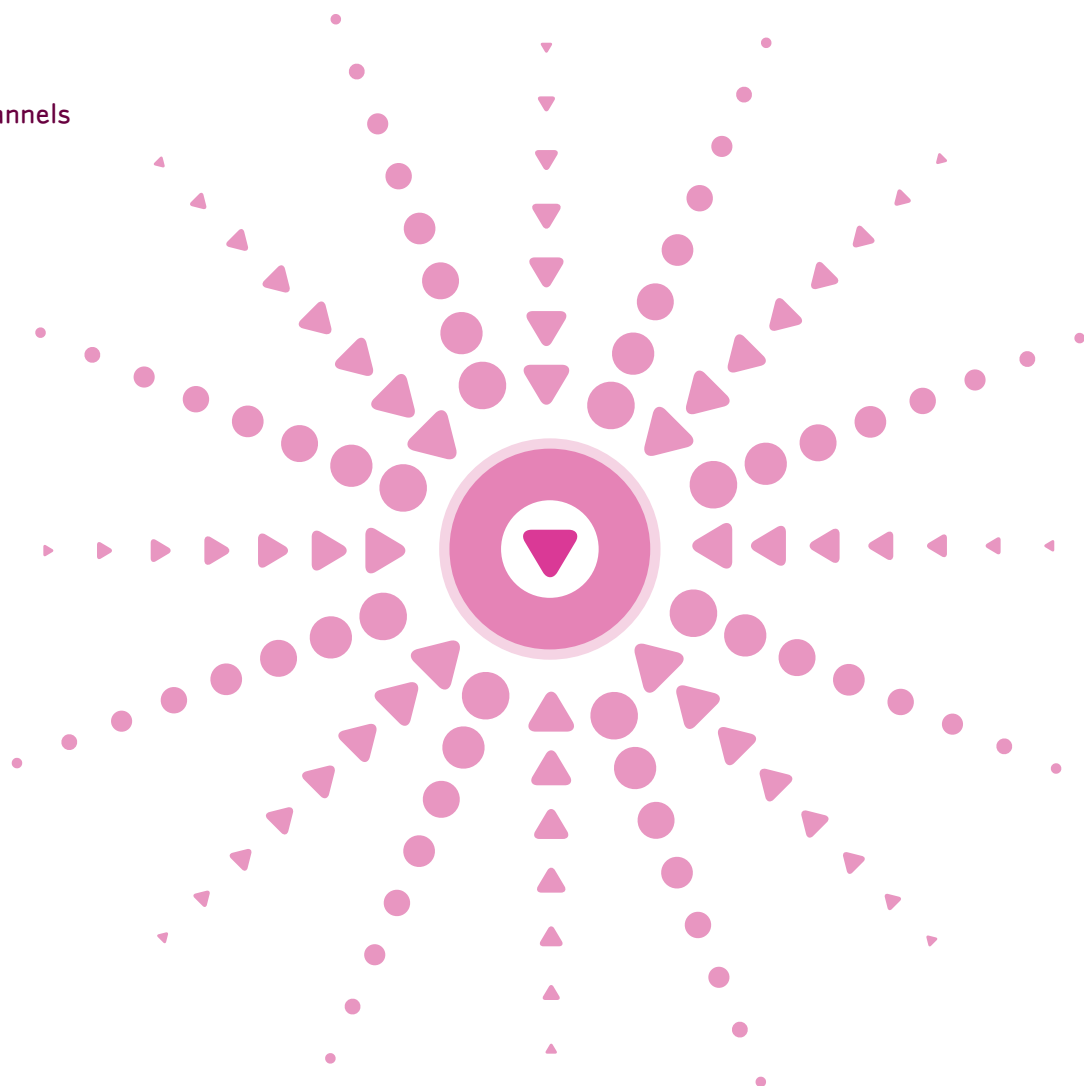




Beyond hERG:

The Role of Ion Channel Profiling in Cardiac Safety Assessment

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INTRODUCTION

Safety assessment has long been a critical step during drug development. Yet, cardiac safety issues have received heightened emphasis recently due to a growing awareness that non-cardiovascular drugs can carry a potential risk of rare but life-threatening arrhythmias. This has resulted in relabeling or withdrawal of major drugs, such as terfenadine,

Cardiac safety issues have received heightened emphasis recently due to a growing awareness that non-cardiovascular drugs can carry a potential risk of rare but life-threatening arrhythmias; in particular, torsades de pointes (TdP).

astemizole, grepafloxin and cisapride. The risk arises from the discovery that drugs spanning a range of therapeutic indications, molecular targets and structural classes can induce a particular form of ventricular tachyarrhythmia known as torsades de pointes (TdP). This is characterized in the electrocardiogram (ECG) by a lengthened QT interval followed by a "twisting" of the waveform around the isoelectric line (Figure 1).

