Improving prediction of torsadogenic risk in the CiPA in silico model by appropriately accounting for clinical exposure

Derek J. Leishman

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Title: Improving prediction of torsadogenic risk in the CiPA *in silico* model by appropriately accounting for clinical exposure.

Author Name: Derek J Leishman

Author Affiliation: Eli Lilly and Company

Corresponding Author: Derek J Leishman

Address: Drug Disposition, Toxicology and PKPD, Eli Lilly and Company, Indianapolis IN 46285 USA

Abstract

Any adverse event is reliant on three properties: the appropriate pharmacology to trigger the event, the appropriate exposure of compound, and intrinsic patient factors. Each alone is necessary but insufficient to predict the event. The Comprehensive *in vitro* Proarrhythmia Assessment (CiPA) initiative attempts to predict the risk of torsade de pointes (TdP) by focusing on an *in-silico* model with thresholds determined at modest multiples of the therapeutic exposure for the parent molecule. This emphasizes the pharmacologic properties necessary for TdP but does not account for situations where clinical exposure may be higher, or where hERG potassium channel active metabolites are involved. Could accounting for clinical worst-case scenarios and metabolites, as is already standard practice in thorough QTc studies, improve the prediction algorithm?

Terfenadine, a drug classed as "Intermediate" risk by CiPA, was assessed differently in the *in-silico* model validation. The clinical concentration of terfenadine used for the model was the exposure in the presence of metabolic inhibition representing a 14 to 40-fold increase in exposure compared to the therapeutic plasma concentration. However, several other "Intermediate" risk compounds are also known to be sensitive to metabolic inhibition and/or to have therapeutically active major metabolites, some of which are known to block hERG. Risperidone and astemizole are relevant examples. If only parent exposure is used to calculate a therapeutic window, risperidone has a relatively large multiple between clinical exposure and the hERG potency. Using this exposure of risperidone, the drug borders the "Intermediate" and "Low/No" risk categories for the CiPA *in-silico* model's TdP metric. The desmethyl metabolite of

astemizole likely contributes significantly to the effects on cardiac repolarization, being equipotent on hERG but circulating at much higher levels than parent. Recalculating the TdP metric and margin values for terfenadine, risperidone and astemizole using the unbound concentration normally associated with treatment and a clinical worst case changes the qNet metric to higher risk values and illustrates the potential benefit to the algorithm of consistently using a clinical high exposure scenario accounting for all "hERG-active species". This exercise suggests repeating the model qualification accounting for clinical exposures and metabolites under 'stressed' scenarios would improve prediction of the TdP risk.

Keywords: hERG, CiPA, safety margin, torsade de pointes, *in silico* model, model qualification

Abbreviations:
TdP – torsade de pointes,
QSP – quantitative systems pharmacology,
I_{Kr} – rapidly activating inward rectifier potassium current,
CiPA – Comprehensive in vitro Proarrhythmia Assessment,
qNet – net current flowing during cardiac action potential – a metric described as predictive of torsadogenic potential,
TQT – thorough QTc,
DDI – drug-drug interaction

Introduction

Determining whether a potential new drug has the properties to cause the ventricular arrhythmia, torsade de pointes (TdP), has been a key activity in drug development for two decades. TdP was first described as an effect of regulatory interest in a 'Points to Consider' paper from the European Medicines Agency in 1996 (CPMP/986/96 Points to Consider: The assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products. Adopted December 1997). Since the "Points to Consider" document, it has been recognized that the existing methods of *in vitro* hERG potassium channel assessment and the *in vivo* preclinical evaluation along with the clinical assessment of effects on the electrocardiogram QTc interval are sensitive in detecting torsadogenic liability but may lack adequate specificity (Sager et al., 2014).

In assessing the potential to delay cardiac repolarization in thorough QT studies (TQT) it is common practice to examine both therapeutic exposures and a 'high clinical' exposure representing situations where exposure may be increased under the influence of intrinsic and extrinsic factors such as drug-drug interaction and disease states. Where active metabolites are involved the effect of each analyte is explored (Garnett et al., 2018). In early evaluations of the margin between hERG potency and therapeutic unbound plasma concentrations it was clear that terfenadine was an anomaly (Redfern et al., 2003; Webster et al., 2002). Despite being associated with clinical cases of TdP the margin between free therapeutic plasma concentration for efficacy and the concentrations necessary for antagonism of hERG was larger than the 30-fold rule of thumb which developed from these initial publications. This anomaly was

reconciled when the effects of metabolic inhibition on terfenadine exposure were considered. Increased free plasma terfenadine exposure, in the presence of metabolic inhibition was associated with dramatically delayed cardiac repolarization (Honig et al., 1993; Honig et al., 1992).

