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REVIEW

Integrated risk assessment and predictive value to humans of non-clinical repolarization assays

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The potential for drugs to be associated with the life-threatening arrhythmia, Torsades de Pointes (TdeP), continues to be a topic of regulatory, academic and industrial concern. Despite being an imperfect biomarker, prolongation of the QT interval of the surface ECG is used to assess the risk of a drug being associated with TdeP such that a thorough examination of drug effects on the QT interval is required for all new chemical entities. Numerous studies have investigated the relationship between non-clinical findings and the risk of TdeP and QT prolongation in the general population. There are many literature references supporting the strong correlation between the clinical safety margin over human *ether-a-go-go* (hERG) inhibitory potency and the risk of drug-induced arrhythmia and sudden death. A quantitative analysis of the relationship between non-clinical studies and the outcome of a human Thorough QT study has also been reported. In the current manuscript, based on the outcome of the non-clinical assays the sensitivity and specificity of each assay and an integrated risk assessment for predicting the outcome of the human Thorough QT study has been conducted. The data suggest that for QT prolongation mediated through inhibition of the hERG current the non-clinical assays are highly predictive of drug effects on the QT interval. Based on the literature review and specific quantitative analysis reported above it is concluded that non-clinical assays predict the risk of compounds to prolong the QT interval and cause TdeP in humans if the mechanism is through inhibition of the hERG current.
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Abbreviations: APD, action potential duration; APD_{90}, action potential duration at 90% repolarization; hERG, human *ether-a-go-go*; TdeP, Torsades de Pointes

Introduction

Within the last decade many drugs have been withdrawn from the market because of their association with the life-threatening ventricular arrhythmia, Torsades de Pointes (TdeP). These agents include antihistamines, antimicrobials, antipsychotic and gastrointestinal stimulants (De Ponti *et al.*, 2002). It is widely accepted that compounds that are associated with TdeP also prolong the QT interval of the ECG. Although QT interval prolongation is an imperfect biomarker for TdeP, regulatory guidelines have been adopted that describe studies required to evaluate the potential for a new chemical entity to prolong the QT interval. These include guidance for both non-clinical (ICH S7A and S7B http://www.ich.org) and clinical (ICH E14) evaluations.

The non-clinical guidance detailed in ICH S7A/B suggests that the potential of compounds to interact with selected cardiac ion channels and to prolong the QT interval in non-rodent species should be evaluated prior to studying the effects of compounds in humans. Of particular interest is the potential of compounds to block voltage-dependent K^+ channels and in particular the rapid delayed rectifier current, I_{Kr}, which specifically plays a key role in ventricular repolarization. The molecular correlate of this channel is the human *ether-a-go-go*-related gene (hERG) that encodes the pore-forming α-subunit of the channel (Sanguinetti *et al.*, 1995). An electrophysiological assessment of the potential of compounds to inhibit this channel and thus prolong the action potential in tissues such as the canine Purkinje fibre make up the core studies recommended in ICH S7B. The species of

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choice to evaluate the potential of compounds to prolong the QT interval *in vivo* tends to be the conscious dog, although non-human primates may also be used.

The focus of ICH E14 provides guidance on how to evaluate drug effects on cardiac repolarization in humans, in particular the design of the Thorough QT (TQT) study. This study is designed to detect threshold effects on the QT interval of approximately 5 ms and should include a positive control to confirm the study sensitivity. If this study is positive extensive ECG monitoring will be required in Phase III clinical trials, while if the trial is negative standard ECG monitoring will be sufficient.

The goal of non-clinical and clinical studies is to provide an integrated risk assessment for the liability of the drug to prolong the QT interval. However, a consensus has not been reached on the concordance between the non-clinical and clinical data and how the former can be used to reduce the demands for a human TQT study. Thus the objectives of this review are to evaluate the available literature that has attempted to assess the concordance between non-clinical and clinical QT data.

