Reverse engineering of drug induced QT(c) interval prolongation : towards a systems pharmacology approach

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Chapter 9:

Reverse engineering of drug-induced QT(c) interval prolongation: Summary, conclusion and perspectives.

1. Introduction

Cardiovascular safety and in particular pro-arrhythmic drug effects remain a major concern in drug development. More than two decades ago, reports on the occurrence of life threatening ventricular arrhythmias in association with the use of terfenadine [1] drove the attention of regulators, clinicians and drug developers to the arrhythmogenic potential of novel drugs. These events have led to the requirement to assess QTc interval prolongation as a biomarker for the risk of drug-induced Torsades de Pointes (TdP) during both the preclinical and clinical phases of development, as described in the guidelines ICH-7A/B and ICH-E14, respectively.

A key element in the evaluation of the arrhythmogenic properties of new drugs has been the mandatory "thorough QT study" (TQT) in healthy subjects or eventually in patients as a tool to prevent drugs which cause QTc interval prolongation from reaching the market. In a TQT study, the risk of QTc interval prolongation is evaluated using a descriptive statistical technique (the so-called "double delta" method) to analyse the change of the QTc from baseline following the administration of supra-therapeutic doses. Due to the relatively large number of subjects that needs to be included and the associated high costs, a TQT study is typically conducted at the end of phase 2 clinical development. There are several limitations to this approach. The first limitation is that drugs are eliminated at a late stage in drug development (i.e. at the end of phase 2 clinical development), while at the same time healthy subjects are exposed to potentially toxic drugs. In addition, since TQT studies are conducted at supra-therapeutic doses, there are a large number of false positives. Furthermore, the reliance on traditional statistical techniques for the evaluation of the results precludes the use of the data for extrapolation to other doses or other populations. Finally, no information is obtained on co-variates which may explain intra- and inter-individual variation in the sensitivity to the QTc prolonging effects.

In order to identify drugs with an effect on QTc at the earliest possible stage during development and to prevent healthy subjects from being exposed to potentially toxic compounds, a variety of tests has been proposed to evaluate QTc interval prolongation in preclinical studies. Three types of approaches can be distinguished: a) *in vitro* assays quantifying the binding or the effect on ion fluxes at the hERG channel, b) *in vitro* assays measuring effects on action potentials in Purkinje fibres and c) *in vivo* studies in non-clinical animal species. However, to date, in their present form, none of the proposed non-clinical models has been proven predictive, in a quantitative manner, of the cardiovascular effects in man.

At present, there are two important developments regarding the evaluation of the arrhythmogenic properties of novel drug molecules. The first is the research into the mechanisms of the generation

of arrhythmias. These efforts focus on the interactions between the drugs and the diversity of ion channels that are involved in the generation and conduction of action potentials in the heart [1–3]. Theoretically, further understanding of such interactions may yield novel biomarkers with much improved predictive value in terms of the prediction of the risk of drug-induced life threatening arrhythmias. The second development concerns the translation and scaling in a strictly quantitative manner of pertinent information from pre-clinical species to humans. The research described in this thesis focused on the second of these two developments, in particular the scaling from pre-clinical conscious dog studies to humans using the known, standard ECG endpoints in cardiovascular safety assessment, i.e., QT prolongation. Here we have capitalised on the availability of a unique data set in which relevant information on the pharmacokinetics and pharmacodynamics of 10 different drugs was shared with a consortium. Undoubtedly, it can be anticipated that the same principles applied throughout the different chapters for the evaluation of QT interval prolongation can be used for the translation and scaling of other biomarkers.

In **chapter 1** of this thesis an overview was presented of the limitations of the experimental protocols currently used for the evaluation of cardiovascular safety during the screening of drug candidates. It is shown that there is often a mismatch between the experimental conditions in non-clinical and clinical settings. An important factor in this respect is that most studies on QTc interval prolongation do not consider drug concentrations in the evaluation of the effects. As a result, evidence of concordance between species has often relied on qualitative, rather than quantitative criteria.

One of the first documented attempts on the use of drug concentrations to predict drug-induced changes in QTc in man from preclinical study results, is the study by Redfern *et al*. [7], in which they examine the value of preclinical cardiac electrophysiology data (*in vitro* and *in vivo*) for predicting risk of TdP. In this analysis, the cardiovascular effects of a wide range of drugs were evaluated in relation to the free plasma concentrations attained upon the administration of therapeutic doses in man. The authors concluded that if a drug does not display a significant effect in a preclinical model at concentrations ranging from 3 to 30 times the therapeutic concentration in man, it is unlikely to display a clinically relevant QTc effect. Although this approximation may be useful to eliminate potentially cardiotoxic drugs from development, an important question is whether the relatively high threshold may result in the loss of potentially useful drugs with an acceptable cardiovascular safety. This raises the question how the cardiovascular safety evaluation can be refined.

In recent years progress has been made in the area of population PKPD modelling. Briefly, by analysing the time course of the drug effect, in conjunction with the time course of the drug concentration and on the basis of a suitable PKPD model, relevant drug concentration-effect

relationships can be derived. In principle PKPD modelling can be applied to data arising from preclinical as well as clinical studies. It has been demonstrated for a considerable number of efficacy and safety endpoints that concentration-effect relationships constitute a strong basis for extrapolation and prediction [8–10]; both from *in vitro* to *in vivo*, from animal to humans and from healthy subjects to patients.

As outlined in **chapter 2**, the objective of the investigations described in this thesis was to evaluate the utility of PKPD modelling in the cardiovascular safety evaluation of drugs, with emphasis on the prediction of the QT prolonging effects in man from non-clinical investigations. In this regard it is important to notice that, despite the relevance of PKPD relationships being highlighted in the ICH guidelines, details about its implementation are not clearly presented. In the most recent Q&A document on the ICH-E14 it is now even suggested that using phase I clinical trials with a more dense ECG monitoring to support a concentration-QTc analysis can potentially be sufficient evidence to apply for a TQT waiver[11]. The methods however, still remain very open. An important limitation remains the absence of stronger quantitative pharmacology rationale for the proposed analyses. The lack of clarity in the evaluation of clinical data seems to have propagated into preclinical protocols.