The Use of *In Silico* Models to Predict TdP Risk

The desire for improved specificity in torsadogenic liability prediction, coupled with the increased understanding of cardiac electrophysiology gained over the past 20 years, has spurred the use of newer technologies. Notable among these newer approaches is the use of a quantitative systems pharmacology (QSP) model of human ventricular myocyte electrophysiology. These QSP models take advantage of the fact that while early in drug discovery, knowledge on the overall properties of a molecule may be sparse, the field is 'knowledge rich' in terms of understanding of the underlying physiology. The ventricular myocyte QSP model has evolved since the original description in 1962 (Noble, 1962) and has been tested and improved over the intervening years. Using only data on potency at ion channels and a clinical concentration as inputs in the QSP model a prediction of effects on cardiac repolarization or of proarrhythmia liability can be generated (Mishra, Polak, Jamei, & Rostami-Hodjegan, 2014; Leishman, 2014; Li et al., 2017; Passini et al., 2017). It is well recognized however that these *in silico* models will simply and consistently reflect the inputs to the model in the model outputs. If the model outputs are to improve the sensitivity and specificity of torsadogenic risk prediction the model inputs need to be appropriate.

In order for any drug to have an adverse event, such as TdP, there is a hierarchy of properties which need to be satisfied. Firstly, the drug and/or its metabolites need to have the right pharmacologic profile. In the case of TdP this is a dominant effect of the drug and/or its metabolites on the rapidly-activating inward rectifier potassium (I_{Kr}) current in ventricular cells. Secondly, the drug and/or its metabolites need to achieve sufficient exposure at the site of action. In the case of TdP this would be in the cardiac tissue. Lastly, there are concomitant risk factors, unique to the patient, which contribute to the realization of the adverse event when appropriate concentrations are achieved. These include things like hypomagnesemia, hypokalemia, sex, recent myocardial ischemia and genetic differences in cardiac ionic currents and electrogenic pumps. The exposure to drug and/or metabolites and the patient dependent concomitant risk factors are influenced by intrinsic and extrinsic factors such as drug-drug interaction (DDI; extrinsic), underlying disease state (intrinsic) and sex (intrinsic). Alone each of these components, pharmacology, exposure, and patient-dependent risk factors, is necessary but insufficient to cause the adverse event. All must be aligned for generation of an adverse event. Any model seeking to determine relative risk of being associated with TdP would intuitively then need to take in to account each of these properties. The model described by Passini and colleagues (Passini et al., 2017) has started to add a capability to examine genetic variation in ion channel and electrogenic pump expression and function. The prediction task can however be simplified by focusing on a model to predict the relative risk of being associated with TdP in even a single patient, thus ignoring the contribution of many individual risk factors that might otherwise have identified which patients might be at risk. This simpler

objective then requires that the model only needs to appropriately address pharmacological properties and the expected or achieved exposure. These are the critical inputs for the model.

In one early report of a predictive model for torsadogenic potential Redfern and colleagues (Redfern et al., 2003) subclassified drugs in to five categories: “antiarrhythmic drugs”, “withdrawn drugs”, “probable TdP”, “possible TdP” and “no TdP”. The lists of drugs associated with TdP at the website www.CredibleMeds.org also classify drugs as having “Known TdP risk”, “Possible TdP Risk”, or “Conditional TdP Risk”. More recently the CiPA initiative (Li et al., 2018) used a classification of drugs into “High”, “Intermediate” and “Low/No” risk TdP categories. All these classifications suggest that rather than a simple dichotomous classification there are differences in the extent to which compounds are torsadogenic and these differences are a reflection of the three properties necessary for an adverse event to be manifest described above. Since the exposure attained in patients and patient-dependent risk factors are influenced by intrinsic and extrinsic factors it seems reasonable that these aspects are critical in determining possible, conditional or intermediate risk relative to probable, high or no risk.

Three Exemplar Drugs Illustrative of the Need to Systematically Evaluate Exposure

Three drugs from the CiPA “Intermediate” risk group are exemplars to illustrate the contribution of exposure in risk classification. The first example is terfenadine. Terfenadine was an effective and extensively used non-sedating anti-histamine (Honig et al., 1993). In terms of clinical exposure terfenadine undergoes extensive first pass metabolism at the small intestinal wall and in the liver by CYP3A (Honig et al., 1992). Terfenadine effectively has a very

low oral bioavailability. The primary metabolite of terfenadine, fexofenadine, is also active as a histamine antagonist and was likely responsible for some efficacy of the drug as an anti-histamine. When the metabolism of terfenadine is blocked, by a CYP3A inhibitor, the exposure to terfenadine increases dramatically (Honig et al., 1993). Terfenadine is a relatively potent hERG blocker, while fexofenadine is much less potent (Rampe et al., 1993). Honig and colleagues demonstrated clearly that in the presence of ketoconazole, an inhibitor of CYP3A, the plasma concentrations of terfenadine are considerably higher (Honig et al., 1993). The extent of the increase varied across the subjects examined. They further demonstrated that there was a concentration dependent increase in the electrocardiogram QTc interval well in excess of 10 ms, a commonly used threshold. Honig and colleagues describe that although terfenadine was extensively prescribed, the total prescription rate for ketoconazole and terfenadine together was only 0.2% of terfenadine prescriptions in 1992. The extensive use of terfenadine, the variable extent of concentration increases in the presence of ketoconazole and the relatively low probability of prescription with a strong CYP3A inhibitor may explain why terfenadine was not initially identified to pose a TdP risk during drug development but has since been classified as “Intermediate” risk and not “High” risk.

