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THEMED SECTION: QT SAFETY

REVIEW

An introduction to QT interval prolongation and non-clinical approaches to assessing and reducing risk

CE Pollard, N Abi Gerges, MH Bridgland-Taylor, A Easter, TG Hammond, J-P Valentin

Safety Assessment UK, AstraZeneca R&D, Alderley Park, Macclesfield, Cheshire, UK

Owing to its association with Torsades de Pointes, drug-induced QT interval prolongation has been and remains a significant hurdle to the development of safe, effective medicines. Genetic and pharmacological evidence highlighting the pivotal role the human ether-a-go-go-related gene (hERG) channel was a critical step in understanding how to start addressing this issue. It led to the development of hERG assays with the rapid throughput needed for the short timescales required in early drug discovery. The resulting volume of hERG data has fostered *in silico* models to help chemists design compounds with reduced hERG potency. In early drug discovery, a pragmatic approach based on exceeding a given potency value has been required to decide when a compound is likely to carry a low QT risk, to support its progression to late-stage discovery. At this point, the *in vivo* efficacy and metabolism characteristics of the potential drug are generally defined, as well its safety profile, which includes usually a dog study to assess QT interval prolongation risk. The hERG and *in vivo* QT data, combined with the likely indication and the estimated free drug level for efficacy, are put together to assess the risk that the potential drug will prolong QT in man. Further data may be required to refine the risk assessment before making the major investment decisions for full development. The non-clinical data are essential to inform decisions about compound progression and to optimize the design of clinical QT studies.
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Keywords: QT interval prolongation; hERG; drug discovery; drug safety

Abbreviations: AP, action potential; APD, action potential duration; CD, candidate drug; ECG, electrocardiogram; GLP, Good Laboratory Practice; hERG, human ether-a-go-go-related gene; MAD, Multiple Ascending Dose study; SAD, Single Ascending Dose study; TdP, Torsades de Pointes; TQTS, Thorough QT/QTc Study

QT prolongation/Torsades de Pointes & its impact on drug discovery

Using the data summarized by Shah (2006), prolongation of the QT interval on the electrocardiogram (ECG) was the reason for around one-third of all drug withdrawals between 1990 and 2006. Although this effect on cardiac electrophysiology is not in itself a safety risk, QT interval prolongation is associated with a potentially fatal arrhythmia called Torsades de Pointes (TdP). Indeed, even though TdP is extremely rare, for the agents withdrawn there was evidence of TdP (see Redfern *et al.*, 2003).

Given the cost of bringing a new drug to the market [estimated in the year 2000 at approximately US$800m (DiMasi *et al.*, 2003)], the proportion of drugs withdrawn owing to QT prolongation/TdP was a significant concern for pharmaceutical companies. The headline drugs withdrawn from sale were just the tip of the iceberg, however, as the development of many more potential drugs was halted following evidence of a QT prolongation risk and some drugs remain on sale despite carrying a QT risk but cannot be prescribed to certain patient groups. Such was the level of concern in the pharmaceutical industry that the 'QT issue' was jokingly dubbed 'Pharmageddon' (WS Redfern, pers. comm.).

Correspondence: Chris Pollard, 19B78 Mereside, AZ R&D Aldereley Park, Macclesfield, SK10 4TG, UK. E-mail: chris.pollard@astrazeneca.com
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All in all this was, and remains, a significant hurdle to the development of effective but safe medicines in an industry needing to focus on improving its productivity by reducing safety-related attrition. This overview aims to introduce the topic and summarize non-clinical strategies to assess and reduce QT interval prolongation risk. It builds on a previous review of the topic (Pollard *et al.*, 2008) and complements more specific case studies described by Valentin *et al.* (2010) and is considered from a broader perspective in the commentary by Guth and Rast (2010).

Background biology

When the prevalence and implications of drug-induced QT interval prolongation and TdP first became apparent, it may have seemed a complex and intractable problem. However, a number of factors have gradually come together to enable the issue to be tackled in a rational way.

The fundamental biology of the QT interval was already known. Specifically, underlying the Q and T waves of the ECG are predominantly the depolarization and repolarization phase of ventricular myocyte action potentials (AP) respectively. Therefore, ECG measurements of the time from the start of the Q wave to the end of the T wave provide a quantitative assessment of AP durations in ventricular myocytes. Allied to this, the molecular mechanisms generating the single-cell cardiac AP had been identified (see, for example, Roden and George, 1996). As shown in Figure 1, however, even a superficial, non-exhaustive summary of the key ionic currents driving the ventricular cell AP illustrates the complex interaction of six different types of voltage-gated cation channel. As the membrane potential changes during the AP reflect shifts in the balance of inward and outward ion fluxes, a drug-induced increase in AP duration could theoretically result from modulation of any of these channel types. Fortunately, the complexity of the problem was somewhat reduced when the ion channel pharmacology of the withdrawn drugs was investigated, as most of them were found to be relatively potent inhibitors of I_{Kr} [a current carried by the channel encoded by the human ether-a-go-go-related gene (hERG)], for example, terfenadine (Rampe *et al.*, 1993), astemizole (Suessbrich *et al.*, 1996), cisapride (Rampe *et al.*, 1997) and grepafloxacin (Bischoff *et al.*, 2000). This, allied with the finding that mutations of hERG were responsible for one form of congenital long-QT syndrome (Curran *et al.*, 1995), was sufficient to focus initial efforts on understanding and combating hERG-mediated QT interval prolongation.