In addition to the proposal of a model-based framework for the evaluation of dromotropic drug effects (QT interval prolongation), we have also assessed the impact of the current practice and decision making regarding the cardiovascular safety profile of candidate compounds in R&D, which still relies on a sequential, fragmented process. It is shown how PKPD modelling can be used to integrate the different phases of drug development, by propagating information from one phase to the next. In this manner the probability of QTc prolongation at therapeutic levels is characterised as early as possible during drug development.

Our research has therefore focused on identifying intrinsic differences in the sensitivity to the QT-prolonging effects of compounds known to block the hERG and other relevant ion channels in preclinical species and in humans. The approach was aimed primarily at disentangling drug-specific properties from (system-specific) physiological differences. Clearly, the possibility of describing between-species differences by systems-specific parameters may support better scaling of drug effects from animals to humans. As depicted in Figure 9.1, efforts in translational pharmacology should provide insight into pharmacokinetic-pharmacodynamic relationships. Parameters describing PKPD relationships can be used in conjunction with simulation scenarios to assess the probability of QT interval prolongation under clinically relevant conditions, thereby eliminating the need for a Thorough QT study.

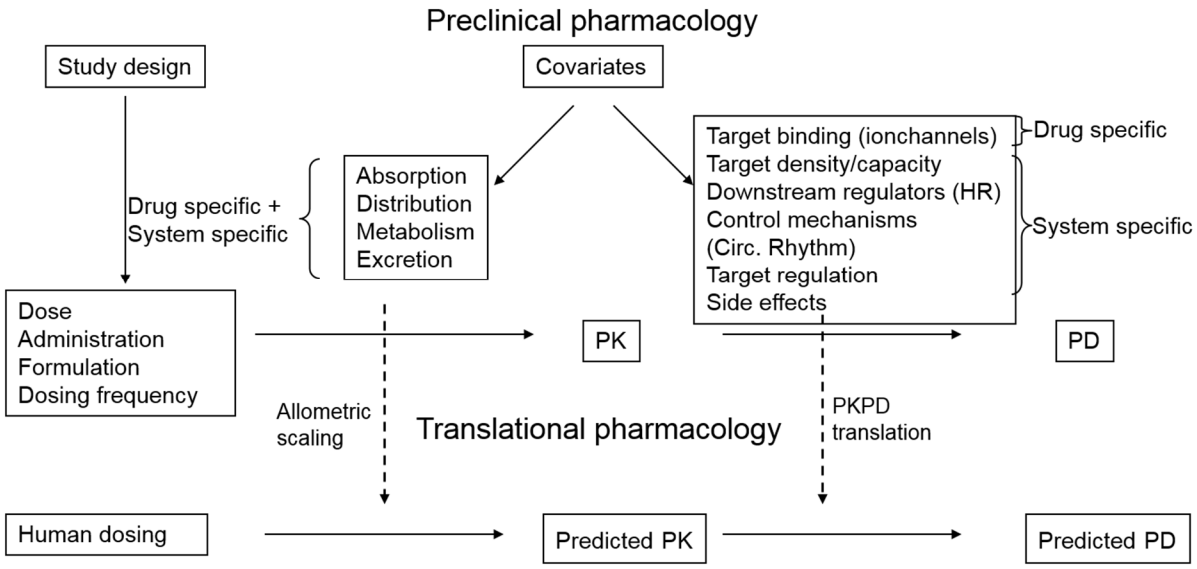

Figure 9.1. Diagram describing the integration of preclinical data for the translation of drug effects from animals to humans. *Each box represents sets of data which need to be generated to minimise the uncertainty in the scaling and prediction of the QT-prolonging effects in humans.*

To re-engineer the evaluation of QT interval prolongation, i.e. starting from clinical data backwards to *in vitro* experimental data, we have explored the advantages of a common model parameterisation throughout the non-clinical and clinical phases of drug development. The ultimate goal of such an approach is to facilitate the integration of the data and subsequently the translation and interpretation of findings, taking into account *in vitro* and *in vivo* differences. In fact, the choice of the PKPD model parameterisation was central to the work presented here, in that it allowed us to discriminate between drug-specific and systems-specific properties. Three important translational issues have been identified, which represent the pillars for the framework used throughout this thesis, namely, **data integration**, incorporation of **pharmacokinetic modelling** as the first step towards the characterisation of PKPD relationships and the **parameterisation of drug-specific and system-specific parameters** describing QT interval prolongation. From an experimental perspective, this work revealed the limitations and advantages of existing *in vitro* and *in vivo* protocols, which were used to characterise the concentration-effect relationship of a range of compounds with known and unknown QT-prolonging effect.

Our approach was implemented to ensure that evidence on safety pharmacology is generated in an efficient, informative manner. Five central questions were identified, which formed the basis for the work described in the subsequent chapters in this thesis:

1. Are there intrinsic differences in the sensitivity to the dromotropic (QT-prolonging) effects of compounds known to block the hERG channel in non-clinical species and in humans?
2. If intrinsic differences exist between species, can a model-based approach disentangle drug-specific properties from (system-specific) physiological or biological characteristics?
3. Assuming that intrinsic differences between species can be described by systems-specific parameters, can correlations be identified that enable scaling of the effects from animals to humans?
4. Can the magnitude of QT prolonging effects at therapeutic concentrations in humans be predicted in a strictly quantitative manner from findings in non-clinical species?
5. Are *in vitro-in vivo* correlations specific and sensitive enough to allow prediction of the QT prolonging effects at therapeutic concentrations in humans?

The answers to those questions are presented along with the results and conclusions from our investigations in the next paragraphs. Our findings are summarised along with some of key requirements as well as advantages and limitations of *in vitro* and *in vivo* protocols aimed at the evaluation of pro-arrhythmic effects and QT interval prolongation.

2. Results and conclusions of the investigations described in this thesis

2.1. The quantitative evaluation of drug effects on QTc is difficult

Firstly, the magnitude of the change in QTc that needs to be quantified is small compared to the relatively high baseline (e.g. 10 ms for a basal QT interval of ~ 400 ms). This is further compounded by high intra- and inter-individual variability. Among other factors, variability is determined by circadian variation in the QT interval resulting from intake of food and water as well as by the intrinsic effect of changes in heart rate. Noise is further increased by measurement issues, which include electrode lead placement and movement.

