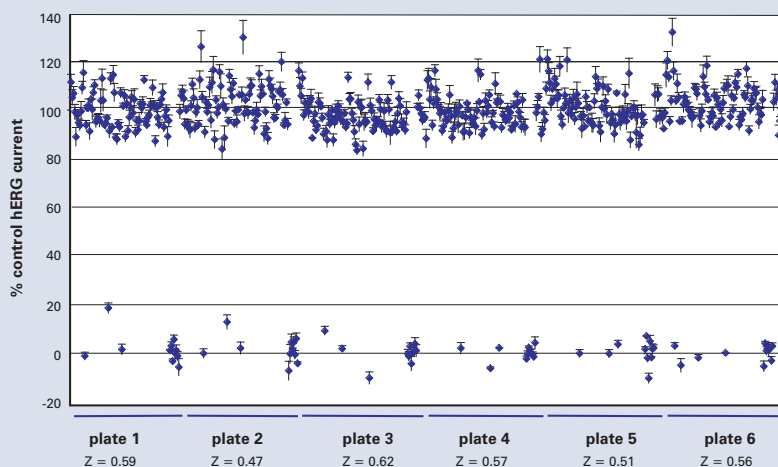
## CONCLUSIONS

The IonWorks HT system is optimal for performing safety screens of candidate pharmaceuticals, and indeed has been successfully used in a pharmaceutical setting for hERG studies.<sup>7</sup> IonWorks HT has daily throughput levels up to 3,000 successful recordings, or 100 eight-point IC curves at n=4; and can be operated by technician-level personnel. For these reasons, the IonWorks HT system is a profound improvement over conventional electrophysiological methods for screening hERG channel blockers.

## INTERNATIONAL CONFERENCE ON HARMONISATION (ICH)

For up-to-date information on Safety Pharmacology Studies For Assessing the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals (S7B), visit: <http://www.ich.org>.

**Z-factor statistical analysis of mock safety screening experiment** (figure 4)



Mean (±SD) values for mock unknowns were plotted as percent of mean control currents (n=4 per compound) and Z-factor values were determined for each PatchPlate. Positive controls and mock hits were readily identified. Z' factors for each plate are shown at bottom.

## REFERENCES

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