The second example drug is risperidone. Risperidone is metabolized in man by CYP2D6 (US Label, Risperdal), an enzyme known to have genetic polymorphisms impacting extent of metabolism. The primary metabolite is 9-hydroxyrisperidone (Mannens et al., 1993). The levels of 9-hydroxyrisperidone achieved in man are appreciable (as high or higher than parent) and the molecule has similar but not identical pharmacology to the parent molecule (Corena-

McLeod, 2015). The extent of exposure and the pharmacologically similar properties of 9-hydroxyrisperidone are well recognized since the product label for risperidone describes the exposure to the risperidone active moiety and combines both parent and metabolite in that calculation (US label, Risperdal). The metabolite 9-hydroxyrisperidone has also been developed as an antipsychotic compound, paliperidone. The distribution of risperidone and metabolite in the plasma also involves binding to α_1-acid glycoprotein (Mannens et al., 1994). These metabolic and protein binding complexities mean that levels of unbound risperidone and paliperidone can be influenced by genetics, concomitant medication and disease states. The US label for oral risperidone states "Increased plasma concentrations of risperidone and 9-hydroxyrisperidone occur in patients with severe renal impairment (creatinine clearance <30 mL/min/1.73 m^2), and an increase in the free fraction of risperidone is seen in patients with severe hepatic impairment. A lower starting dose should be used in such patients". Some patients have appreciable exposure to parent and metabolite, for example in a group of female Chinese patients steady state exposures of nearly 100 ng/ml risperidone and over 100ng/ml paliperidone were observed (Zhou et al., 2006). Both risperidone and paliperidone are hERG blockers (Vigneault et al., 2011; Kramer et al., 2013). Although, the US label describes no QTc prolongation being observed in many analyses, post-marketing experience and a clinical thorough QT study have demonstrated a modest QTc interval prolongation with both risperidone (Harrigan et al., 2004) and paliperidone (US label, Invega Sustenna). The QTc prolongation following administration of risperidone has been described as being largely driven by the metabolite paliperidone (Suzuki et al., 2012). The impact of intrinsic and extrinsic factors on exposure to risperidone and paliperidone may explain why some authors described this

compound as not associated with TdP (Redfern et al., 2003) yet it was recently classified as an "Intermediate" risk drug and a "Possible TdP Risk" drug.

The third example is astemizole. The astemizole case mirrors the terfenadine case in many ways. Oral astemizole undergoes extensive first pass metabolism (Heykants et al., 1986) and resulting plasma exposures are relatively low. However, the principal metabolite, desmethylastemizole, circulates at appreciable levels and has a long terminal half-life (13 days). The pharmacological properties of the metabolite are similar to parent and this accounts for the anti-histamine efficacy of astemizole despite the extensive first pass metabolism. Where this differs from terfenadine is in that both astemizole and desmethylastemizole are potent hERG blockers. The IC_{50} values are 0.9 nM and 1 nM for astemizole and desmethylastemizole, respectively (Zhou et al., 1999). The long terminal half-life of the metabolite contributes to the appreciable steady state exposures well in excess of parent exposure if the drug is taken regularly. Cases of torsade de pointes with astemizole have been reported in overdose, usually associated with very long QTc intervals (Rao et al., 1994), and when dosed with CYP3A inhibitors ketoconazole and erythromycin. A case report of torsade de pointes with astemizole treatment illustrates that the patient was on multiple medications and that the exposure to the desmethyl metabolite on hospital was still appreciable (Vorperian et al., 1996). Astemizole was withdrawn from the market in 1999. Unfortunately, most plasma concentration data for astemizole is described in terms of combined parent and metabolite and plasma protein binding data for the metabolite is not available although it might be assumed the unbound fraction is the same as that for parent if not larger. The National Medical Services consider

combined levels of astemizole and its metabolite of around 20 ng/ml (approximately 1.5 nM unbound concentration; Vorperian et al., 1996) to be toxic.

Other drugs in the CiPA "Intermediate" risk category have metabolites which circulate at appreciable levels that contribute to the therapeutic effect (e.g. clarithromycin; Rodvold, 1999), are known substrates for CYP enzymes, are subject to inhibition of metabolism by other drugs or influenced by genetic polymorphisms. Overall, this suggests that the drugs in the "Intermediate", "Possible TdP Risk" and "Conditional TdP Risk" groups need a systematic evaluation of their pharmacological and pharmacokinetic properties if they are to be appropriately evaluated in an *in silico* or other predictive model.