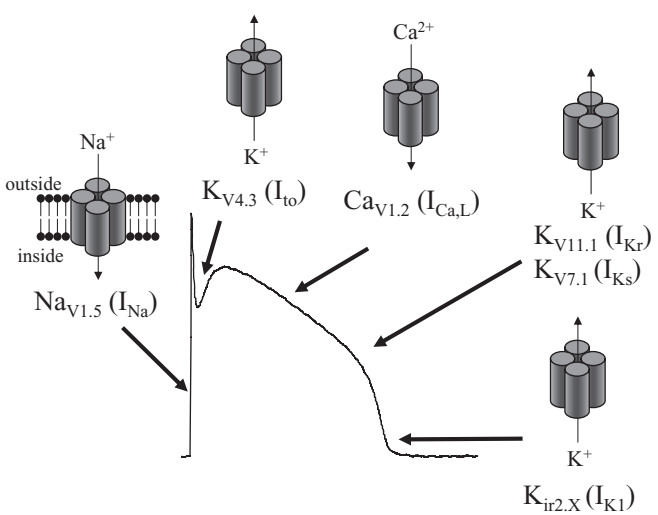

Figure 1 A summary of some of the key ion channel types underlying the ventricular cardiac action potential (AP) along with an indication of the phase of the AP where they are most dominant. The I_{K1} current maintains the resting membrane potential between APs.

In summary (Figure 2), a simple scenario became apparent. Under normal circumstances, potassium efflux via hERG is a dominant component in the repolarization phase of the ventricular myocyte AP and is therefore a key factor in determining its duration. Inhibition of this channel type decreases potassium efflux, slows repolarization and prolongs AP duration. As the QT interval reflects ventricular AP durations, as described above, hERG block prolongs the QT interval. Although this is an oversimplification of complex biology, it was the critical element enabling an initial approach to the problem to be developed as it linked a known and testable molecular entity (hERG) to a significant adverse drug reaction (TdP).

There was a sting in the tail, however. Owing to an atypical amino acid sequence in the trans-membrane-spanning domain of hERG termed S6 (Mitcheson and Perry, 2003) the

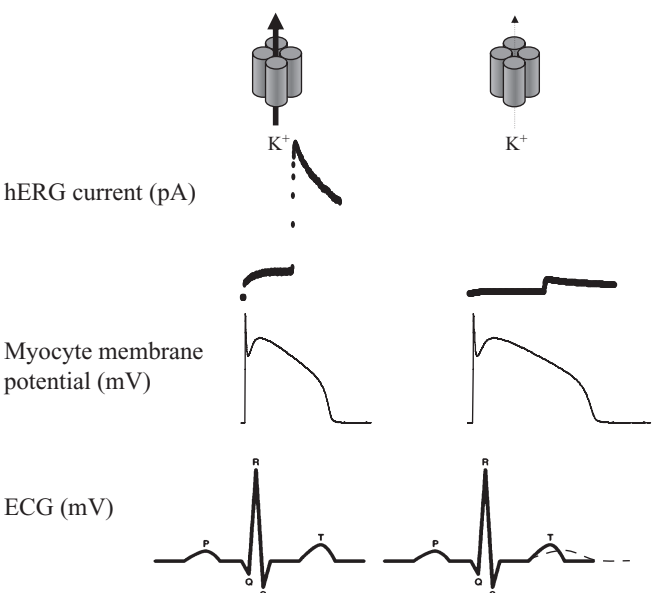

Figure 2 A simplified view of human ether-a-go-go-related gene (hERG) and QT interval prolongation biology. Under normal circumstances (left-hand panel), potassium efflux via the hERG-encoded channel (top) is a key factor in the repolarization phase of ventricular myocytes. This ensures normal action potential durations (middle) and this is reflected in a normal QT interval (bottom). In the presence of a hERG blocker (right-hand panel), potassium efflux is reduced (top), repolarization is slowed and ventricular action potential duration increases (middle) and this is seen as prolongation of the QT interval (dashed line, bottom). Note that the hERG current shown is from a cell line and evoked by a square-wave voltage protocol, as distinct from the form of the current during the action potential in a ventricular cardiac myocyte. ECG, electrocardiogram.

channel was pharmacologically promiscuous, binding to a broad range of drug-like molecules. This was bad news, as avoiding hERG activity was a new and frequent burden for already fractious medicinal chemists trying to design molecules with good efficacy and drug absorption and metabolism properties.

As the hERG/QT/TdP story evolved and was relayed by safety pharmacologists to those trying to discover new drugs, the most common question was: If QT interval prolongation *per se* does not represent a safety risk, should not we be assessing the risk of TdP as early as possible? At the time, the late 1990s, owing to its extremely low incidence, the only definitive way of determining the risk of TdP was (and is) to prescribe the drug to a very large, diverse patient population; clearly, a less than ideal approach. A lot of effort has since been expended trying to understand the fundamental mechanisms of TdP in order to develop non-clinical models to predict TdP risk. However, although work continues to identify definitive non-clinical or early clinical markers of TdP superior to that of QT interval prolongation, the latter index of risk remains the only surrogate end-point acceptable to regulatory agencies; this is not unreasonable given that TdP is generally accompanied by QT interval prolongation. Tangible evidence of regulatory focus on the QT interval as a surrogate marker was the finalization of ICH E14 (Anon, 2005a), which stipulates the need for the so-called 'Thorough QT/QTc Study' (TQTS) to be conducted in man for all systemically bioavailable drugs prior to marketing authorization. Drugs found to prolong the QT interval in the TQTS may not be approved or may carry warnings on the drug label that result in prescribing restrictions and a potential loss in commercial value. Thus, significant effort has been put into developing non-clinical strategies to assess and minimize QT prolongation risk before giving a compound to man. In this relatively early (discovery) phase of the whole process, discontinuation of a potential drug can occur when, in relative terms, only small amounts of time and money have been committed (the so-called, 'fail early, fail cheap' philosophy).