QT PRODACT and ILSI-HESI initiatives

Following the publication of ICH S7B, pharmaceutical companies belonging to the Japan Pharmaceutical Manufactures Association conducted a series of prospective studies to investigate the concordance between non-clinical findings and clinical outcome with respect to QT prolongation and TdeP – 'QT Interval Prolongation: Project for Database Construction, QT PRODACT'. A series of compounds known to cause QT/TdeP changes in humans (astemizole, bepridil, cisapride, disopyramide, E-4031, haloperidol, MK-499, pimozide, quinidine, terfenadine and thioridazine) and compounds known to be devoid of such effects (amoxycillin, aspirin, captopril, ciprofloxacin, diphenhydramine, flecainide, lidocaine, nifedipine, propranolol and verapamil) were evaluated in a range of non-clinical assays. Seven out of the 11 positive controls caused a robust prolongation of APD_{90} (action potential duration at 90% repolarization) in the isolated guinea pig papillary muscle (Hayashi *et al.*, 2005) while two compounds (pimozide and terfenadine) were without effect and astemizole, bepridil and quinidine had only marginal effects. In contrast only one compound from the negative controls, flecainide, prolonged APD_{90}. In addition to studying the effects of compounds on APD the effect on the shape of the action potential was also investigated through calculating $APD_{30–90}$. The authors found that this measure correlated better with human outcome for the positive controls with only terfenadine being inactive. However, 50% of the negative controls were also found to be active in this assay suggesting a limited ability to discriminate between true positives and false positives. Nevertheless, the authors conclude that the guinea pig papillary muscle may be useful to identify compounds that prolong the QT interval in humans.

The same compounds were studied for their effects on QTc in conscious telemetered dogs following oral dosing (Toyoshima *et al.*, 2005). All of the positive controls prolonged the QTc interval at plasma concentrations similar to those that prolonged QT in humans. Importantly, with the exception of nifedipine, none of the negative controls prolonged the QTc interval in dogs, and this 'false positive' effect was attributed to the failure to appropriately correct for the increase in heart rate. Although these findings strongly support the predictive value of the dog for QT changes in humans, one of the limitations of this study was that often the maximum effects on QTc did not correlate with the peak plasma exposure. For example the peak QTc effects of the low and mid-dose of bepridil occurred at 16–20 h post dose whereas the peak plasma concentrations occurred at 1–1.5 h post dose. Thus the ability to define a safety margin from these studies was limited.

Studies have also been conducted in the cynomolgus monkey, and in this species none of the negative control compounds prolonged QTc suggesting that the specificity (i.e. a low false negative rate) of this assay was good (Ando *et al.*, 2005). However, haloperidol, terfenadine and thioridazine all failed to significantly prolong QTc in the monkey; the reason for this is unknown, but it is evident from the data that all compounds increased QTc by at least 25 ms. This suggests that these studies were insufficiently powered to detect the desired effects – group size numbers were only four animals per group and greater effort needs to be taken to reduce inherent data variability. Studies in our own laboratories demonstrate that larger group sizes are required to design an appropriately powered study.

A similar prospective programme of work was sponsored by the International Life Sciences Institute and Health Environmental and Sciences Institute (ILSI-HESI). In this study six compounds associated with TdeP in humans (bepridil, cisapride, haloperidol, pimozide, terfenadine and thioridazine), and six compounds believed to be devoid of such effects (amoxycillin, aspirin, captopril, diphenhydramine, propranolol and verapamil) were tested in hERG, canine Purkinje fibre and conscious telemetered dog assays (Hanson *et al.*, 2006). All of the positive controls were relatively potent inhibitors of the hERG current (IC_{50} values less than 300 nM) and, with the exception of verapamil (IC_{50} 180–253 nM), the negative controls were either inactive or weak inhibitors (with IC_{50} values greater than 1866 nM). Consistent with the PRODACT study, prolongation of APD_{90} in the Purkinje fibre repolarization assay only detected two out of six positive controls (cisapride and haloperidol) while one of the negative controls (amoxycillin) was active. However, again as in the PRODACT study a measure of the shape of the action potential, in this case, $APD_{40–90}$, was more sensitive and detected five of the six positive controls. Consistent with PRODACT, all of the positive controls prolonged the QTc interval (when corrected using Fridericia's correction factor) in the conscious telemetered dog. This was not observed with the negative controls. An important, yet perhaps expected, observation from this study was the impact of heart rate correction factors on the concordance between the dog and human outcomes: the predictive value being considerably less if Bazett's formula was used. Thus, it is essential that the choice of correction factors is considered when correcting for heart rate with cardiovascularly active compounds and that each laboratory must justify the heart rate correction factor in their colony of dogs.