Secondly, the distinction between drug-specific and system-specific parameters is challenging. This is important, since the translational value of parameters or parameter estimates depends on the correlations between non-clinical and clinical conditions. The predictive value of a model or parameter therefore depends on its biological, physiological or clinical meaning. Identifying and

quantifying systems-specific vs. drug-specific properties into distinct parameters provides the basis for using the same model and its parameters in a prospective manner, i.e., to simulate and predict drug effects before experimental data are generated. In addition, evidence of a common parameter describing system-specific properties across species offers the opportunity to translate findings across species. While the same model was used to describe the probability of QTc-interval prolongation across species, we have been able to conduct the analysis independently for each drug. The approach therefore warrants the prospective use of the model for the evaluation of novel candidate molecules.

The proposed framework relies not only on the evidence generation from new experimental protocols, but also on existing knowledge, which can be formally incorporated into a PKPD model. From a statistical point-of-view, the use of Bayesian inference to support the estimation of system-specific parameters, namely, the individual correction factor for RR interval (heart rate), the oscillatory component describing the circadian variation and the truncated Emax model to capture maximum physiological response, represents an important opportunity for protocol design optimisation, as historical data can be integrated into *prior* parameter distributions. It should also be noted that despite the apparent simplicity of the model, our choice of parameterisation is aimed at physiological plausibility. When viewed from the physical rather than the statistical perspective, the formulas for QT as a function of RR are equivalent to the duration of systole as a function of heart rate. The overall equation used to estimate and predict drug effects throughout our investigations shows how drug-induced effects can be captured by similar parameters in preclinical species and humans.

In contrast to other examples in the published literature, where other more empirical PKPD indices have been used to compare drug effects across species (e.g., percentage increase from baseline) [12,13], our approach relied on a discrete value or threshold of 10 ms. However, any other threshold value can be used for the purposes of establishing the liability of QT interval prolongation. The rationale for our choice was based on the clinical relevance of 10 ms. This threshold also takes into account experimental noise. It was assumed to yield better precision in parameter estimates, as QT values tend to decrease with increasing heart rates, a phenomenon which reflects differences in basal metabolism in smaller animals. The use of normalised values, such as percentage increase from baseline, imposes the assumption of linearity in the transduction and amplification processes across species. Thus far, there is limited data to support this assumption, especially if one conceives that physiologically, action potential duration and sinus arrhythmia may result from the contribution of different ion channels [14].

2.2. There are interspecies differences in PKPD relationships

The first step in the endeavour to identify interspecies correlations was to characterise the pharmacokinetic-pharmacodynamic relationship of a reference compound with known QT prolonging effects in humans. Assuming that the proposed model parameterisation has effectively disentangled drug-specific from system-specific properties, in **Chapter 3** it was shown that significant species differences exist in the PKPD relationship of moxifloxacin, as defined by the probability of QT interval prolongation ≥ 10 ms as a pharmacodynamic endpoint. The concentration – probability curve differed in conscious dogs, cynomolgus monkeys and humans. The concentrations associated with a 50% probability of QT prolongation ≥ 10 ms (Cp50) varied from 20.3 to 6.4 and 2.6 µM in dogs, monkeys and humans, respectively. Such differences could not be normalised by taking into account plasma protein binding. An important, yet unnoticeable aspect of this investigation is that the use of PKPD relationships allows one to evaluate drug effects across a similar, clinically relevant exposure ranges. From a methodological point of view, a striking conclusion which can be drawn from this experiment is that the effects of moxifloxacin in the cynomolgous monkey are not the same as in humans. Despite the views that PKPD parameters ought to be compared in logarithmic terms, the differences between monkeys and humans should not be seen as noise. Despite the marked variation in pharmacokinetics across species, which is accounted for during modelling, it is likely that intrinsic differences in cardiac physiology also contribute to the magnitude of the QT prolongation. This answered the question that there are intrinsic differences between species.

2.3. Drug-specific parameters can be identified using a model-based approach

To answer the question if a model-based approach could disentangle drug-specific properties from (system-specific) physiological or biological characteristics we have explored the effects of a range of compounds with varying affinity and potency for hERG channels. Given the importance of devising an approach that can be implemented for prospective use in R&D [15,16] and the evidence regarding the intrinsic interspecies differences in PKPD relationships, dogs were selected as the reference species for further evaluation of compounds with known QT prolonging effects (positive control), minor QT prolonging effects (borderline effects which are often associated with false positive and false negative results) and no QT prolonging or QT shortening effects (negative control).

In **Chapter 4,** we assessed whether the differences between dogs and humans are systematic by re-testing moxifloxacin and two additional compounds with known QT prolonging effects in humans, namely cisapride and sotalol. A systematic difference of approximately ten-fold was observed for all three compounds when comparing the estimates of Cp50, i.e., the concentration which corresponds

with a 50% probability of QT prolongation ≥ 10 ms. Moreover, we showed that the use of a linear relationship between concentration and effect was sufficiently robust to describe the pro-arrhythmic effects of the drugs in dogs and in humans. Whereas it is theoretically possible that drugs might cause different maximum QT prolongation in a given species, clinically this is not the parameter of interest, as pro-arrhythmic effects arise from small changes in action potential duration, i.e., from considerably small increases in QT interval. It is the pharmacodynamic parameter (slope) in dogs that differs from humans.

These findings prompted us to further explore the relevance of the slope of PKPD relationship in conscious dogs as the basis for the prediction of drug-induced QTc prolongation in humans. In addition, our investigation has shed light on points to consider regarding the experimental protocol design. It became evident from the comparison between clinical and nonclinical data that dose selection, pharmacokinetic and pharmacodynamic (ECG) sampling as well as statistical design and number of animals per dose level need to be revisited and optimised. Current practice in safety pharmacology does not support the generation of data with sufficient quality and precision to ensure the characterisation of PKPD relationships.

A natural question that arises from the results obtained so far is whether they can be generalised, i.e., whether systematic differences in the PKPD relationships observed between dogs and humans can be detected for compounds with minor or no prodromic effect at therapeutic concentrations in humans. Evidence of systematic differences would eventually enable the use of a scaling factor to predict drug effects in humans based on experimental data in dogs. This concept is particularly appealing for novel compounds which have not been tested experimentally in patients, as well as those with known activity on different ion channels, for which *in vitro* experiments cannot provide an accurate estimate of the drug effects *in vivo*.