Risk assessment strategy in the context of drug discovery

The top part of Figure 3 shows a generic drug discovery and development process and an estimate of the number of compounds at each stage for a single project. After identifying and validating a molecular target believed to play a role in the disease of interest, lead compounds are often found by running a high-throughput screen. This could involve compound numbers in the order of millions. Lead compounds are optimized for parameters relating to efficacy, drug absorption and metabolism and, ideally, safety. This phase is dependent on the iterative, synthesis-screening cycle, and therefore involves mainly *in vitro* assays producing data in a short period of time to influence chemical design. It may take synthesis of thousands of compounds before identification of a small number (<10) with the appropriate balance of properties to warrant progression to the candidate selection phase, where the focus switches to *in vivo* models. This detailed analysis in integrated systems hopefully confirms the optimism generated from *in vitro* information and yields data that allow the best compound to be selected for progression to human studies.

A non-clinical strategy to assess and reduce QT interval prolongation risk in the context of drug discovery is also shown in Figure 3 and described in more depth below.

hERG assays

As hERG is the most likely, although not the only, mechanistic basis for drug-induced QT interval prolongation, a hERG assay is pivotal to the lead optimization phase. To be of maximum value it is essential that the assay is positioned very early in the optimization phase so that any hERG liability in the chemical area of interest is identified as soon as possible, thus giving time for the activity to be designed out. This

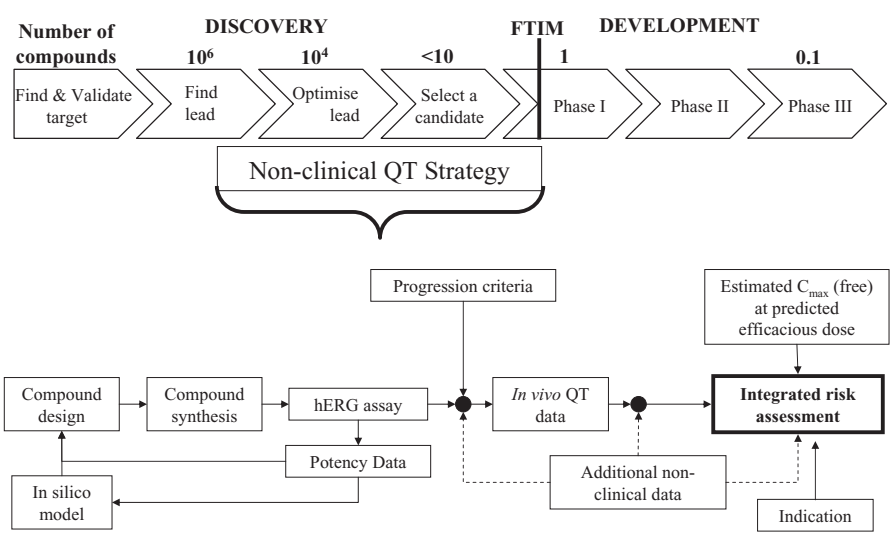

Figure 3 A generic drug discovery and development process (top) in relation to a non-clinical QT strategy (bottom). hERG, human ether-a-go-go-related gene.

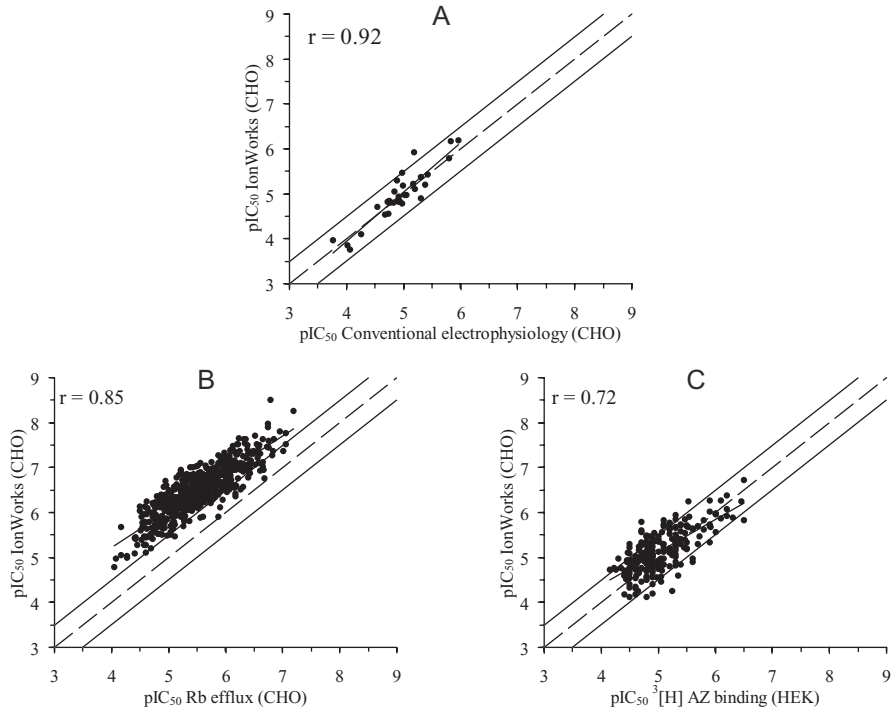

Figure 4 Comparative potency data from different kinds of hERG assay. Potency data (pIC_{50}) from IonWorks are shown on the ordinate of each graph relative to conventional voltage clamp electrophysiology (A), rubidium efflux (B) and ligand displacement (C) assays. For each plot, the lines shown are as follows: the solid line is the correlation between data from the two assays; the dashed line indicates the line of identity, and either side of this are lines minus and plus a half log-unit away from the line of identity. Correlations between assays [Pearson's coefficient (*r*)] are shown for each comparison. CHO, Chinese hamster ovary; HEK, human embryonic kidney; hERG, human ether-a-go-go-related gene.

requirement effectively means that the hERG assay needs to have a throughput capable of testing hundreds of compounds per day and a data reporting time within a few days of running the assay. This is for the simple reason that until medicinal chemists know the consequences of a given chemical change (i.e. has it increased or decreased hERG potency or left it unchanged?) they cannot plan which changes to make next. This requirement to fit into the synthesis-screening cycle initially limited hERG assays to those indirectly assessing potency (e.g. binding and rubidium efflux assays), but the 'gold standard' assay – which is based on voltage clamp electrophysiology – gradually has become a viable alternative owing to the development of high-throughput electrophysiology devices (Priest *et al.*, 2007). This led to debates about which of the three assay types was most appropriate, a debate that could be termed: the binders versus the fluxers versus the clampers.