Both the PRODACT and ILSI-HESI studies support the concordance for the hERG electrophysiological assay and conscious dog to predict clinical outcomes for QT prolongation/TdeP. However, one of the weaknesses of these studies was the limited analysis of the pharmacokinetic/pharmacodynamic (PK/PD) relationships in the *in vivo* studies and the limited correlation with human exposure data.

Analysis of safety margin approach

Although not prospective, a number of authors have attempted to define the value of a safety margin approach to better predict human QT prolongation/TdeP liability from non-clinical studies. This approach has been driven by the recognition that the hERG channel is very promiscuous and will bind a very wide range of chemotypes at high concentrations (Stanfield *et al.*, 2006). Thus simply assessing inhibitory potency of a compound against the hERG current and QT prolongation in animals with no reference to human exposure has the potential to identify a high false positive rate (i.e. low specificity) for the non-clinical assays.

In an analysis of 15 compounds, Webster *et al.* (2002) demonstrated a strong correlation (R^2 = 0.8111) between a compound's potency to inhibit the hERG channel and it's free plasma concentration in humans, which was associated with QT prolongation/TdeP. Furthermore, all compounds associated with QT prolongation/TdeP were found to have *in vitro* safety margin (hERG IC_{50} value/free therapeutic concentration) of less than 30-fold while most compounds devoid of QT/TdeP liability had a safety margin of 50-fold or greater. There were exceptions to this: terfenadine (93-fold) and tacrolimus (700-fold). However, in contrast to the hERG safety margin, both of these compounds had much smaller safety margins (one- to threefold) when evaluated for QT prolongation using *in vivo* assays. In the case of terfenadine this can best be explained by the observation that plasma concentrations following therapeutic doses of terfenadine are very low, hence the high safety margin over hERG inhibition. However, in the presence of metabolic inhibition the levels of terfenadine are elevated to concentrations that inhibit hERG, prolong the QT interval and are associated with TdeP (Monahan *et al.*, 1990). Thus this small safety margin correctly predicts the TdeP risk of terfenadine in clinical use. The data for tacrolimus suggest that this compound prolongs the QT interval through a non-hERG-mediated mechanism that can be detected *in vivo* (Minematsu *et al.*, 1999). Thus, Webster *et al.* propose that safety margins based on free drug plasma concentrations may be an effective way to identify the risk of compounds causing TdeP in humans.