Drug-induced TdP can revert spontaneously without serious symptoms, but it can also cause syncope or degenerate into ventricular fibrillation and cause sudden death. This

phenomenon had previously been recognized in a very rare inherited condition, known as long QT syndrome (LQTS), which is caused by mutations in any of a number of different genes that predominantly encode ion channels (Table 1).

At a cellular level, the prolonged QT interval results from lengthening of the action potential in ventricular myocytes, which at a molecular level (as indicated by LQT mutations), can be caused by functional modulation of a number of the ion channels that mediate this. To date, the vast majority of clinical cases of drug-induced (or acquired) LQTS have been found to be due to blockade of the hERG channel (human ether-a-go-go-related gene). This underlies the rapid delayed rectifier potassium current (I_{Kr}) that has a major role in terminating the plateau phase of the action potential (Figure 4, phase 2). Although the events leading to TdP and sudden death have been established, the mechanistic links between these processes are not well understood. As more and more examples of drug-induced TdP are uncovered, it is apparent that the correlations of hERG blockade with QT prolongation and TdP are imperfect, and other risk factors are important. Thus, the development and interpretation of definitive assays for predicting pro-arrhythmic risk remain problematic.

Table 1. Ion Channel Genes Associated with Inherited Long QT Syndrome

Gene	Channel	Frequency
LQT1	KCNQ1	30-35%
LQT2	hERG	25-30%
LQT3	NaV1.5	5-10%
LQT5	minK	1%
LQT6	MIRP1	Rare
LQT7	Kir2.1	Rare
LQT8	CaV1.2	Rare
LQT10	NaV1.5	Rare

THE DEBATE OVER REGULATORY GUIDELINES FOR "FRONTLINE" SCREENING

Acquired LQTS has been identified as a major concern to the pharmaceutical industry worldwide and associated regulatory agencies. The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) has set out several documents containing guidelines for assessing this risk. The ICH S74A document provides guidelines for a core battery of *in vivo* safety pharmacology studies including cardiovascular measurements, such as blood pressure, heart rate and ECGs. The issue of QT prolongation is specifically addressed by documents ICH 74B and E14, which provide guidelines for non-clinical and clinical evaluation, respectively.

ICH 74B focuses on the non-clinical testing requirements needed to make an "integrated" assessment of risk and on the knowledge base necessary for this. Electrical activity in the heart and the ion channels underlying this are recognized as key factors, though only *in vitro* hERG assays and *in vivo* QT prolongation studies in animal models are emphasized for "frontline" screening.

However, the predictive value of these studies for human risk is increasingly being debated. Numerous

examples of "false positives" have been documented, where hERG block is not associated with QT prolongation and/or where the latter is not correlated with TdP, e.g. verapamil (Zang et al., 1999), clomiphen (Yuill et al., 2004), clemastine (Ridley et al., 2006), fluoxetine (Pacher et al., 2000), citalopram (Witchell et al., 2002), moxifloxacin (Lu et al., 2007) and ranolazine (Antzelevitch et al., 2004). Examples of "false negatives," e.g. where QT prolongation is associated with only weak or no blockade of hERG, are also being reported, e.g. alfuzosin (Lacerda et al., 2008) and chloroquine (Rodriguez-Menchaca, et al., 2008).