In practical terms, the binding assays are the cheapest, have the highest throughput and are the most technically simple to run. They are, however, based on displacement of an affine, radio-labelled ligand from the channel in a preparation of membrane fragments from a hERG-expressing cell line. This inevitably leads to concerns about the physiological relevance of the assay and the fact that only one binding site is being investigated.

Going to the other extreme, the automated voltage clamp-based assays are relatively expensive, relatively low throughput and, despite being automated, still require some technical expertise to run successfully. They do, however, use whole cells and, crucially, force the channel protein through approximately the same complex state changes it undergoes in the ventricular myocyte of a beating heart. Hence inhibition via any binding sites will be detected and the data are likely to have high physiological relevance.

The rubidium efflux-type assays can be considered intermediate in terms of cost and throughput. Although whole cells are used and activity at any binding site could potentially be detected, their relevance to normal physiology is undermined by the need to add high extracellular potassium concentrations to evoke channel activation and the fact that rubidium is the charge carrier through hERG; these features of the assay may account for potency underestimates relative to voltage clamp assays (Yang and Roden, 1996; Rezazadeh *et al.*, 2004).

The advent of high-throughput voltage clamp electrophysiology has begun to allow individual prejudices to be objectively explored, as a significant volume of electrophysiology data can now be generated for comparative purposes. Our own data are shown in Figure 4 using voltage clamp electrophysiology data from IonWorks, a 384-well plate-based device first reported by Schroeder *et al.* (2003). The automated electrophysiology data correlate well with those generated in the same cell line using conventional, microelectrode-based experiments (see also Bridgland-Taylor *et al.*, 2006). While there is a good correlation between IonWorks data and rubidium efflux results, the relationship is offset such that the efflux assay under-predicts IonWorks potency by around 10-fold, on average. Finally, a comparison of IonWorks data with binding assay potencies suggests a weaker correlation but no consistent potency offset.

Such comparative data and the practicalities of each assay type provoke lengthy discussions about which assay or combination of assays is the best way of meeting the requirements of the synthesis-screening cycle. One opinion is that the costs and reduced throughput of doing voltage clamp experiments dictate the need for a two-step process where binding or rubidium efflux assays act as a pre-filter, thus reducing the burden on electrophysiology assays. In this case, the speed, simplicity and low cost of a binding assay probably have the edge overall. The key factor to bear in mind, however, is that automated electrophysiology can provide enough data so that for each chemical series the concordance or otherwise with an indirect assay can be determined on a case-by-case basis. In other words, if binding and electrophysiology data for chemical series X correlate well, the main screening burden could be confidently placed on the binding assay, with occasional checks using electrophysiology to ensure that this correlation is maintained as the chemistry in a given area evolves. If there is a poor correlation, the needs of that chemical area may need to be more dependent on electrophysiology. As a 'rule of thumb', a binding to electrophysiology potency correlation where 95% of the data points lie within minus and plus a half log-unit away from the line of identity would be a good justification to make full use of the binding assay. Otherwise, more reliance on electrophysiology would probably be required. The same generalization could be applied to rubidium efflux data but, because of the offset in potency, would need to be applied around the line of correlation rather than the line of identity.

Virtual hERG screening

There is also another reason to generate quantitative hERG potency information. Developing a database containing each structure tested and its potency provides the 'substrate' for computational chemists to build and refine predictive models of hERG activity that permit 'virtual screening'. This is an important bonus feature of the screening data as it enables better decisions to be made about which chemical structures to make next. For example, if the medicinal chemist is considering making compound E or F, but the computational model predicts that E will be a potent hERG blocker based on its similarity to compounds A, B, C and D (which have already been made and tested at hERG), then they can focus their limited resources on making compound F. This means that while medicinal chemists desire rapid screening, their computational cousins lobby for chemical diversity in the database to increase the chances that a compound being 'virtually' screened will have some close analogues that have actually been screened. While this diversity may take time to develop, it can lead to '*in silico*' models of significant value (Gavaghan *et al.*, 2007).

Progression criteria

The ideal scenario with respect to hERG is that the chemical leads are not active, even at high concentrations. However, given the promiscuity of hERG this may only be the case in certain chemical areas (e.g. acids). Hence in many drug discovery projects there will be multiple iterations of the screening loop in Figure 3, attempting to lower hERG potency while retaining desirable properties. This leads inevitably to the question: What potency needs to be achieved before a compound is considered to carry a low enough QT prolongation risk to be progressed to the later stages of the discovery process?

Several literature-based exercises have confirmed what is intuitively obvious about the relationship between plasma exposure levels for clinical efficacy, hERG potency and the risk of TdP. Namely, that in general, the larger the safety margin between systemic exposure in man and potency at hERG, the lower the risk of TdP (Cavero *et al.*, 2000; Kang *et al.*, 2001; Webster *et al.*, 2002; Redfern *et al.*, 2003; De Bruin *et al.*, 2005). Furthermore, these authors have tried to quantify a suitable safety margin in order to provide a target value for those aiming to screen out hERG activity. Overall, these reports suggest that a minimum safety margin of 30-fold is required. However, the safety margins are based on measured C_{max} free values for efficacy while drug discovery projects usually only have predicted estimates of this figure. Also, the clinical end point in these reviews is TdP, not QT interval prolongation. Although TdP is the relevant end point, the TQTS defined in ICH E14 (Anon, 2005a) effectively means that QT interval prolongation is the dominant index for judging risk. The effect of this on non-clinical decisions about suitable safety margins is unclear, but the disparity between the statistical power of the TQTS (which aims to detect QT interval increases in the region of 2.5%) and that achievable to detect significant effects in *in vivo* non-clinical assays (conventionally around 10%, Hammond *et al.*, 2001; Tattersall *et al.* (2005) has obvious implications (i.e. *in vivo* non-clinical studies are likely to underestimate the risk of a 'positive' TQTS).