To that purpose, in **chapter 5** we have evaluated the sensitivity of the approach to detect drug effects for compounds which show low or borderline QTc interval prolonging effect. Three investigational compounds (NCE01, NCE02 and NCE03), for which experimental data was available in dogs and healthy subjects, were included in the analysis. The comparison of these three compounds was of interest because they differed not only in potency (the concentration range in which they act), but also in intrinsic efficacy (the maximum effect that may be reached). It was demonstrated that the new chemical entities have distinctive pro-arrhythmic activity, with QT-prolonging, QT-shortening and borderline QT-prolonging effects. Specifically, our analysis revealed that while NCE01 has similar properties to moxifloxacin (i.e. QT-prolonging effect), NCE02 is a compound with 'borderline' effect on QTc and NCE03 appears to cause a shortening of the QTc interval rather than a prolongation. Our

investigation also shows that some knowledge about the expected therapeutic concentration range of the drug is required to ensure an accurate interpretation of preclinical findings.

Their PKPD relationships were successfully described by a single structural model with acceptable precision in prediction. The probability of an increase in QT/QTc interval ≥ 10 ms was evident for NCE01, whilst this threshold was not achieved for NCE02 and NCE03 in either species. Together with the evidence of model performance for compounds with known pro-arrhythmic effects (**chapter 4**), our approach seems to offer the basis for the screening of novel molecules, using the slope of the PKPD relationship, i.e., a pharmacodynamic parameter. Undoubtedly, we acknowledge that the evidence from three compounds with unknown mechanisms of action is not sufficient to establish the sensitivity and specificity of the method. These results provide, however, further motivation to explore the feasibility of an *in silico* framework for future evaluation of the pro-arrhythmic potential of candidate molecules, in which drug specific parameters are used to scale up drug effects in humans.

Conceptually, the proposed approach consists in the use of simulation scenarios in which predicted pharmacokinetic profiles in humans are analysed in conjunction with (predicted) estimates of the slope of the PKPD model in humans based on estimates obtained in dogs. From a drug development perspective the availability of such a framework overcomes one of the main limitations of earlier PKPD models in preclinical research, i.e., their use as a screening tool for new compounds. In addition, it enables early assessment of the clinical relevance of the pro-arrhythmic effects of a drug. For instance, simulation scenarios can be used to assess correlations between drug exposure and abnormal or extreme QT values. In this context one could also generate probability curves for observing QT intervals > 500 ms.

2.4. A pharmacodynamic parameter enables scaling of the effects from animals to humans

An alternative approach was proposed that ensures reduction in the uncertainty about the propensity of non-antiarrhythmic drugs in prolonging QT/QTc interval before progressing into clinical development. In **Chapter 6**, it was shown that model parameters describing QT interval prolongation in dogs can be correlated with drug effects in humans. Data on the pharmacokinetic-pharmacodynamic relationships of nine compounds with no or varying QT-prolonging effect (cisapride, sotalol, moxifloxacin, carabersat, GSK945237, SB237376 and GSK618334, and two anonymised NCEs) were included in this analysis. Our findings show that the slope of the concentration-effect relationship in dogs correlates with the same parameter in humans (Figure 9.2). Whilst the magnitude of the slope estimates clearly differs between species on average by a factor of

11.6-fold, evidence of a linear correlation indicates that the larger its value in dogs the stronger the QT interval prolonging effect will be in humans. This was observed for cisapride, sotalol, moxifloxacin, NCE03 and GSK6183343, where a distinct QT-interval prolonging effect was detected within the (putative) therapeutic concentration range of these compounds. On the other hand, if the slope was found to be around zero, there was no or borderline QTc-prolonging effect, as shown for carabersat, SB237376 and GSK945237. In addition, our results revealed that a negative value for the slope parameter is associated with a shortening of the QT-interval. This phenomenon was observed for NCE04 in dogs and humans. As most compounds have not been used in clinical practice, confirmatory data from clinical practice is not available to corroborate the predictive performance of the findings. Nevertheless, these results strongly suggest that in conjunction with pharmacokinetic data, extrapolation of the slope parameter in dogs can be used to predict the probability of QT interval prolongation in humans.

Our approach clearly contrasts with previous efforts exploring PKPD relationships of drugs with known pro-arrhythmic activity [8,10]. We have shown that a common model parameterisation can be used across species. This implies parameter re-estimation, rather than model building each time a new molecule is evaluated. Another interesting finding that appeared to be constant across compounds was the difference in precision of parameter estimates in dogs, as compared to clinical data. Obviously, the continuous sampling of ECG parameters in a limited number of telemetered animals does not eliminate the uncertainty arising from differences in pharmacokinetics and other sources of variability.

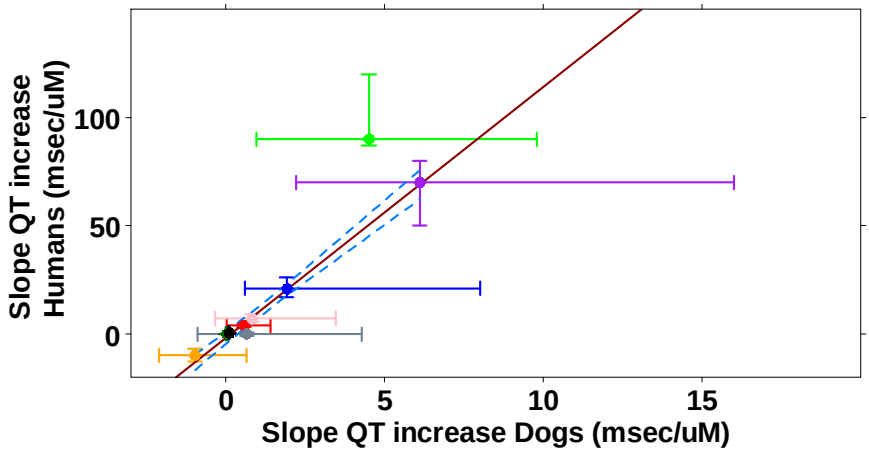

Figure 9.2. Unweighted linear correlation of the slope (ms/µM) in dogs and humans excluding cisapride. *Compounds are shown with different colours: cisapride (green), moxifloxacin (red), sotalol (blue), NCE04 (orange), NCE03 (purple), carabersat (grey), GSK618334 (pink), GSK945237 (dark*

green), SB237376 (black). Dashed lines represents the 95% confidence interval around the mean, linear correlation (red line) = y=-1.709+11.586x, R^2=0.989

Whereas further investigation is required to establish the generalisability of the correlation for a wider range of compounds, the use of clinical trial simulations in conjunction with PKPD estimates from conscious dogs appear to be sensitive and specific enough to support the extrapolation of the probability of QT interval prolongation in humans. These findings have guided us to evaluate the prospective value of the approach in R&D and thereby answer the fourth of the five questions, i.e., whether the magnitude of QT-prolonging effects at therapeutic concentrations in humans can be predicted in a strictly quantitative manner from findings in preclinical species.