The CiPA *In Silico* Model

The Comprehensive *in vitro* Proarrhythmia Assessment (CiPA) initiative used an *in silico* QSP model for TdP liability prediction and described the model qualification process has been described (Li et al., 2018). The qualification describes a model output metric used for torsadogenic potential, qNet, based on the model inputs of ion channel potencies for inhibition of key ionic currents, kinetics of block at the hERG channel and unbound therapeutic plasma concentration. Some measure of output variability is incorporated in the model algorithm by accounting for variability in the inputs. A Bayesian estimator is used to incorporate some variability in the hERG potency input and some exposure variability is incorporated in the concentration input by using the mean of 1- to 4-times the efficacious unbound plasma concentration. Two threshold qNet values separating compounds in to "High", "Intermediate"

or “Low/No” risk groups were described and would be used to ‘bin’ new compounds in to the different risk categories. The qNet metric is the net current during a simulated action potential triggered at a rate of 0.5 Hz (equivalent to a heart rate of 30 beats per minute). The metric is only available as an output from the *in silico* model and cannot be experimentally derived *in vitro* or *in vivo*. As this output from the model is the chosen metric of torsadogenic risk (Li et al., 2018) it is critically important to examine the quality of the inputs; as inputs determine the output in a fashion sometimes described as ‘garbage in, garbage out’.

In the CiPA *in-silico model* qualification the exposure level of terfenadine used is not the therapeutic level (Li et al., 2018), rather the exposure used reflects the average elevated exposures observed under metabolic inhibition representing a 14 to 40-fold multiple over therapeutic unbound concentration (compare Redfern et al., 2003). Furthermore, in the CiPA model assessment the qNet metric is determined as an average of the qNet values determined at multiples of 1- to 4-fold of this utilized exposure value. In contrast, the exposure to risperidone and astemizole used in the model qualification reflect only the therapeutic levels of parent and do not account for the circulating levels of metabolites. Neither are conditions of possible elevated exposure to parent and/or metabolite under DDI or disease state considered. Terfenadine was the only drug in the model qualification where the therapeutic exposure was not used, and in no cases were the contributions of metabolites considered. For *apriori* decisions, the *in-silico* model cannot be considered qualified when drug exposure is not consistently applied and when the ‘high-clinical’ exposure scenarios are not systematically explored.

Could Systematic Consideration of 'High Clinical' Exposure Influence Predictions?

The potential impact of appropriately accounting for both drug and metabolites and 'high clinical' exposure scenarios is illustrated in the current analysis by examining the impact on both the hERG margin and the qNet torsadogenic potential metric for terfenadine, risperidone and astemizole. The intent of the current analysis is to highlight, through the three exemplar drugs, how to use the exposure data for torsadogenic risk prediction in a manner consistent with how it is used in assessment of the QT interval in a TQT study. It is the author's hope that this would trigger qualification studies for *in silico* torsadogenic prediction models which better take in to account both the ion channel pharmacology and the pharmacokinetics of drugs.

Methods

The *in vitro* hERG potency estimates for terfenadine, risperidone and paliperidone were all obtained from a single literature report (Kramer et al., 2013). The hERG potency estimate for fexofenadine was from a different study (Rajamani et al., 2002). The hERG potency values for astemizole and desmethyastemizole come from a single study (Zhou et al., 1999). There was no consistent source for the hERG potency values for all compounds relevant to the current analysis. Literature values for therapeutic and potential 'high clinical' exposure were used alongside published values for plasma protein binding. These exposure and plasma protein binding values were used to estimate unbound exposure of all drugs and metabolites (See Table 1. legend for more information on data sources). The exposure to "hERG-active species" for each parent-metabolite pair was determined by adjusting the unbound exposure of the metabolite in a manner reflective of the parent : metabolite hERG potency ratio. The calculation effectively turns the unbound metabolite concentration in to an unbound parent concentration so that these can be combined. If the parent and metabolite are equally potent then the unbound concentration of parent and metabolite would be simply added together. In the cases of terfenadine, risperidone and astemizole the metabolite is numerically less potent at hERG than the parent and so a proportion of the metabolite concentration, consistent with the potency ratio, is added to the parent concentration to determine the overall concentration of "hERG-active species". This method assumes that the effect of parent and metabolite are of similar kinetics and are additive. The parent hERG potency and the unbound "hERG-active species" exposure were used to determine therapeutic margins in both therapeutic and 'high clinical' exposure ranges (Margin = Parent hERG IC_{50} / ["hERG-active species"]). Where there

was a range of plasma protein binding values available, the calculations were based on the hERG potency and the exposure in the presence of the lowest plasma protein binding value. This represents the more conservative approach. The same hERG potency and exposure values were used in the publicly-accessible ApPredict tool from the University of Nottingham (https://cardiac.nottingham.ac.uk/; Williams & Mirams, 2015) to determine qNet values under both exposure scenarios. A Hill slope of 1 was assumed and was used as an input value in the calculation of qNet. Only the hERG potency, and no other ion channel data, was used in determining the qNet value since values for the other ion channel potencies were not consistently available for astemizole, desmethylastemizole, fexofenadine, paliperidone, risperidone and terfenadine. In the available ion channel selectivity data (see Kramer et al., 2013) astemizole, paliperidone, risperidone and terfenadine appear relatively selective for the hERG channel over the cardiac sodium and calcium channel. Fexofenadine is also weak at the hERG channel. Thus, using only hERG potency is not a significant issue in the current analysis. The ApPredict model uses the hERG potency, the Hill slope and the concentration information supplied to determine the amount of hERG inhibition at the input concentration and adjusts the conductance of the hERG channel in the *in silico* model. The core model is the same as the O'Hara-Rudy based model used in the CiPA *in silico* model and the qNet values match those derived from an in-house version of the CiPA model (data not shown). The ApPredict model was chosen for this analysis as it would be readily accessible to readers for independent verification of the results.