Unfortunately, in early lead optimization there is usually no credible way of estimating the likely drug exposure to give efficacy in patients. Thus, for hERG the only pragmatic way of making a decision to progress a compound to more complex models may be to assign an absolute potency value that must be achieved (i.e. the IC_{50} must be higher than x $\mu mol \cdot L^{-1}$). Inevitably, this leads to charges of imposing arbitrary hurdles, a criticism that is only partly valid as some common sense can be applied. For example, anti-infectives such as the withdrawn drug grepafloxacin (hERG IC_{50} 50 $\mu mol \cdot L^{-1}$; Kang *et al.*, 2001) generally need high plasma levels for efficacy so in such circumstances the absolute potency hurdle would need to be set much higher than for compound classes that historically are active in the $nmol \cdot L^{-1}$ range *in vivo*. Hence, based on what is known about the efficacy mechanism and the prior art in a given area, some judgements can be made as to the most rational choice of absolute cut-off for hERG potency.

Beyond direct hERG interactions

The box in Figure 3 called 'Additional non-clinical data' encompasses a variety of *in vitro* and *in vivo* assays that add to the hERG and *in vivo* QT data. These can be run on a case-by-case basis at various stages of the process, including after the

compound has been tested in man (e.g. testing human-specific metabolites at hERG). The need for this category of assays, in effect, highlights the fact that hERG inhibition, although pivotal to early screening, may not be the only aspect of the topic needing investigation.

In vitro *AP assays*

One of the *in vitro* assays is AP recording from single cardiac cells, the most commonly used preparation being the Purkinje fibre, owing to the relative technical ease of making stable intracellular recordings in these large, non-contractile fibres responsible for rapid AP conduction to the ventricular muscle mass. This model was the *in vitro* mainstay of the first regulatory QT guidance (Anon, 1997) before the central role of I_{Kr}/hERG was widely appreciated. AP assays were also prominent in the draft versions of the latest non-clinical regulatory guidance [ICH S7B (Anon, 2005b)], where they appeared as a so-called 'core assay' before being relegated to a 'follow-up' study. Owing to these regulatory influences, a lot of Purkinje fibre data have been generated. As reported by others (Guth *et al.*, 2004; Martin *et al.*, 2004; Lu *et al.*, 2008), an examination of our own dog Purkinje fibre data (Easter *et al.*, 2006) exposes the deficiencies of the simple scenario outlined in Figure 2, on which the strategy thus far is based. If the biology really was that simple, when hERG blockers spanning a range of potencies were tested in the Purkinje fibre assay, there should be a reasonable correlation between, for example, hERG IC_{50} and the concentration giving a 10% prolongation of Purkinje fibre AP duration (usually quantified as APD_{90} – the duration at 90% of repolarization). In reality, as shown in Figure 5, there are a variety of outcomes in the Purkinje fibre. Some compounds (23 out of 38) have hERG IC_{50}s the same as or greater than the concentration causing a 10% APD_{90} increase (filled, black circles). This is the expected outcome for a selective hERG blocker and for these data there is a good correlation [Pearson's coefficient (r) = 0.88]. The fact that for many of these 23 compounds the hERG IC_{50} is 3–10-fold greater than the concentration causing a 10% APD_{90} increase is not surprising, as although hERG IC_{50} is pharmacologically the most appropriate value to quote, physiologically only around 10% hERG inhibition is enough to give significant increases in AP duration (Jonker *et al.*, 2005). From the data set in Figure 5, it is clear, however, that some hERG blockers are also likely to be inhibiting inward current. Although we have not experimentally proven this, they may be inhibiting calcium influx via $Ca_{V1.2}$ – the L-type channel. These compounds range from those where the hERG IC_{50} is exceeded before seeing a 10% APD_{90} increase (filled, grey circles), to those where APD_{90} decreases significantly (open, black circles). It is also worth bearing in mind the Purkinje fibre data for hERG inactive compounds that we have tested (which cannot be plotted on Figure 5). Of 12 compounds that were inactive at hERG (tested at 10 $\mu mol \cdot L^{-1}$ or above), they neither increased nor decreased APD_{90} significantly in dog Purkinje fibres even though they were tested in the same concentration range as they were at hERG.

It would be a logical step to insert a Purkinje fibre assay before the *in vivo* assessment as the data are rich in useful information. As can be inferred from Figure 1, by measuring parameters characterizing AP morphology other than APD_{90}, like resting membrane potential, upstroke velocity and APD_{50}, reasonable inferences can be made about drug effects on $K_{ir2.X}$, $Na_{v1.5}$ and $Ca_{v1.2}$ respectively. It is also an ideal preparation to investigate the influence of different stimulation frequencies on the effect of a drug. As bradycardia is a TdP risk factor, if the degree of drug-induced APD_{90} prolongation increases at low stimulation frequencies [so-called 'reverse use-dependence' (Hondeghem and Snyders, 1990)] this would be considered an additional negative characteristic of a compound.