In an extension of this work, Redfern *et al.* (2003) conducted a literature review of 52 drugs classifying them into five categories: category 1 were agents that prolonged repolarization as the primary pharmacology (e.g. class Ia and III antiarrhythmics), category 2 were drugs withdrawn or suspended from the market because of a known TdeP risk (e.g. cisapride), category 3 were drugs that had measurable incidence of TdeP in humans (e.g. thioridazine), category 4 were drugs for which there were isolated reports for TdeP in humans (e.g. fluoxetine), and category 5 were drugs that had no published reports of TdeP in humans could be found. All compounds in categories 1 and 2, for which there were literature reports of QT prolongation in animals, had small safety multiples (<30-fold) for the clinical therapeutic free plasma concentration when compared with concentrations that prolonged QT in animals. Unfortunately there was a paucity of published non-clinical data for the category 5 compounds to demonstrate that these compounds were devoid of effects on the QT interval in animals. The most robust data set was obtained by comparing published hERG inhibitory potency with the clinical therapeutic plasma concentrations. All compounds in categories 1, 2 and 3 had hERG safety margins of less than 30-fold, with the exception of tedisamil (31-fold) and amiodarone (1400-fold). Despite a large safety margin over inhibition of hERG amiodarone had a small safety margin for QT prolongation *in vivo* suggesting that this compound also prolongs QT in humans through a non-hERG mechanism. In contrast to categories 1, 2 and 3, compounds in category 5 tended to have a hERG safety margin of greater than 30-fold. Notable exceptions to this were verapamil (<10-fold) and ketoconazole (11-fold). Verapamil is an interesting case study because the compound potently inhibits the hERG current but does not prolong QT *in vivo* in animals and man following oral dosing at therapeutic doses, although supratherapeutic plasma levels have been shown to prolong QTc in cancer patients (De Cicco *et al.*, 1999). This is believed to be due to its potent calcium channel blocking activity that will reduce APD and will negate the QT prolonging effects of I_{Kr} current block (Chouabe *et al.*, 1998).

A further more sophisticated approach to investigating the value of using safety margins to predict human TdeP liability has been conducted by De Bruin *et al.* (2005). Data from the International Drug Monitoring Program of the World Health Organisation were analysed to determine the 'reporting odds ratios' (RORs) for a composite end point of cardiac arrest, sudden death, TdeP, ventricular tachycardia and ventricular fibrillation for 49 drugs. This was compared with the ratio of the effective free therapeutic clinical concentration and hERG IC_{50} value. The authors found that ROR for compounds with a safety margin of 10-fold or less was sixfold greater than that for compounds in which the safety margin was 1000-fold. Furthermore, there was a strong correlation between the hERG safety margin and the logarithm of case events (cases/non-cases).

Taken together these studies demonstrate a good relationship between the hERG safety margin and TdeP risk in humans when the data are supported by *in vivo* QT data. However, given safety margins are a continuum it would be inappropriate to suggest that a given safety margin, for example 30-fold, is able to distinguish between compounds that cause TdeP and those that do not. For example, verapamil has a modest hERG safety margin and low risk of TdeP, whereas amiodarone has a very large safety margin but a well-defined TdeP risk (Sager, 2008). However, in contrast to amiodarone, verapamil does not prolong the QT interval in animals. Thus, a safety margin based on a combination of hERG and *in vivo* data would appear to provide a guide to the QT prolonging potential of new chemical entities.

Concordance between non-clinical and human clinical QT studies

The above studies reviewed the outcomes of a wide range of divergent drug classes based on the exposure of millions of patients with the focus on defining the relationship between non-clinical findings and the clinical outcome of TdeP. With the adoption of ICH E14 another critical question to emerge is to understand the relationship between non-clinical findings and the outcome of a clinical QT study. This is particularly important given the cost of conducting these studies and more importantly the impact of the study outcome; for example if the study is positive this may lead to discontinuation of the drug from development or would necessitate the need for intensive ECG monitoring in phase III clinical trials. This is also a very challenging question to address because an increase in QT of approximately 5–10 ms constitutes a positive effect in the clinical QT study and yet there are no accepted criteria for a positive effect in the non-clinical studies. Based on a detailed PK/PD model of the effects of dofetilide in humans it has been suggested that a 10% inhibition of hERG currents by dofetilide corresponds to 20 ms of QT interval prolongation (95% confidence interval, 12–32 ms) (Jonker *et al.*, 2005). Thus, rather than the traditional 50% inhibitory concentration being considered a positive effect in the hERG assay, these data suggest that a 5–10% inhibitory concentration may more accurately predict a QT change in humans of 5–10 ms. Likewise, although the alometric scaling from animals to humans for QT changes has not been robustly defined, it is evident that a commonly used change of 10% prolongation of the QT interval in animals (~25 ms; Hanson *et al.*, 2006) may not have adequate sensitivity to predict 5–10 ms (~1.5–3%) change in humans and that a change of ~10 ms (~4%) in animals may be more appropriate.