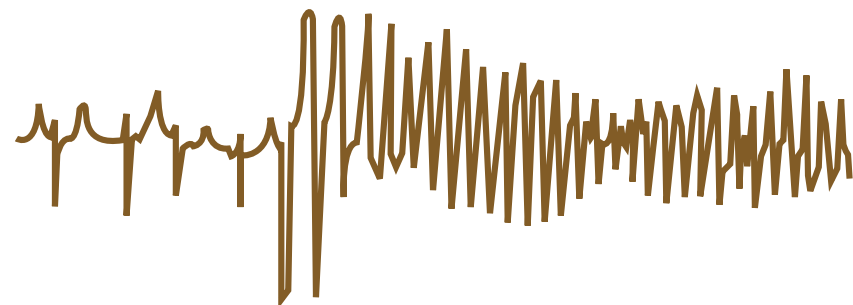
Follow-up studies are recommended where discrepancies do occur between hERG and non-clinical QT prolongation assays. Though no specific guidance is given by ICH S7B, several other *in vitro* and *ex vivo* assays are in use, e.g. action potential duration (APD) measurements using dog purkinje fibres (Carlsson et al. 1997), rabbit ventricular wedge (Yan et al., 2001) and Langendorff perfused heart (Hondeghe et al., 2003). APD studies offer a stepping stone between reductionist hERG assays and the complex integrated responses measured by *in vivo* QT studies but, like the latter, they are subject to discrepancies arising from species-related differences in pharmacology and physiology (Gintant et al. 2001). Clearly, in addition to this, they do not take into account pharmacokinetic

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factors. It is worth noting that in a recent study comparing four different torsadogenic antibiotics, the purkinje fibre and perfused heart assays were less successful than *in vitro* hERG data in ranking the relative risk of TdP, which was only marginally less predictive than the ventricular wedge (Lu et al., 2007).

On the other hand, clinical evaluation of torsadogenic risk using thorough QT/QTc studies (where QTc refers to the QT interval corrected for changes in heart rate) as advocated by document E14 is also problematic. The "normal" QT interval for humans is dependent on gender (typically around 440 msec for males and 460 msec for females) and is subject to significant variation, for example, according to heart rate, physical activity and diurnal rhythm. Against this natural variability, the

Figure 1. Electrocardiogram from a Patient with Torsades de Pointes



extent of QT prolongation that is torsadogenic can be as little as 10 msec or even less. This presents major challenges for reliable detection of drug-induced QT effects and dictates that clinical studies require large numbers of subjects which are very expensive. While different formulas have been advocated for correction of heart-rate dependent variation of QT interval, these are not definitive and the choice of correction method can have a bearing on the outcome of the study.

Finally, as with non-clinical studies, the predictive value of QT interval as a marker for TdP is also debatable. A pragmatic view of safety testing for torsadogenic risk is that a range of *in vitro* and *in vivo* data, the latter both non-clinical and clinical, needs to be gathered and considered in parallel to derive a truly integrated assessment of risk.

AUTOMATED ELECTROPHYSIOLOGY PROVIDES THE OPPORTUNITY FOR WIDER, EARLIER ION CHANNEL SCREENING

Screening for drug safety has traditionally been employed at a relatively late phase of drug development,

Recent advances in automation and information technology have provided the opportunity for translating key clinical liabilities into fast and cost-effective *in vitro* assays that are amenable to much wider screening at earlier stages of the drug discovery process.

typically in the advanced stages of lead optimization. Liabilities uncovered at this stage can easily clog development pipelines and cause high attrition rates with escalated costs.

However, recent advances in automation and information technology have provided the opportunity for translating key clinical liabilities into fast and cost-effective *in vitro* assays that are amenable to much wider screening at earlier stages of the drug discovery process. It should be emphasized that the primary aim of these screens is to raise flags which could trigger follow-up studies, such as mechanistic or *in vivo* studies to further investigate any potential liability, or simply be used to rank and prioritize compounds for further progression along the screening cascade. A good example of this is the use of radioligand binding assays

for high throughput screening against hERG (Figure 2).

The emergence of automated electrophysiology platforms has enabled the extension of broad hERG liability screening to higher fidelity, information-rich functional assays that were previously only feasible for small numbers of compounds late in development. The IonWorks® instrument is highly suited to this type of screening since it has the highest capacity of those currently available. Though electrical access to the cell is enabled somewhat differently (e.g. perforated patch versus whole cell patch) the correlation with traditional "gold standard" manual electrophysiology assays is excellent (Figure 3A). This enables it not only to flag potential liability, but also to support SAR chemistry aimed at eliminating this activity from key

chemical series. Other automated electrophysiology instruments have lower capacity for screening but can also be used for this application (e.g. the PatchXpress™ system, Figure 3B and 3C). Although having lower throughput than the IonWorks system, these instruments use the whole-cell patch clamp technique and are capable of running more complex protocols also suitable for more detailed studies.