Despite the undoubted value of AP assays for a detailed understanding of a small number of compounds, there are some issues worth bearing in mind. First, this kind of assay is extremely low throughput. Second, for compounds where the AP-prolonging effect of a hERG blocker is offset by its coincident L-type calcium current inhibition, the resulting relative lack of effect on APD_{90} can generate false optimism that such a compound could progress into clinical studies with the expectation that this self-cancelling effect will mean a trouble-free development phase. It is essentially assuming that across a range of people and exposures, this electrophysiological balancing act will be maintained. While this is may be an acceptable position for a drug targeting a serious disease, there is a danger that it is used to justify compound progression for benign indications. There is also a fundamen-

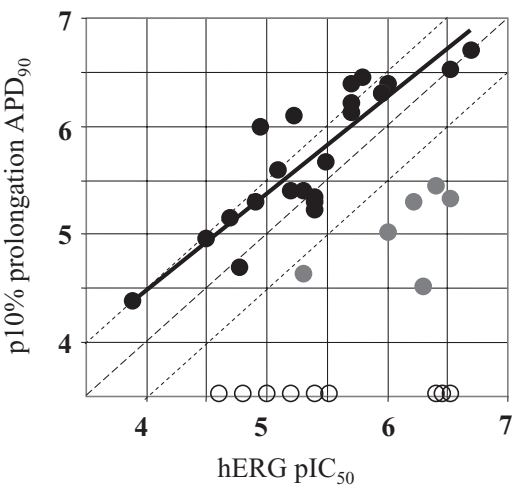

Figure 5 Effect of human ether-a-go-go-related gene (hERG) blockers on dog Purkinje fibre action potential (AP) duration. The hERG potency (pIC_{50}) of 38 compounds tested using conventional electrophysiology according to the method described by Bridgland-Taylor *et al.* (2006) is shown relative to the effect of each compound on APD_{90} using the method described by Abi-Gerges *et al.* (2004). In order to make the data from the two assays comparable, the 'potency' for each compound in the Purkinje fibre is expressed as the negative log_{10} of the concentration that increased APD_{90} by 10%. The black, filled circles show compounds most likely to be selective hERG inhibitors. The grey, filled circles show compounds that the data suggest may also inhibit inward current. The black, open circles show compounds that caused a decrease in APD_{90}, so while being hERG blockers, these compounds are highly likely to also inhibit inward current over the same concentration range. The lines shown are as follows: the solid line is the correlation of the data indicated by black filled circles; the dashed line indicates the line of identity, and either side of this are lines minus and plus a half log-unit away from the line of identity.

tal disadvantage in using Purkinje fibres as their electrical activity makes a negligible contribution to the T wave, the end of which is believed to be defined by APs in myocytes from the mid-myocardial region (Yan and Antzelevitch, 1998).

There are examples of combined hERG/L-type blockers that have become marketed drugs without any significant QT/TdP issues (e.g. verapamil). However, the precision with which the QT interval is now assessed for potential new drugs has increased dramatically, most notably in the form of the TQTS. In addition, even though it is likely that L-type inhibition has a protective effect in preventing TdP [as L-type activity is believed to be a key driver of the early after depolarizations that may be the substrate for TdP development (Belardinelli *et al.*, 2003)], the commercial value of having a completely 'clean' drug, as opposed to one with hERG/L-type 'baggage', may be significant – all other properties being similar.

Given that cell lines are now available expressing all the channel types in Figure 1, and as the automated electrophysiology platforms enable high-volume testing (e.g. Harmer *et al.*, 2008), putting additional channel types into the synthesis-screening loop may be an alternative to single cardiac cell assays. Aside from reducing animal use, this 'molecularization' of the AP provides the medicinal chemist with the most helpful data: a potency value at channel X that they can work with in terms of trying to find compounds with a reduced potency at that specific molecular entity. In early discovery, this is more helpful than reporting a 10% decrease in APD_{90} from a single cell assay that could be the result of an interaction with more than one channel type.

Effects on hERG channel expression

Whether ion channel interactions are investigated *in vitro* using heterolously expressed channels or cardiac tissues, both are acute assessments of a compound's ion channel pharmacology and are only likely to detect direct modulation of the channel protein. Significant evidence has emerged, however, that some compounds [e.g. pentamidine (Kuryshev *et al.*, 2005)], although inactive as hERG blockers, can modulate the cardiac AP by inhibiting trafficking of the hERG channel protein to the plasma membrane. By decreasing channel density, this indirect effect on cardiac electrophysiology nevertheless has the same effect on AP duration such that drugs like pentamidine can lead to TdP in man (Eisenhauer *et al.*, 1994). The matter is further complicated by the fact that while compounds like pentamidine inhibit hERG channel trafficking but are inactive as direct channel inhibitors, others show both activities (see van der Heyden *et al.*, 2008 for a recent summary). The degree of risk from this additional mechanism is difficult to quantify, but as a high-throughput assay measuring hERG trafficking has been reported (Wible *et al.*, 2005), such an assay can be used to check for this effect. Two general strategies could be adopted in a drug discovery context. If, in a chronic *in vivo* model, QT prolongation is seen for a late-stage compound that does not block hERG, a trafficking assay could be used to test whether the compound is active. If it is, the assay could be incorporated into the early discovery phase of that particular project to try to remove this liability. Alternatively, every discovery project could routinely test a small number of representative compounds early in the process. Given the ease and low cost of adopting the latter approach, it may be the preferred option given that it is likely to reduce the chances of having to halt progression of a compound late in the discovery phase after considerable resource investment, as in the former approach.

Data from pro-arrhythmia models

From our own experience, the most controversial data type that could contribute to the category of 'Additional non-clinical data' in Figure 3 is that from pro-arrhythmia models, which aim to predict the risk of TdP in man (as opposed to QT risk). Detailed coverage of these models is outside the scope of this article (but see, for example, Lawrence *et al.*, 2008). If a well-validated pro-arrhythmia model is available, it would be logical in our view to use it if there is clear evidence of a high QT prolongation risk from other non-clinical data, but only if the compound is targeting a very poorly met, serious indication. At such an early stage of the whole process (i.e. before first administration to man) the data would only help internal decision-making. That is, it would be one element of an overall risk–benefit analysis aiming to decide whether to invest in a compound carrying significant QT risk is wise, knowing this is likely to slow down and increase the cost of the development programme. In short, if there was strong evidence that a candidate drug (CD) was likely to have good efficacy in, for example, a currently untreatable oncology indication, even with a QT risk the prediction of a low pro-arrhythmic (i.e. TdP) risk may be a factor to gain support for compound progression. Although it is obvious that it would be far better to avoid the QT risk in the first place given that the regulatory arbiter of risk is the TQTS.