Based on the above criteria for an effect in non-clinical studies (10% inhibition of hERG, 10% prolongation of APD_{90} in canine Purkinje fibres and 10 ms prolongation of QTc in dogs), in this manuscript the author has reviewed 19 compounds that have been evaluated in clinical QT studies that were designed to detect a 7–10 ms QTc change. Although all of these studies have included a positive control, to demonstrate assay sensitivity, some were conducted prior to the adoption of ICH E14 and therefore may not meet all the criteria for a clinical TQT study. Nevertheless they were all prospective studies dedicated to investigating compound effect on the QT interval. Thirteen of the compounds were studied in healthy volunteers and the remaining six compounds studied in subjects with psychotic disorders. Eleven compounds produced a QTc prolongation that exceeded the predefined study sensitivity while eight compounds were considered negative.

Of the 11 compounds active in the clinical QT study, nine of these caused 10% inhibition of the hERG channel at approximately the same (up to twofold) unbound drug concentrations achieved in the clinic and were therefore considered 'true positives' for predicting the clinical outcome (Table 1). In contrast, two compounds were negative and considered 'false negatives'. Of the eight compounds negative in the clinical study, six were negative in the hERG assay (true negatives) and two were positive (false positives). From this analysis the sensitivity (true positives divided by the sum of true positives and false negatives) and specificity (true negatives divided by the sum of the true negatives and false positives) can be calculated for each assay. In addition, the analysis was repeated by comparing the free drug levels achieved in the clinic with 10- and 30-fold higher concentrations in the non-clinical assays. Data are shown in Table 2.

Specific examples include moxifloxacin that prolonged the QTc interval in a number of healthy volunteer studies by 14–19 ms following a single dose of 400 mg. Peak plasma levels following this dose were 4 μM free drug and at this concentration the compound inhibited the hERG current (0.5 μM), prolonged the APD (7.5% at 10 μM) and prolonged QTc in the dog following exposure to free drug levels of 8 μM. Thus in the case of moxifloxacin the non-clinical data predicted the clinical outcome. In contrast, risperidone had no effect on the QTc interval in patients with a psychotic disorder (Harrigan *et al.*, 2004) following exposures of approximately 30 nM free drug, but inhibited the hERG current (10% inhibition at 8 nM) and prolonged APD (26% at 320 nM). Thus by comparing exposures in non-clinical and clinical

Table 1 Concordance between human *ether-a-go-go* inhibitory activity and clinical QT study outcome

	Human –ve			*Human +ve*		
	×2	*×10*	*×30*	*×2*	*×10*	*×30*
Non-clinical –ve	6	3	1	2	1	1
Non-clinical +ve	2	5	6	9	9	9

Tables show number of studies that demonstrated positive or negative effects in non-clinical and in clinical QT studies based on non-clinical concentrations up to 2- (×2), 10- (×10) and 30- (×30) fold higher than clinical free plasma concentrations.

Table 2 Sensitivity and specificity of non-clinical QT assays to predict the outcome of a clinical QT study

Clinical exposure multiple	*Human* ether-a-go-go		*Canine Purkinje fibre*		In vivo *dog*	
	Sensitivity	*Specificity*	*Sensitivity*	*Specificity*	*Sensitivity*	*Specificity*
2	0.82	0.75	0.2	1.0	0.83	0.86
10	0.90	0.38	0.33	0.80	0.83	0.33
30	0.90	0.14	1.0	0.40	0.83	–

Sensitivity is defined as the number of true positives divided by the sum of true positives and false negatives and specificity as the number of true negatives divided by the sum of the true negatives and false positives.

studies it would appear that hERG current inhibition by risperidone was a false positive.