MILLIPORE'S CARDIACPROFILER™ PANEL – A COMPREHENSIVE CARDIAC SCREENING SERVICE

Automated electrophysiology has dramatically improved the tractability of many ion channel types. Now, Millipore has employed these advances, offering the opportunity for earlier

Automated electrophysiology has dramatically improved the tractability of many ion channel types. Now, Millipore has employed these advances, offering the opportunity for earlier and broader liability screening for other channels in addition to hERG.

and broader liability screening for other channels in addition to hERG. Millipore's CardiacProfiler panel is a collection of screening assays to enable profiling of key ion channels mediating the cardiac action potential and/or that

are genetically associated with LQTS. This includes the sodium channel that initiates the action potential (Nav1.5), the calcium channel that maintains the plateau phase (Cav1.2), the potassium channels involved in repolarisation (Kv4.3, Kv1.5, KCNQ1, hERG) and the leak or hyperpolarisation activated currents that maintain the resting potential of myocytes (Kir2.1, HCN4) (Figure 4, page 5).

The Millipore CardiacProfiler panel can be used to support different screening approaches. As already noted, inherited LQTS can be caused by alteration of function of a number of different channels that mediate the action potential independently of hERG. Researchers are uncovering examples of drug-induced effects on QT interval which are associated with modulation of cardiac channels other

Figure 2. Radioligand Binding Assays for hERG Screening

2A. Competition binding data for hERG ligands using Millipore membrane preparations from hERG expressing HEK293 cells (Catalogue No. CYL3039) were incubated with 3.0 nM [³H]-Astemizole and increasing concentrations of different compounds.

2B. K_i values obtained compared to published data.

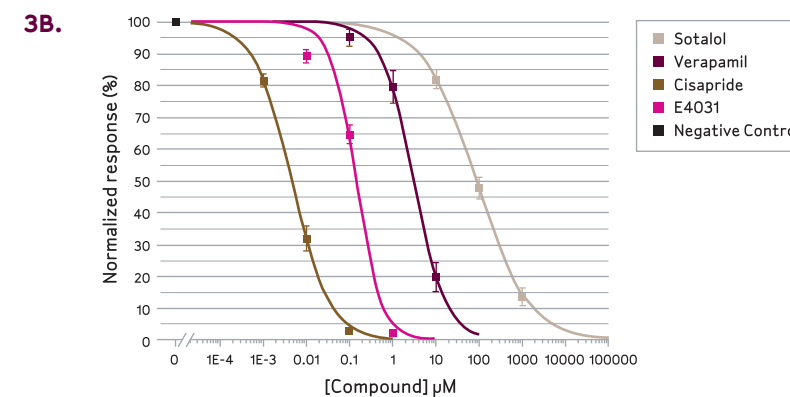
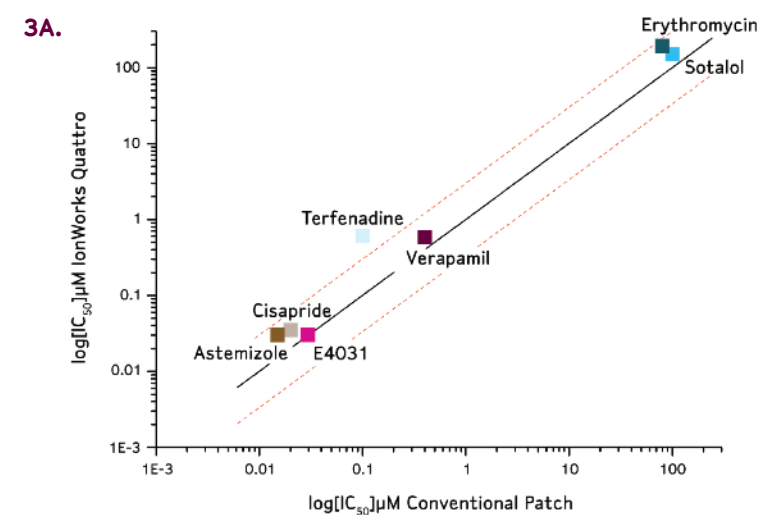
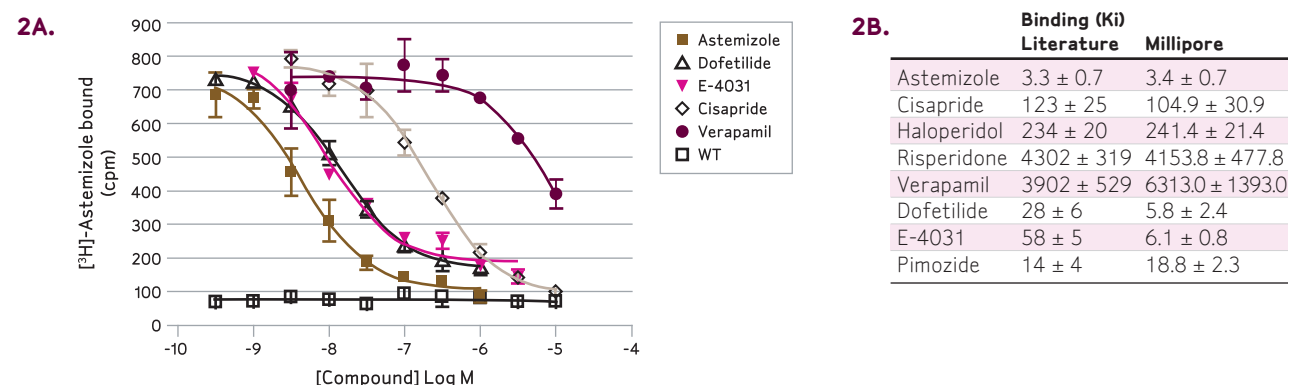