Results

The concentration (Table 1) of "hERG active species" compared to the concentration of parent alone differed by 1.33- to 37-fold for the three compounds under therapeutic conditions. The most dramatic difference being observed with astemizole where at steady state the metabolite is in vast excess compared to parent (Heykants et al., 1986). The difference between concentrations of parent alone under normal efficacy conditions and "hERG active species" in the 'high clinical' scenario ranged from 18- to 60-fold. The exposure values used in the CiPA assessment were 4 nM, 1.81 nM and 0.26nM for terfenadine, risperidone and astemizole, respectively. The therapeutic "hERG active species" concentrations used in the present study were 0.4 nM, 4.5 nM and 0.335 nM for terfenadine, risperidone and astemizole, respectively. The exposure values used to determine margin and qNet under therapeutic conditions are shown in Table 1. The values for potential 'high clinical' scenarios are also shown in Table 1 representing plasma exposure to terfenadine under the influence of ketoconazole (Honig et al., 1993), plasma exposure for risperidone at steady state in Chinese female patients (Zhou et al., 2006), and the plasma exposure to desmethylastemizole in a case of torsade de pointes (Vorperian et al., 1996). The hERG block potency used in the CiPA model was based on a kinetic model and cannot be easily infered relative to the static IC_{50} values used in the current study.

Therapeutic margins calculated under normal therapeutic plasma exposures were 115, 57 and 2.7 for terfenadine, risperidone and astemizole, respectively (Table 1). Under 'high clinical' exposure scenarios these margins were 9, 5 and 1.7 for terfenadine, risperidone and

astemizole, respectively. The qNet values for terfenadine, risperidone and astemizole under normal therapeutic concentrations were 0.0693 μC/μF, 0.0689 μC/μF and 0.057 μC/μF, respectively. The terfenadine and risperidone values under normal therapeutic conditions are close to the current border between the "Intermediate" and "Low/No" risk categories (Figure 1). The astemizole value is in the "High" risk area. Under 'high clinical' exposure scenarios the qNet values all migrated towards higher risk values. The values were 0.0653 μC/μF, 0.0624 μC/μF and 0.0513 μC/μF for terfenadine, risperidone and astemizole, respectively. The hypothesis underlying the current analysis is that a 'high clinical' exposure should be used, as well as any contributing metabolite effect. In the original CiPA analysis a 'high clinical' exposure was used for terfenadine and as fexofenadine is a very weak hERG blocker it has no or little impact on the margin or qNet value. The terfenadine qNet value derived under similar 'high clinical' conditions in the current analysis is close to that derived in the original CiPA assessment (Figure 1). The difference between the values is likely a product of the different hERG potencies used in this assessment and the CiPA assessment, since the relative exposure values used 5.5 nM versus 4 nM (Li et al., 2017) would suggest the margin should be slightly smaller and the qNet value slightly higher risk. The issue of different hERG values is one which should be corrected by systematic data collection but does not interfere with the observation in this study that the margin shrinks and the qNet value migrates towards higher risk values when a 'high clinical' scenario is explored or when metabolites are considered.

==========. Table 1. Near here ==========

Discussion

The differences in exposure between parent only and the total "hERG active species" under therapeutic conditions and 'high clinical' scenarios are appreciable. The differences are certainly far greater and more variable than could simply be accounted for by using a 1- to 4-fold multiple of the therapeutic exposure of the parent alone as used in the CiPA assessment (Li et al., 2018). This suggests there could be a significant advantage to accounting for parent and metabolite pharmacology and pharmacokinetics in a torsadogenic risk prediction.

In this analysis, when terfenadine and risperidone were treated similarly the margin and qNet values (see Figure 1) were similar in both the normal and 'high clinical' exposure scenarios. This is in contrast to the current CiPA *in-silico* model qualification study (Li et al., 2018) where the qNet value for risperidone is distinctly different from terfenadine. In the case of astemizole, owing to the large exposure to a potent hERG blocking metabolite, the qNet values are smaller (higher risk) than those observed in the CiPA study for both clinical scenarios. The margins for astemizole are also very modest. In the CiPA study the qNet values, and presumably the margins, for astemizole and terfenadine were similar. The current analysis suggests that the interpretation of risk for the three drugs relative to each other may be different from the impression derived from consideration of only parent concentration under therapeutic conditions.