This type of analysis has demonstrated that when the same concentrations are compared between the non-clinical and clinical assays the hERG and *in vivo* assay have good sensitivity and specificity. When an integrated risk assessment is undertaken, that is using a combination of *in vitro* ion channel and *in vivo* non-clinical data, to predict the risk of QT prolongation in man (data not shown) the sensitivity (0.90) and specificity (0.88) is increased, because one of the false negatives in the hERG assay was active *in vivo*. In contrast, and consistent with the ILSI-HESI and PRODACT studies the canine Purkinje fibre was shown to have poor sensitivity, but high specificity. This implies that compounds that prolong the APD_{90} in the Purkinje fibre have a high probability of causing QT prolongation in humans. When high concentrations in the non-clinical assays are compared with the clinical outcome it is apparent that the sensitivity of hERG and *in vivo* assays does not change although the specificity is decreased. This can be explained by the aforementioned hERG promiscuity (Stanfield *et al.*, 2006) in that a large number of compounds will bind to the hERG channel at concentrations that are not achieved in humans.

Within this present data set there is one compound that caused a modest and poorly dose-related prolongation of QTc in humans that was devoid of effects on hERG, Purkinje fibre and in the conscious dog even up to 30-fold higher concentrations achieved in the clinic. This compound had a mild vasodilator effect and is believed to prolong QTc through mechanisms similar to sildenafil and vardenafil (Morganroth *et al.*, 2004) that also prolonged QTc in humans at concentrations below those required to inhibit the hERG channel.

The overall conclusion from this analysis is that by comparing the same concentrations observed in non-clinical and clinical studies the effects on the hERG channel and *in vivo* are predictive of the clinical outcome for QT prolongation. This is strengthened if an integrative analysis is undertaken rather than using each assay in isolation.

Improving the predictive value of non-clinical QT assays for human outcome

Following this review of the available data assessing the concordance between non-clinical and clinical QT data it is evident that further work can be undertaken to improve the confidence in translation. The first is to recognize that small changes in the QT interval (5–10 ms) represent a change of regulatory concern. Thus if the non-clinical assays are to minimize the risk of compound attrition due to QT issues (changes of this magnitude *per se* do not represent a risk to healthy volunteers) they need to be able to predict, with confidence, these small changes in humans.

The conscious telemetered dog appears to have good predictive value for human outcome. It is quite possible that the monkey also has the same predictive value, but this was not convincingly demonstrated with statistically significant changes in the PRODACT study. This may in part be related to the low animal numbers per group and high variability in the data and therefore a low power to detect an effect. Analysis of Pfizer in-house studies suggests that group sizes of six monkeys are required for the study to have an 80% power to detect a 10 ms QT change (data not shown). Although regulatory guidance is available for clinical QT study designs, no such standards exist for non-clinical studies. Regulatory guidance for specific study designs is not appropriate, but studies need to be designed with the appropriate power to detect the small changes in the QT interval that would be predictive of an effect of concern in humans. It is also important to consider how best to communicate the study power when describing non-clinical studies to ensure that if no drug effect has been described then this represents a genuine no-effect and not the consequence of an insufficiently powered study.

The emerging scientific data do support the use of safety margins to determine the liability of new chemical entities to prolong the QT interval. However, in order to define a safety margin a detailed analysis of the exposure response relationship for a drug effect is required. There is an increasing interest in the application of PK/PD modelling to better define safety margins in safety pharmacology (Cavero, 2007). Such approaches are able to take into account any time delays for an effect to be observed relative to a change in drug plasma concentrations and to attempt to predict the human PK/PD response. This has been applied to the effects of dofetilide on the QTc interval in the dog in which a time delay of 11 min was described with a maximum response of 59 ms (Ollerstam *et al.*, 2006). A wider application of PK/PD modelling in non-clinical studies will undoubtedly improve the predictive value of non-clinical studies, especially when attempting to predict relatively small effects on, for example, the QTc interval at a given human exposure that may be at the efficacious concentration or supratherapeutic concentration to be tested in a clinical TQT study.