In vivo *QT data*

Once a small number of compounds have exceeded the progression criteria for hERG and those relating to simple efficacy and drug absorption and metabolism assays, they are likely to progress to more complex models in order to select a CD. For QT risk assessment, the CD of choice has to be tested *in vivo*, usually in dogs. The choice of dogs is based on a variety of factors. The canine equivalent of hERG (cERG) plays the same critical role in AP repolarization in dogs, as demonstrated by the QT-prolonging effect of highly selective hERG blockers in dogs (e.g. dofetilide; Haushalter *et al.*, 2008). The amino acid sequence of the putative drug binding site for cERG shares 100% homology with hERG (Zehelein *et al.*, 2001). Acceptable drug absorption and metabolism properties of the CD are usually established in dogs before the QT study. This means that doses can confidently be selected that are likely to give plasma exposure levels that 'include and exceed the primary pharmacodynamic or therapeutic range', as recommended by ICH S7A (Anon, 2000). Finally, the dog is the traditional, large animal, general toxicology species. From a QT perspective this is important, as with the emerging use of non-invasive telemetry (McMahon *et al.*, 2007), high-quality QT data can now be obtained from chronic-dosing toxicology studies and added to the *in vivo* data that will already have been obtained from an acute, safety pharmacology study, usually using the same

species and strain (see below). There are, however, cases where dogs are not used, usually because the primary therapeutic target is not present or, in the case of biologics, that the CD does not cross-react with the canine target receptor. In these cases, primate studies tend to be conducted.

In the final version of ICH S7B (Anon, 2005b), only a hERG assay and an *in vivo* non-rodent QT assessment constitute 'core' assays from a regulatory perspective – both should be compliant with Good Laboratory Practice (GLP). The hERG data generated for the synthesis-screening cycle can easily be repeated to GLP standards (using conventional electrophysiology) for compounds destined for clinical studies. Two general approaches exist for generating GLP-compliant *in vivo* QT data. One perspective is to run a single study to GLP that provides data both for internal decision-making and for regulatory submissions [not just for QT measurements but for all the cardiovascular end points stipulated by the general safety pharmacology regulatory guidance (ICH S7A; Anon, 2000)]. Alternatively, a non-GLP study (sometimes termed a pilot study) is performed to generate data for internal decision-making and, if the CD is progressed, to aid optimal design for the subsequent GLP study.

It is beyond the scope of this review to go into detail about this study type (but see, for example, McMahon *et al.*, 2007). In brief, the CD's effect on the QT interval (usually after correction for heart rate effects – QTc) is measured following dosing of the compound via the intended clinical route. At key time points plasma samples are collected to establish exposure levels of the parent molecule. The effect on QTc can then be plotted relative to the free plasma level alongside the hERG data. There is debate about whether the free or total plasma levels should be plotted on the abscissa but see Wallis (2010) for evidence of the value of using free levels.

Integrating the data

By the time there is enough belief in a compound to warrant the investment in a cardiovascular safety pharmacology dog