2.5. Prediction of the magnitude and clinical relevance of QT prolonging effects in humans

An application of the proposed framework is illustrated in **Chapter 7**, where we have analysed in a blinded manner the experimental data of methadone in conscious dogs. Numerous publications had described the potential link between QT prolongation and methadone use in patients on oral treatment across a dose range of 10-1200 mg/day. However, as this synthetic opioid compound was developed before the introduction of the current ICH-E14 guidelines, limited evidence on cardiovascular safety was available at the time of approval by the regulatory agencies.
In this prospective exercise, the slope parameter estimates from conscious dogs were used combined with pharmacokinetic information from a hypothetical dose escalation, phase I study in healthy subjects. The main goal of our investigation was to demonstrate the advantages of using extrapolated PKPD parameters in conjunction with clinical trial simulations to explore the potential effects at therapeutically relevant concentrations. This analysis revealed that drug-induced QT interval prolongation is very small in dogs, as expressed by shallow slope and the wide confidence intervals for the slope parameter (ms/µM). Results from simulation scenarios did not predict the same effect size described previously in the published literature [17], which includes reports of a 10ms increase after administration of a 200-mg dose of methadone. By contrast, at this dose level the predicted effect in healthy subjects was marginal; a 50% probability of ≥ 5msec increase was predicted only after a 500-mg dose of methadone. Such a discrepancy does not necessarily represent inaccuracies in the estimates of the drug-specific parameters. It seems to reflect the differences in methadone disposition in humans as compared to dogs. Given that interspecies differences in pharmacokinetic disposition are not a unique feature of methadone, it becomes evident that attention to the dose rationale in experimental protocols is crucial for accurate extrapolation, translation and interpretation of prodromic effects in preclinical experiments.

The striking discrepancies in the pharmacokinetics of methadone make this case a very interesting point for discussion from a drug development perspective. We have thus far endorsed the views that characterisation of PKPD relationships in animals in conjunction with early pharmacokinetic-pharmacodynamic data in humans should provide sufficient evidence about the probability of QT interval prolongation ≥ 10 ms in humans, without relying on the TQT study as a confirmatory step. The findings in this prospective exercise seem to support this recommendation, but also raise a major concern about the role of quantitative pharmacology principles in safety evaluation. Whereas the current ICH-S7A guidelines suggest that plasma exposure levels should 'include and exceed the primary pharmacodynamic or therapeutic range', this requirement is often overlooked, as ADME properties may not have been fully characterised at the time when these experiments were performed. Therefore, the answer to the question whether we can predict the clinical effect from strictly non-clinical data is no, if the ADME is not fully understood and metabolite profiles in humans are not known. The answer to that question is also yes, as the results showed that there could be a concern and if the compound was in development, further investigations and caution in the process would have been most likely suggested.

2.6. In vitro hERG binding and inhibition are not predictive of the magnitude of the QT prolongation in humans

The investigations described in this thesis were not limited to establishing the value of pharmacokinetic-pharmacodynamic relationships *in vivo* as the basis for predicting drug effects in humans. The last question we set was still to be answered: are *in vitro-in vivo* correlations specific and sensitive enough to allow prediction of the QT prolonging effects at therapeutic concentrations in humans?

From a drug discovery perspective, the relevance of *in vitro* data to support the screening of candidate molecules was also investigated. Data from hERG assays, which currently represent a primary screening filter, were considered as input parameters for the proposed PKPD modelling framework, aiming at the characterisation of *in vitro-in vivo* correlations. Whereas different functional assays are available for screening, the use of *in vitro* hERG inhibition relies on the assumption that any strong signal will be predictive of potential QT prolongation *in vivo*. In addition, it has been shown that estimates of drug potency in functional assays depend on experimental protocol characteristics. In **Chapter 8**, we have therefore attempted to explore the role of experimental protocols that provide insight into drug-specific properties, such as affinity, which may be less prone to variations in experimental conditions. Data of the effects of cisapride, sotalol and

moxifloxacin in a hERG functional patch clamp assay, in a hERG radio-labelled dofetilide displacement binding assay and QT interval in conscious dogs were analysed in parallel to identify potential correlations between pharmacological activity *in vitro* and *in vivo*. Based on receptor occupancy concepts, it was anticipated that a systematic correlation might be found between hERG binding and changes in action potential duration *in vitro* and consequently QT prolongation *in vivo*. Unfortunately, both *in vitro* assays showed discrepancies in parameter estimates, which prevented the identification of *in vitro-in vivo* correlations. In addition, modelling of drug effects using a typical Emax model, which is plausible for *in vitro* experiments, was not possible due to large variability in the functional patch clamp assay. Parameter precision was too poor for meaningful use of the results in a subsequent step i.e., as input for the prediction of drug effects *in vivo*. Dofetilide displacement suggested that binding curves are unrelated to the *in vivo* potency estimates for QTc interval prolongation in dogs and humans. These results corroborated our initial findings, indicating that the high sensitivity of these assays for small changes in the experimental conditions and the lack of standardised protocols for *in vitro* assays make it impossible to achieve sufficient precision for subsequent extrapolation or scaling purposes. Screening and ranking procedures based on binding or potency estimates for inhibitory activity on single ion channels may be very misleading. Clearly, integrative approaches are needed that account for the multifactorial nature of the pro-arrhythmic effects *in vivo*. Among the available options is the use of human cardiomyocytes, as proposed by the comprehensive *in vitro* proarrhythmia assay (CiPA) consortium [18]. This initiative has ambitious goals in order to adapt the Guidelines taking into account the advancement in *in vitro* methodologies, but this will imply a significant paradigm shift. An overview of the conceptual and experimental differences between CiPA and current ICH-S7B guidelines is summarised in Table 9.1.