Figure 3. Use of Automated Electrophysiology Platforms for Functional hERG Profiling

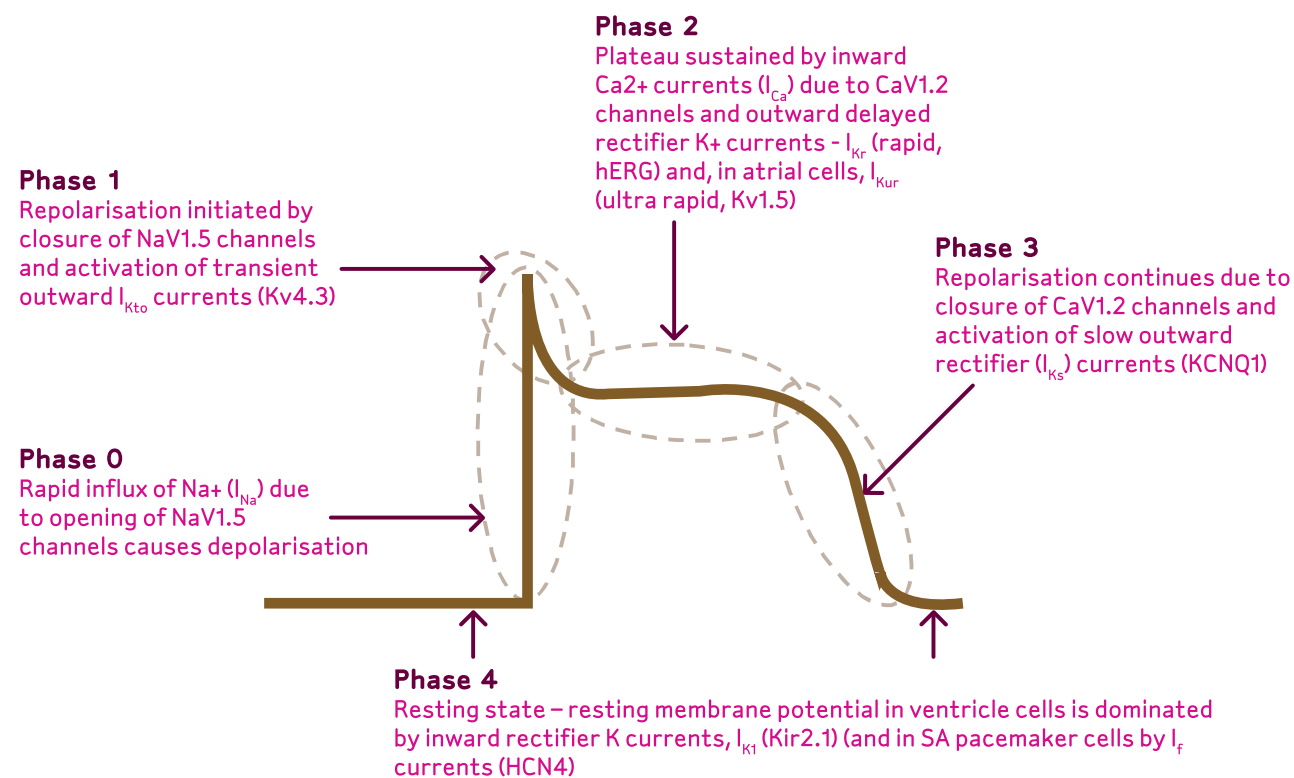
3A. IonWorks hERG assay: correlation between IonWorks IC₅₀ values and published values obtained using manual patch clamp for seven reference compounds. Most fall either on the line of equivalence (shown in black), or within a 3-fold range of this (red dotted lines), indicating good correlation between methodologies.