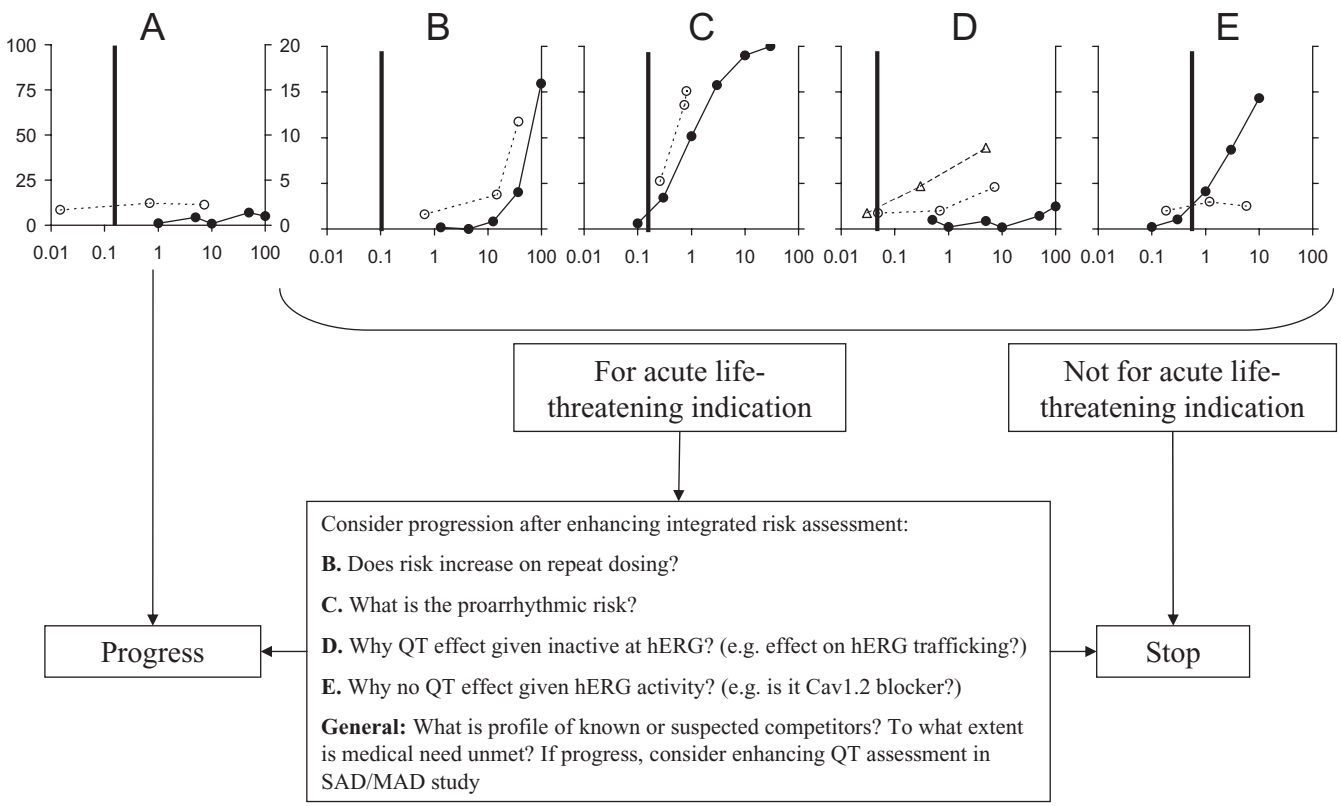

Figure 6 Some typical data sets and possible decisions resulting from an integrated risk assessment approach. Human ether-a-go-go-related gene (hERG) data are shown as filled circles and plotted on the left-hand ordinate as % inhibition of tail current. Acute-dose *in vivo* QTc data are shown as open circles and chronic-dosing *in vivo* QTc data as open triangles; both are plotted on the right-hand ordinate as % increase in QTc relative to the free plasma level of drug. The estimated therapeutic C_{max} free is shown as a vertical line. For each scenario, following an integrated estimate of the QT risk based on hERG and *in vivo* data, the options are to progress (A) or to stop or reassess the risk after refining the risk assessment by generating additional data (B–E). For relatively benign indications, data sets B–E may all prompt a stop decision without the collection of further data. For more serious indications, data sets B–E would prompt further work, as indicated for each data set. There would also be general considerations around competitor compound profiles and to what extent the intended indication was already relatively well treated. Finally, if compounds with profiles B–E were progressed to human studies, the non-clinical data could be used to decide if enhanced early clinical electrocardiogram (ECG) monitoring would be worthwhile in order to get an early assessment of whether the perceived risk from non-clinical data translated to man. For example, if compound C was progressed to man, extra ECG monitoring in the first human study [the Single Ascending Dose (SAD)] study, may be justified. For compound D, more emphasis may need to be placed on additional ECG monitoring in the Multiple Ascending Dose (MAD) study that would follow the SAD study. Such increased monitoring in early development would be a means of generating decision-making human data without waiting until later in development for the outcome of the Thorough QT/QTc Study.

study, the project is likely to have some idea about the peak free plasma level that may be seen in man at the estimated therapeutic dose (therapeutic C_{max} free). That is, the compound will have been tested in *in vivo* models of efficacy and plasma exposures measured. In addition, the drug absorption and metabolism properties will have been established, thus providing information for allometric scaling to man. Although this figure will necessarily still be of uncertain accuracy, it is essential for the QT-related data to be put into context for the internal bodies that decide whether a CD has the quality to justify the quantum leap in investment required to take the CD into man. At this point in time, the literature reviews cited above concerning safety margins become invaluable. As the absolute risk of TdP for QT-prolonging drugs is very low, the non-clinical data need to be interpreted from a risk–benefit perspective; therefore, the intended indication is required to complete the basic integrated risk assessment.

For a proportion of these integrated QT risk assessments, clear-cut decisions can be made to stop or continue CD development. There are, however, a host of intermediate scenarios that require further data to be generated to aid decision-making, hence use of the 'Additional non-clinical data' box in Figure 3. Both simple and complex scenarios with associated additional study types are summarized in Figure 6. The actual data shown are not real but do represent the kind of scenarios that have been seen in practice. The profiles and associated options should not be over-interpreted though: that would imply an unrealistic level of certainty as the number of factors to take into account make 'rules' not only difficult to draft but also wishful thinking. For example, while it may be logical to progress an oncology compound carrying a QT risk from a risk–benefit perspective, if competitor compounds are known and have no QT risk, then all other things being approximately equal, it may not be logical to move forward. In short, the scenarios shown are simply a broad perspective on some of the most obvious non-clinical data sets.

Regulatory value of non-clinical integrated risk assessments

It would be true to say that the impact of non-clinical QT-related data on regulatory thinking has varied over time and across territories, ranging from the high point of a draft version of ICH S7B (Anon, 2005b) that incorporated the concept of a safety margin, to the low point when ICH E14 (Anon, 2005a) was finalized without reference to the potential value of non-clinical data. However, non-clinical integrated risk assessments may after all have a role in regulatory judgements in some regions: Shah and Morganroth (2008) state, 'There is little doubt that the three ICH regions place a different emphasis on the relative merits of nonclinical studies . . .'. In particular, if it were not feasible to conduct a TQTS (e.g. for oncology drugs that cannot be tested in healthy volunteers), it would be logical, in our opinion, for a high-quality, non-clinical data set to be an essential part of the overall risk assessment for man.

Conclusion

By adopting a hERG-focused strategy, the risk of drug-induced QT interval prolongation is being addressed early in drug discovery and minimized before progressing compounds to clinical development. There are a number of aspects of this topic that remain to be addressed, however. In the QT arena, the provision of widely accepted non-clinical or early clinical markers of TdP risk remains an important topic for serious indications if the QT prolongation risk cannot be screened out. Thinking beyond QT risk, in our opinion, extending safety strategies to include other cardiac ion channels and related ECG changes will be of significant value in drug discovery and development. Although validation of the relevant non-clinical assays will need to be carefully considered, as outlined by Pugsley *et al.* (2008). The learning points from the hERG/QT experience will be invaluable in this regard.

Conflict of interest

The authors state no conflict of interest.

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