The translational aspects of drug-induced QT interval prolongation have focused on block of the hERG channel as the molecular target. Although this appears to be the case for a vast majority of drugs there are exceptions to principal, for example vardenafil and sildenafil (Morganroth *et al.*, 2004). It is widely believed that such agents increase the QTc interval through an effect on autonomic tone (Berger *et al.*, 2005). These effects have been difficult to reproduce in animals and further research is required to better understand these mechanisms and therefore improve our understanding of the translation of these effects between animals and man.

Conclusions

Drug-induced prolongation of the QT interval remains an important regulatory and human drug safety issue. The need to understand the translation of effects in animals to humans is essential given the fact that nearly all pharmaceutical companies are screening newly synthesized compounds for their potential to inhibit the hERG current. Frequently compounds are only progressed to human trials if the safety margin over the projected therapeutic exposure is considered adequate for the therapeutic indication. Thus critical decisions are being made on the progression of compounds to the next phase of testing based on data derived from the non-clinical QT assays.

If the non-clinical assays only poorly predict the human QT liability many potential life-saving drugs may be discarded or drugs may be advanced to clinical trials despite having a significant liability to prolong the QT interval.

The available data suggest that a vast majority of compounds that prolong the QT interval do so via inhibition of the hERG current. The data reviewed in this manuscript suggest that the translation of hERG inhibition to QT prolongation in non-rodents is well defined. There are exceptions to this, for example when additional pharmacology properties attenuate the QT prolonging effects of hERG inhibition and these considerations need to be taken into account when interpreting these data. The translation of hERG inhibition to QT prolongation in non-rodents is supported by the observation that the pharmacology of the dog homologue of the hERG channel shares the same pharmacology as hERG (Wang *et al.*, 2003). Furthermore the translation of QT prolongation in non-rodents to humans for hERG blockers is also very good. The importance of a combination of non-clinical assays is demonstrated with compounds such as verapamil that inhibits the hERG current, but because of its additional calcium channel blocking properties does not prolong the QT interval in animals or in humans (Chouabe *et al.*, 1998). Although verapamil is often quoted as an 'outlier' because it does not prolong the QT interval *in vivo* despite potent hERG blocking properties, it does reinforce the confidence in translation from non-rodent *in vivo* studies from animals to man as this compound does not prolong the QT interval in animals or humans at therapeutic doses.

In many respects it is not surprising that hERG inhibition translates well from *in vitro*, to *in vivo* and then to humans because this represents translation of human pharmacology. It is also clear that other mechanism can prolong the QT interval and the translatability of these effects from animals to humans is less well defined, and further research is required to better define the translation for these mechanisms. One compound was identified in the TQT data set that caused a modest prolongation of the QTc interval in humans that was not detected in animals. It is significant to note that this compound, similarly to vardenafil and sildenafil, did not prolong the QT interval, but only the corrected QT interval. In addition the effects on QTc were not proportional to dose and both of these characteristics are quite distinct from hERG blockers. Likewise, the risk for causing TdeP is unknown. The α-adrenergic receptor blocker, alfusosin, has also been shown to have a similar modest effect on the QTc interval without being associated with an increased risk of TdeP (Lacerda *et al.*, 2008).

In conclusion, there is strong evidence for translation of hERG inhibition to QT prolongation in man. However, given the promiscuity of the hERG channel it is critically important that effects on the hERG channel are placed in context with the human efficacious plasma concentrations and such an approach can be used to minimize the risk of QT prolongation being observed with new chemical entities. However, further research is required to understand the translation of non-hERG-mediated QT prolongation both in animals and in man, for example through changes in autonomic tone or through the modulation of other cardiac ion channels. This will better inform us of the translation from animals to man and to design new assays with the potential to test for mechanism that appear to prolong the QTc interval in humans, but not in animals.

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