========== Figure 1. Near Here ==========

Terfenadine, as already described, was commonly prescribed (Honig et al., 1993; Honig et al., 1992). It was ultimately withdrawn from the US market by the sponsor in agreement with the FDA in 1997. Initially the very modest effect of therapeutic plasma concentrations of terfenadine on the QTc interval was not considered a cardiac repolarization risk (Pratt et al., 1996). Overall this is consistent with a therapeutic margin of 115-fold. However, this margin is dramatically reduced in the 'high clinical' scenario. Relative to the 30-fold margin rule of thumb (Redfern et al., 2003; Webster et al., 2002) the therapeutic plasma concentration of terfenadine leads to a margin up to 4-fold larger than the 30-fold rule of thumb. However, under the 'high clinical' scenario the margin is 3 times less than the 30-fold margin. Previous reports have demonstrated how the relative odds of reporting TdP and related adverse events correlates with diminishing hERG margin, especially inside the 30-fold margin level (De Bruin et al., 2005). Thus, reinforcing for terfenadine that the 'high clinical' exposure value is the one relevant to torsadogenic risk and risk prediction. Use of this scenario in the original CiPA assessment is clearly warranted.

The case of risperidone is similar to the terfenadine case, although overall margins are smaller for risperidone under both exposure scenarios. Some authors originally classified risperidone as a non-torsadogenic drug (Redfern et al., 2003) and the US label does suggest QTc prolongation has not been routinely observed in all studies. This is consistent with a relatively large hERG margin (>45-fold) under normal therapeutic exposure scenarios (Gintant, 2011). However, there are clearly higher exposure scenarios where the margin is reduced and in the illustrated example the margin is reduced to a similar value to that for terfenadine under

conditions of metabolic inhibition. As there are many on-target side effects with anti-psychotic drugs such as risperidone the dosing advice available in the product labels usually suggests a dose titration to tolerable levels. The dose titration schemes may effectively limit the torsadogenic risk of such compounds, as might some interfering pharmacology.

In the case of astemizole the margins under normal and 'high clinical' scenarios are very modest and would be anticipated to be associated with QTc prolongation. The hERG-blocking potency estimate for astemizole in a different study is 4 nM (Kramer et al., 2013; the same study used for paliperidone, risperidone and terfenadine) which would increase the margin and qNet value (lower risk). This emphasizes the need to determine the hERG potency in a consistent and physiologically-relevant manner. There is also the outstanding question of whether hERG blockade by two species is additive. This is a reasonable assumption but there is very limited data testing the assumption in the literature. It is also the assumption which is captured in drug labels which suggest a drug which prolongs the electrocardiogram should not be taken with another drug which prolongs the QTc interval. The concentrations of the desmethyl metabolite of astemizole are particularly high at steady-state owing to the long terminal half-life. The package label for the antiarrhythmic drug, dofetilide (Tikosyn product label) describes that incidence of torsade de pointes was dose- and exposure-related. It also illustrates that the majority of observed torsade de pointes cases happened within 3 or less days of commencing the particular dose of dofetilide. Clinical concentration-QTc analysis for dofetilide demonstrates that the concentration-QTc relationship is considerably steeper on the first day of dosing compared to the fifth and subsequent day of dosing (Allen et al., 2002; Le Coz et al.,

1995). This likely needs to be taken in to consideration when assessing the effect of steady state exposures of desmethylastemizole.

More broadly, the examples illustrated here suggest that when the 'high clinical' scenario is consistently considered, the CiPA *in-silico* qualification could be quite different (Figure 1). The classification of "High", "Intermediate" or "No/Low" risk was not made based on the qNet value. It was a classification determined by a group of experts although no published account of the methodology is available. Appropriate inputs in the *in silico* model will determine the outputs (qNet or another index of torsadogenic risk), it will not however change the classification. What it will change is the result of qualification, the potential metric chosen and the thresholds for that metric. The thresholds illustrated as vertical lines in Figure 1 were determined by the data and a model training and testing process. It is already clear that the qNet scores for the "Intermediate" risk group would migrate towards the "High" risk group in the current analysis since risperidone was the drug at the lower bound of the "Intermediate" group, and all the qNet values migrated to the left under 'high clinical' scenarios. The qNet values for astemizole described here are more reminiscent of the "High" risk group. As described in the introduction there are other intermediate risk compounds which need their metabolites appropriately accounted for and 'high clinical' scenarios considered. It is likely that their qNet values would also migrate towards the higher risk values. Ion channel neutral compounds (those without ion channel effects at relevant concentrations) will see no change in their qNet score. "No/Low" risk drugs where there is a predominant effect on inward currents may even see their qNet score migrate (get larger) to values even more distinct from the higher

risk groups. The migration towards the "High" risk group of the "Intermediate" risk group qNet values may make the difference in qNet values between these groups less obvious, at the same time as the "Intermediate" risk group becomes more obviously separated from the "No/Low" risk group. This suggests that if the model were re-trained with the new input values the model output may struggle to clearly differentiate between "High" and "Intermediate" risk drugs and could again effectively become dichotomous between drugs with a risk of TdP and those with "No/Low" risk. This would effectively recognize that a label of "possible", "conditional" or "Intermediate" torsade risk in reality is a reflection of the exposure property required to manifest TdP rather than being determined by pharmacology alone. This is supported by a recent publication examining the effects of the CiPA 28 drugs on properties of induced pluripotent human stem cell-derived cardiomyocytes (Blinova et al., 2018). In Supplementary Figure 3 the effects on action potential triangulation of "High" risk and "Intermediate" risk drugs are similar except the effects of "Intermediate" risk drugs are elicited at higher concentrations.