3B. PatchXpress hERG assay: Concentration response curves for selected reference compounds.

3C. IC₅₀ values obtained from the PatchXpress data are shown in the table, together with typical values from conventional patch clamp reported in the literature.

	IC ₅₀ (Patch Express)	IC ₅₀ (Literature)
Sotalol	84.7 ± 4.3 μM	100 μM
Verapamil	3.1 ± 0.4 μM	3 μM
Cisapride	5 ± 1 nM	20 nM
E4031	144 ± 44 nM	30 nM

Figure 4. Ion Channels and Conductances Underlying the Ventricular Action Potential



than hERG (with little or no blockade of the latter). For example, upregulation of NaV1.5 currents by alfuzosin, Lacerda et al., 2008. The capacity of the Millipore **CardiacProfiler** panel assays is compatible with broad screening across multiple programs aimed at helping to identify these compounds early in the drug discovery process. In this way, the panel can be used as an aid to prioritize and rank initial hit or lead series compounds or flag them for later investigation.

The **CardiacProfiler** panel can also be used as a component of any follow-up studies that might be required for analysis of compounds with hERG blocking activity that appear to have no effects on QT interval. This would complement the other more complex approaches described above (e.g. *in vitro* or *ex vivo* APD studies) but would circumvent any species-related differences, since the **CardiacProfiler** assays are all

based on human recombinant channels. To enhance interpretation of these profiling data, we have begun to benchmark this panel of assays by profiling against reference compounds with known therapeutic concentrations and *in vivo* effects. These include “selective” hERG blockers which cause TdP (e.g. sotalol and cisapride) as well as calcium channel blockers (verapamil and diltiazem) and other mixed channel blockers (e.g. amiodarone and flecainide) which have low risk of TdP (Figure 5). Each of these reference compounds shows the expected profile at therapeutically relevant concentrations, confirming the utility of the panel in helping to predict *in vivo* liability.

In conclusion, Millipore’s **CardiacProfiler** panel provides an important new tool for drug developers and safety pharmacologists in assessing the integrated cardiotoxic risk of compounds in development.