Table 9.1. *Comparison between ICH-S7B/E14 and evolving Comprehensive In Vitro Pro-arrhythmia Assay (CiPA) paradigm* [19].

S7B/E14	CiPA
Preclinical/clinical strategy for assessing the potential of a test substance to delay ventricular repolarisation	**Preclinical strategy to evaluate the proarrhythmatic risk of a test substance that delays ventricular repolarisation, or not**
Focused on the ability of a test substance to inhibit I_{kr}/I_{hERG} (S7B)	**Composed of two distinct series of tests:**
Additional preclinical in vitro repolarisation screens are not prescribed, resulting in variability in type and comprehensiveness of complementary data	**In vitro study of drug effects on multiple cardiac ion channels (not just I_{kr}/I_{hERG}) and incorporation of these effects in an in silico model of human ventricular action potential**
	Confirmation of the *in silico* results using human ventricular myocytes derived from human induced pluripotent stem cells
Promulgates the idea that small effects on hERG will lead to adverse regulatory outcome	**Prevents unwarranted attrition due to hERG liability based on mechanistic understanding of proarrhythmatic risk**
Guidelines are not prescriptive, permitting flexibility of user approach, leading to variability of data due to lack of standardised approach	**Established best practices for ion channel and stem cell cardiomyocyte studies: standardised protocols and methods are developed and adopted to minimise intra- and interlaboratory variability**
Guidelines do not suggest *in silico* modelling	**Makes available a single, common and fully validated *in silico* model to quantify the risk of arrhythmia based on ion channel data**
Does not directly address the endpoint of clinical concern (i.e. proarrhythmatic risk)	**Provides a common complete assessment of proarrhythmatic risk early in the drug development process**
Supports well-controlled *in vivo* cardiogram (ECG) assessment in preclinical studies	**Supports well-controlled *in vivo* ECG assessment in preclinical studies**
Supports careful clinical assessment of electrical effects of test substance in phase I ECG studies before progressing to Thorough QT (TQT) study	**Supports robust QT assessment of test substance in early-phase clinical studies (phase I); test substances with a safe CiPA profile do not require TQT study**
Requests that all sponsors submitting a new drug application conduct a TQT study to determine if a drug prolongs QTc	**Scheduled to replace TQT studies**

3. General conclusions

In spite of the limited number of compounds included in the investigations presented throughout this thesis, our findings provide strong evidence supporting the use of a model-based framework for the evaluation of drug-induced QT interval prolongation. Our results show that the assessment of

drug-specific properties becomes central, disentangling noise and variability arising from systems-specific characteristics. In brief, the following conclusions can be drawn:

1. There are intrinsic differences in the sensitivity to the dromotropic (QT-prolonging) effects of compounds known to block the hERG channel in non-clinical species and in humans. Cynomolgus monkeys are less sensitive to the dromotropic effects of moxifloxacin compared to humans and conscious beagle dogs less sensitive compared to cynomolgus monkeys (**Chapter 3**).
2. A model-based approach can distinguish drug-specific properties from (system-specific) physiological or biological characteristics. This enables the use a single model parameterisation with comparable values of the systems-specific parameters to describe the of the effects different compounds tested in dogs (**Chapters 4, 5 and 6**)
3. On the basis of the single mechanism-based PKPD model structure and common parameterisation, a linear correlation was identified between the slopes of the linear concentration-effect relations of nine compounds with varying affinity for hERG channels in dogs and humans. Given that intrinsic differences between species are described by systems-specific parameters, this approach enables scaling of the effects from dogs to humans, with a mean scaling factor of 11.6 (**Chapter 6**)
4. Although the magnitude of the QT-prolonging effects at therapeutic concentrations in humans is predictable in a strictly quantitative manner from findings using the estimates of the slope in dog studies, knowledge of the pharmacokinetic disposition in humans appears to be essential, particularly in relation to biotransformation and the potential effects of metabolites. It is also evident that the effects of more compounds need to be included in this framework to establish the accuracy and precision of the parameter estimates (**Chapter 7**).
5. At the moment, the classical *in vitro* experiments are not specific and sensitive enough to allow the characterisation of *in vitro-in vivo* correlations or prediction of the QT-prolonging effects at therapeutic concentrations in humans (**Chapter 8**).

4. Perspectives – towards a systems pharmacology approach

A new paradigm for the evaluation of cardiovascular safety must emerge in which evidence generation is integrated with *prior* knowledge. In this respect, a systems pharmacology approach is envisaged, but its relevance for the screening of prospective candidate molecules will require one to address some important outstanding issues in cardiovascular safety. First and most important is the need to further understand the link between QT interval prolongation and TdP, which is clearly

multifaceted and is influenced by a number of underlying factors including age, gender, underlying disease state, electrolyte imbalance, concomitant medication, and more [20,21]. Although the majority of compounds that can induce TdP are known to inhibit cardiac potassium channels encoded by hERG, block of this ionic current alone is not always predictive of delayed repolarisation or proarrhythmic risks. Irrespective of the evidence which will arise from drug effects on pluripotent stem cell- and embryonic stem cell-derived cardiomyocytes, clinical and epidemiological insight is required to better define the conditions and covariates that determine the deterioration of supraventricular arrhythmias into TdP. Attention to the causative nature of the correlation between QT interval, its prolongation and arrhythmia is essential if emphasis during drug screening is shifted to electrophysiology and *in silico* modelling. To date, it remains unexplained why verapamil, a calcium channel blocker, prolongs QT interval but is not associated with TdP. Without further understanding of the link between electrophysiological, pharmacological and clinical factors, potentially efficacious and safe compounds will continue to fail screening, which could be otherwise progressed into clinical development.

A second aspect that deserves attention is the lack of standardisation and variability in experimental procedures *in vitro* and *in vivo*, which result in large residual variability and potentially biased estimates. For instance, food and water intake in conscious dogs need to be closely monitored, as they contribute considerably to random noise and consequently to type I error (false positive results). In addition, it is a well-known fact that despite the increased sensitivity of experimental methods *in vitro*, noise remains an important confounder. Whilst variation ranging between 2- to 6-fold may seem acceptable for between laboratory differences, it does considerably inflate the risk of false positive and negative results, especially if findings are evaluated without clear estimates of what represents a clinically relevant exposure range [22]. Consensus must be reached about the need for more stringent experimental conditions, akin to the requirements imposed for good laboratory practice (GLP)-compliant methods to improve precision and minimise type I error. An example of such efforts is illustrated by the Minimum Information for a Cardiac Electrophysiology Experiment (MICEE) website (www.micee.org), which highlights the requirements for storing and in-depth annotation of documents describing cardiac electrophysiological experiments.