The current qNet threshold between "No/Low" risk and "Intermediate" risk corresponds to around 1% hERG block. If a Hill Slope of 1 is assumed this would suggest a hERG margin of around 100. This amount of hERG block and this magnitude of margin suggest a higher sensitivity in this analysis than the amount of hERG block (Jonker et al., 2006) and magnitude of margin (Gintant, 2011) than is associated with a positive signal in a TQT study. It is not clear that this offers more specificity than the current TQT oriented testing paradigm. Again, re-

training and qualifying the model based on high clinical scenarios may change the thresholds for a prediction metric and add the desired specificity.

The analysis, using three exemplar drugs, presented here prompts the question, “what should happen next?” Firstly, the ‘high clinical’ exposure scenarios have to be described in a systematic manner which is consistent with the process used in designing and interpreting TQT studies since the introduction of ICH E14 in 2005. The current example illustrates a rationalization of potential high exposure scenarios for terfenadine, risperidone and astemizole based on unbound plasma exposure as a surrogate for exposure at the ion channels. Ultimately having a method utilizing unbound intracardiac cellular levels would be valuable, these physiologically-based pharmacokinetic (PBPK) models are currently under development (Tylutki et al., 2018). This could eventually be used in systematically defining ‘high clinical’ scenarios. Secondly, based on the exposure assessment the data gaps need to be consistently filled. Just as in the case of the principal 28 CiPA classified compounds, a consistent hERG assay to determine the hERG potency of key metabolites which circulate at unbound levels which make them likely contributors to cardiac effects should be conducted. The additional ion channel studies, on calcium and sodium currents and hERG block kinetics, should also be considered for the metabolites. The question of additivity of ion channel block should also be addressed. Thirdly, the normal therapeutic and ‘high clinical’ exposure information can be used with the ion channel profiles to make *in silico* risk predictions. Lastly, whether or not there should be any distinction between the “High” and “Intermediate” risk groups, on the basis of pharmacological properties beyond consideration of exposure, needs to be determined. At

that time, it may also be possible to test if qNet remains the best risk metric or whether other available indices, especially those also available experimentally, would suffice.

In March 2017 the FDA held an Advisory Committee on Model-Informed Drug Development (https://www.fda.gov/advisory-committees/pharmaceutical-science-and-clinical-pharmacology-advisory-committee/2017-meeting-materials-pharmaceutical-science-and-clinical-pharmacology-advisory-committee). In the morning session the case for Physiologically-Based Pharmacokinetic (PBPK) models was presented. These models allow specific tissue exposure to be explored as well as the impact of intrinsic and extrinsic factors influencing exposure. In the afternoon session the CiPA case was presented with the *in silico* QSP model as the core of the integrated risk assessment. The cases of terfenadine, risperidone and astemizole presented here illustrate that the juxtaposition of these two sessions at the Advisory Committee was prescient. The case made here is that the combination of PBPK and QSP models is potentially very powerful in predicting safety risk. A process combining cardiac PBPK and QSP models would offer a clear advantage in proarrhythmia risk assessment in drug development.

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Table Legend

Table 1. The hERG safety margin and qNet metric for terfenadine and risperidone under therapeutic and 'high clinical' exposure scenarios. The source information (enclosed in [ ]) for the plasma concentrations, plasma protein binding and hERG IC_{50}s is as follows: 1, Redfern et al., 2003; 2, Honig et al., 1992; 3, Allegra US Label; 4, Kramer et al., 2013; 5, Rajamani, Anderson, Anson, & January, 2002; 6, Honig et al., 1993; 7, Schoretsanitis et al., 2018 8, Risperdal US Label; 9, Invega Sustenna US Label; 10, Zhou et al., 2006; 11, Heykants et al., 1986; 12, Drug Bank; 13, Zhou et al., 1999; 14, Vorperian et al., 1996. Margin = Parent hERG IC_{50} / ["hERG-active species"]. The qNet value is an *in silico* model output from the ApPredict tool available on selecting the CiPA model and running the simulation at 0.5 Hz. The numbers in bold are the values used in the calculations, when a range of values were possible. n.a. = not available. * = assumed same as parent.

Figure Legend

Figure 1. Illustrates the relative qNet (units = μC/μF) for 20 of the 28 CiPA drugs (based on Li et al., 2018). The colored circles and lines represent the mean and confidence intervals for the qNet determination. The data from the current analysis for terfenadine (black squares), risperidone (black triangles) and astemizole (black circles) are also shown. The open symbols represent qNet calculations based on therapeutic exposures and the closed symbols represent qNet values calculated under 'high clinical scenarios'. The dashed vertical lines are the CiPA model-derived thresholds determining the risk classification between "Low" and "Intermediate" risk (blue) and between "Intermediate" and "High" risk (red). Drugs are (from top to bottom): Dofetilide, Sotalol, Disopyramide, Domperidone, Astemizole, Terfenadine, Cisapride, Droperidol, Pimozide, Ondansetron, Chlorpromazine, Clarithromycin, Clozapine, Risperidone, Metoprolol, Tamoxifen, Loratadine, Verapamil, Ranolazine, Nitrendipine.