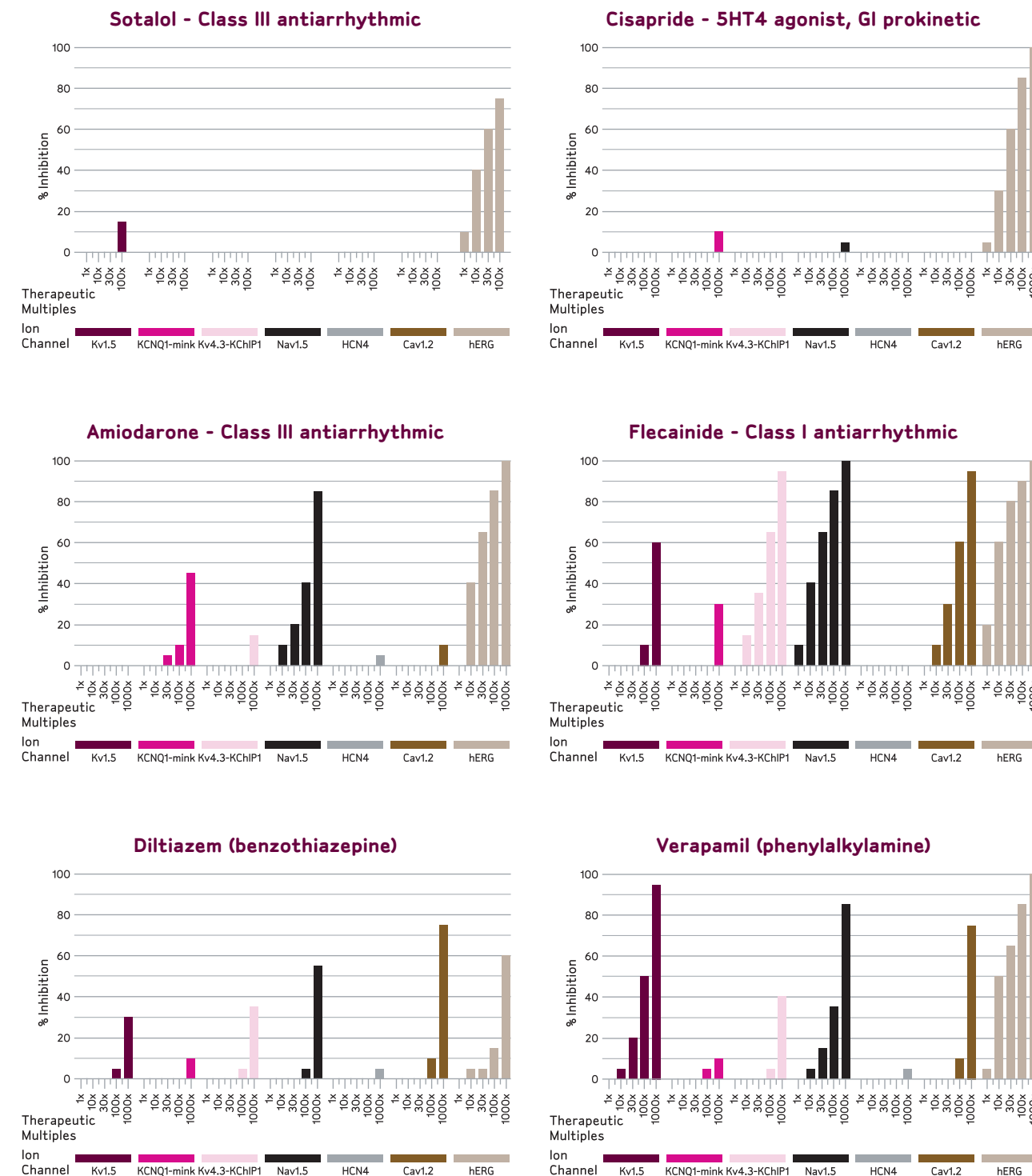
MILLIPORE CARDIACPROFILER PANELS

Highly validated cell lines and protocols for a fully licensed service.

HIGHLIGHTS:

- Only provider licensed to offer an L-type calcium channel service for liability testing
- Validated cell lines and protocols ensure high-quality, reproducible results you can trust
- A plate-based APC platform offers affordable, fast turnaround
- A cost-effective alternative to complex and specialized action potential duration assays
- Flexible services can be tailored to meet requirements for throughput, data quality and cost

Figure 5. Benchmarking the CardiacProfiler Panel with Reference Compounds



About the Author

Jeff Clare has over 20 years experience in the pharmaceutical industry including 14 years in ion channel drug discovery. He obtained his PhD from the University of Kent, and spent five years as a post-doc at UMIST (Manchester) and later at the University of Connecticut. He then joined Wellcome Biotech, developing expression systems for subunit vaccines and biopharmaceuticals, before transferring to Wellcome Research Laboratories to initiate their efforts on ion channel cloning and expression. Subsequently at GlaxoWellcome and then Glaxo-SmithKline, as Head of Ion Channel Gene Expression, he was engaged in numerous drug discovery and research projects for a wide variety of channels. He is currently Director of R&D for Ion Channels at Millipore in Cambridge, UK.

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