In summary, the following points need to be considered for the implementation of a model-based framework in pharmaceutical R&D:

I. Assessment of the clinical relevance of findings from experimental protocols *in vitro*, such as ion channel binding, functional inhibition, and action potential duration, requires caution. Parameter

estimates, such as affinity and potency from *in vitro* assays cannot be translated to *in vivo* animal studies or humans without integrating the contribution of systems-specific parameters *in vivo*.

II. In spite of systematic differences between species, the evidence of a correlation between the slope of the concentration-QT effect relationship in conscious dogs and humans provides the basis for predicting drug effects at therapeutic and supra-therapeutic concentrations in humans. Thanks to the meta-analytical nature of the approach, it is anticipated that the precision of parameter estimates and corresponding predictions will increase as novel compounds are added to the pool of existing compounds.

III. Pharmacokinetics must be characterised in the same animals used for the evaluation of drug-induced effects on ECG (pharmacodynamics). The use of pharmacokinetic data from different animals, as well as the empirical selection of blood sampling times leads to poor precision in PKPD parameter estimates and uncertainty in the prediction of drug effects in humans.

IV. Interspecies differences in drug disposition properties need to be taken into account for accurate translation of drug-specific properties. In this context, detailed information on the concentration vs. time profile of the parent drug and metabolite(s) is essential.

V. Optimality concepts are critical for the design of experimental protocols. Design variables such as dose level, sampling schedule and number of animals can be optimised to minimise uncertainty and improve the precision of parameter estimates. Alternative study designs [23] can be identified with the use allometric scaling/PBPK for improved estimation of PK parameters in dogs and consequently better translation to humans

VI. Evidence of a positive slope (i.e., drug-specific property) in dogs does not determine whether the development of a compound should be stopped or progressed. Estimates of the probability of QT interval prolongation need to be considered in conjunction with clinical trial simulations. Accurate evaluation of the clinical implications of pro-arrhythmic properties requires the use of simulation scenarios in which additional intrinsic and extrinsic factors are integrated, taking into account characteristics of the target population and therapeutic use of the compound.

4.1. In silico modelling and effective data integration

Over the last few years regulatory agencies, academic researchers and pharmaceutical companies appear to have recognised the drawbacks of the existing approach for the evaluation of pro-arrhythmic properties based on a predominant focus on the hERG channel. For the first time an approach is being implemented in which experimental data on key cardiac ion channels *in vitro* are integrated with an *in silico* model with the primary objective of predicting drug effect on action potentials in computationally reconstructed human ventricular cardiomyocytes using the ion channel

dataset, and confirmation of the *in vitro* and *in silico* prediction by determining the electrical activity of human induced pluripotent stem cell- and embryonic stem cell-derived cardiomyocytes (hiPS/hES-CMs) [2,19,24]. The implications of the approach is illustrated by the work of Mirams and collaborators, who have recently characterised the implications of experimental variability and signal stability in electrophysiology for wide range of compounds [25].

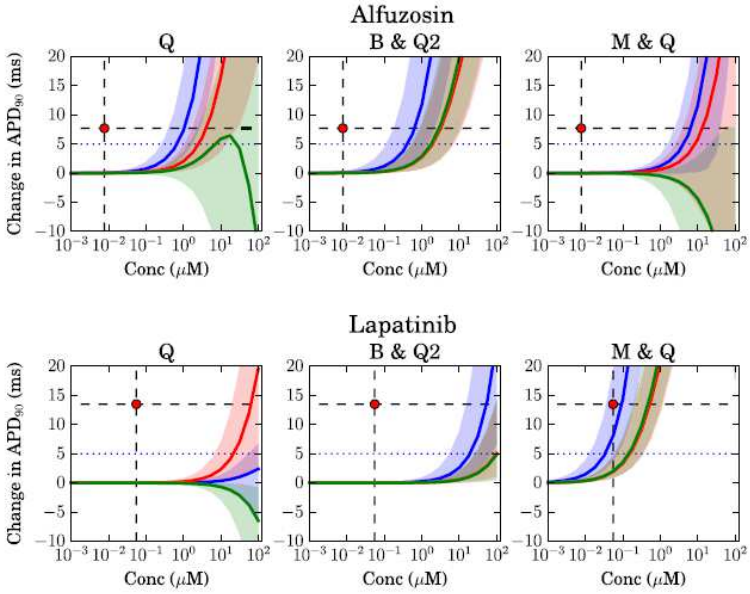

***Figure 9.3**. **Simulated change in action potential duration (90%) plotted against (free plasma) concentrations.** Models: Blue — O'Hara; red — ten Tusscher; green — Grandi. Three data sources are shown for: 'Q' (Quattro); 'B &Q2' (Barracuda & Quattro); 'M&Q' (Manual hERG & Quattro), as per Table 9.1. Estimated 95% credible regions are shown around each line which captures uncertainty due to screening assay variability. The clinical study result is shown with a black dashed horizontal line for the largest dose in the TQT study; the estimated free plasma concentration Associated with this is shown with a vertical dashed black line, and their intersection with a red circle. The 5-ms 'cut-off', used in contingency table calculations, is shown with a horizontal blue dotted line. (Adapted from* [25]*).*

As is depicted in Figure 9.3, differences in parameter estimates from *in vitro* experimental protocols are propagated and amplified in the predictions obtained by different *in silico* models describing changes in action potential duration. Moreover, it is shown how the results from this investigation relate to clinical findings with the same compounds. In a similar investigation, Gotta *et al.* have shown how preclinical cardiovascular safety studies compared between facilities with respect to their sensitivity to detect drug-induced QTc prolongation, based on the changes in QTc interval relative to baseline (ΔQTc). The authors emphasise how uncertainty about the consistency of quantitative ΔQTc predictions prevents accurate translation of findings [12] (Figure 9.4).