Table 1.

Drug	Exposure Scenario	Conc. (ng/ml)		Plasma protein binding (%)		Unbound Conc. (nM)		hERG IC_{50} (nM)		Conc. "hERG-Active" Species (nM)	Margin	qNet
		Parent	Metab.	Parent	Metab.	Parent	Metab.	Parent	Metab.			
Terfenadine	Therapeutic	1.5-**4.5** [1]	245 [2]	97 [1]	**60**-70 [3]	0.3	195.3	50 [4]	65100 [5]	0.4	114.6	0.06 93
	'high clinical'	81 [6]	176-**488** [6]	97	**60**-70	5.2	389.1	50	65100	5.5	9.2	0.06 53
Risperidone	Therapeutic	4.4 [7]	17 [7]	90 [8]	74 [9]	1.1	10.4	260 [4]	780 [4]	4.5	57.4	0.06 89
	'high clinical'	89 [10]	138 [10]	90	74	21.7	84.2	260	780	49.8	5.2	0.06 24
Astemizole	Therapeutic	0.13 [11]	4.87 [11]	96.7 [12]	96.7*	0.009	0.362	0.9 [13]	1 [13]	0.335	2.7	0.057
	'high clinical'	n.a.	7.7 [14]	96.7	96.7*	n.a.	0.54	0.9	1	0.54	1.7	0.0513

Table 1. The hERG safety margin and qNet metric for terfenadine and risperidone under therapeutic and 'high clinical' exposure scenarios. The source information (enclosed in [ ]) for the plasma concentrations, plasma protein binding and hERG IC_{50}s is as follows: 1, Redfern et al., 2003; 2, Honig et al., 1992; 3, Allegra US Label; 4, Kramer et al., 2013; 5, Rajamani, Anderson, Anson, & January, 2002; 6, Honig et al., 1993; 7, Schoretsanitis et al., 2018 8, Risperdal US Label; 9, Invega Sustenna US Label; 10, Zhou et al., 2006; 11, Heykants et al., 1986; 12, Drug Bank; 13, Zhou et al., 1999; 14, Vorperian et al., 1996. The numbers in bold are the values used in the calculations, when a range of values were possible. n.a. = not available. * = assumed same as parent.

Conflicts of Interest

The author is an employee and shareholder of Eli Lilly and Company.

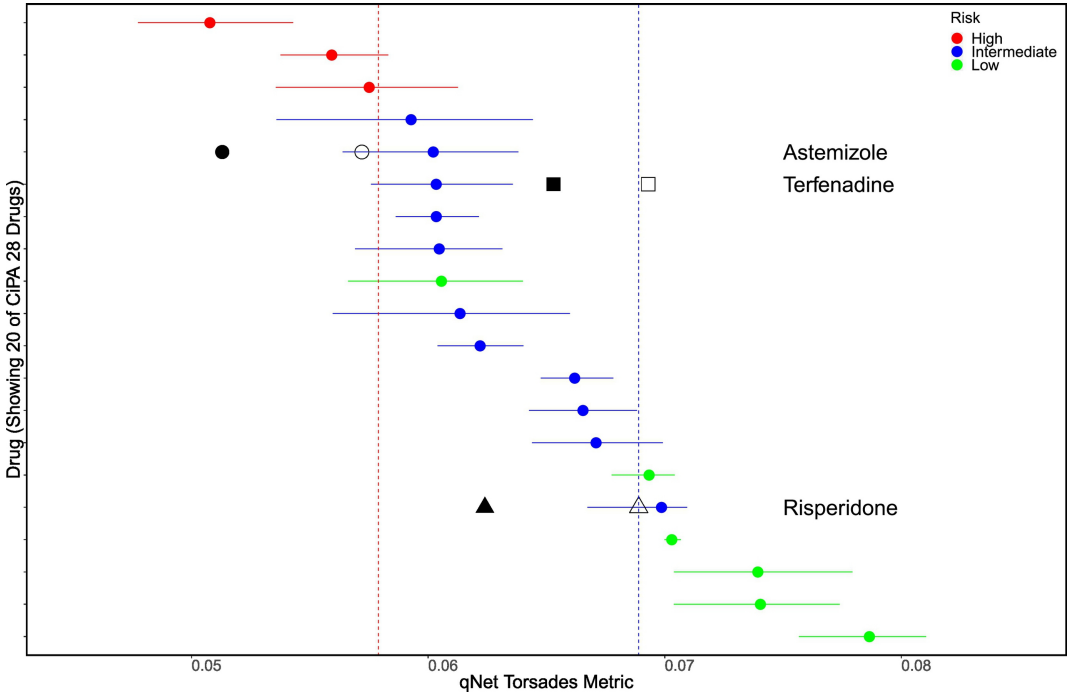
Risk
High
Intermediate
Low
Astemizole
Terfenadine
Risperidone
Drug (Showing 20 of CiPA 28 Drugs)
0.05
0.06
0.07
0.08
qNet Torsades Metric

Figure 1