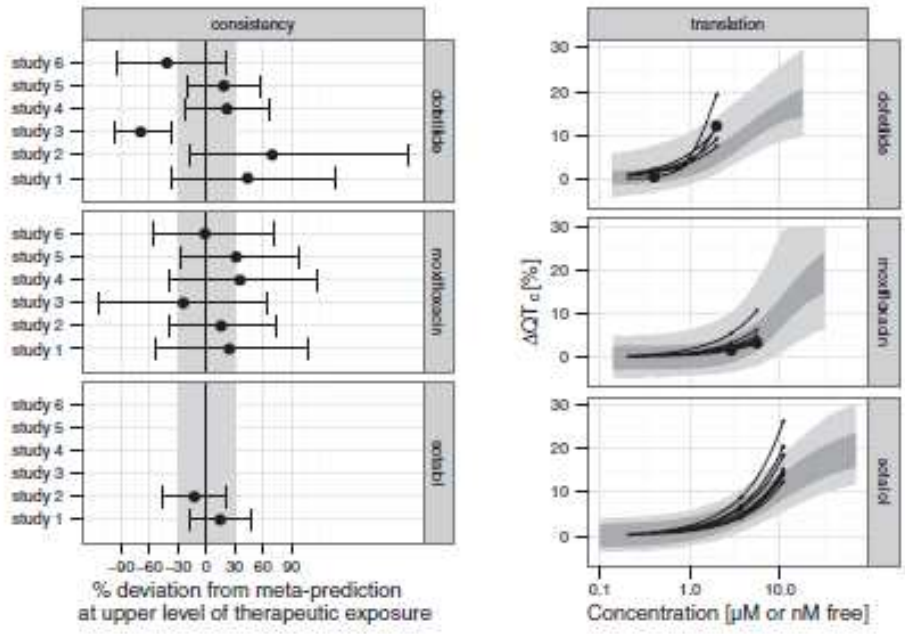

Figure 9.4. Uncertainty about consistency of quantitative predictions prevents accurate translation of findings: *% deviation of individual study predictions from meta-predictions at upper level of therapeutic exposure. Error bars: 95%CI. Vertical shaded area: ±30% = overall mean ISV estimate. Translation: PD relationships in the conscious dog [shaded area: 95%CI of meta-predictions (dark grey) and 90%PI (light grey)] are overlapping with clinical QTc prolongation (thin lines) at the lower and upper levels of therapeutic exposure (dots; clinical meta-predictions are indicated with larger dots) when expressed as %QTc prolongation from baseline (%ΔQTc). Adapted from Gotta et al.* [12].

It is evident from the introduction of *in silico* modelling that evidence synthesis, i.e., integrative approaches that combine existing knowledge start to appear in guidelines. The use of a model-based approach, whether implemented as systems-pharmacology (Figure 9.5), operational or semi-mechanistic stochastic models, will enable further advancement in the field [12,26–29]. However,

attention must be given to the validation procedures and to the contribution of random and non-random sources of variability [25]. If these models are aimed at supporting decision making about the progression of a candidate molecule, a robust evaluation of their predictive performance is needed. Thus far attention appears to be given to parameter identifiability and model stability [30]. Identifiability and stability are not the only factors that need to be considered for establishing the sensitivity, specificity, reproducibility and overall predictive performance of the model.

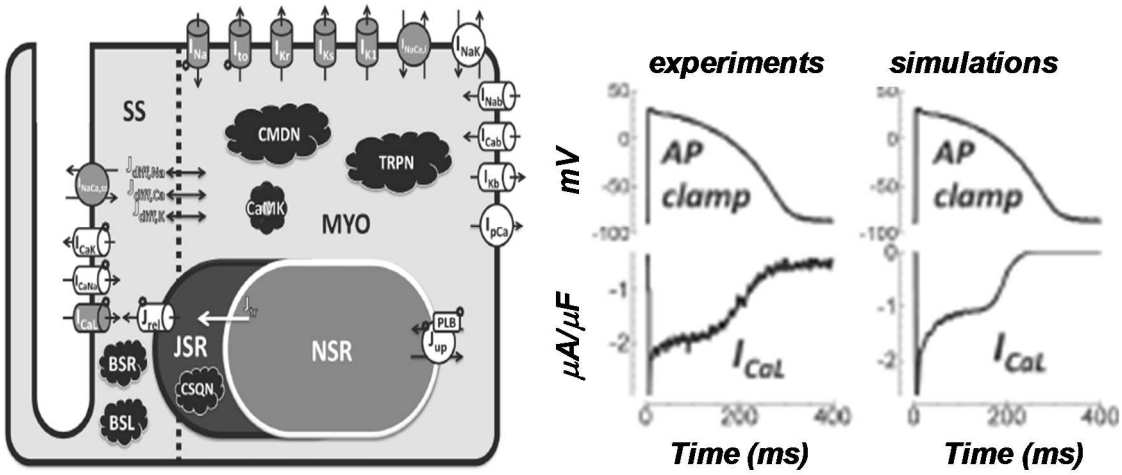

Figure 9.5. Schematic diagram of the human ventricular myocyte model. *Formulations for all currents and fluxes were based either directly (grey) or indirectly (white) on un-diseased or non-failing human experimental data. Model includes four compartments: 1) bulk myoplasm (myo), 2) junctional sarcoplasmic reticulum (JSR), 3) network sarcoplasmic reticulum (NSR), and 4) subspace (SS), representing the space near the T-tubules. Insert panel shows an application of the model in the evaluation of human AP clamp waveform, used to elicit 1 mM nisoldipine sensitive current (ICaL, experiments, left) and comparison to simulations using the same AP clamp (right). Adapted from O'Hara et al.* [27].

4.2. Characterisation of intra- and inter-individual variation in QTc interval prolongation

A last critical factor that prevailed throughout the investigations presented in this thesis and which will be pivotal for the accurate extrapolation and prediction of preclinical findings was pharmacokinetics. More specifically, it has become evident that the choice of the doses used in experimental protocols is critical and that experimental and virtual (*in silico*) protocols need to take into account the anticipated therapeutic exposure as well as the potential interspecies differences in drug disposition. Despite the advancement of methodologies aimed at better screening of candidate molecules, the focus on integration of *in vitro* and *in silico* approaches continue to overlook the

impact of uncertainty about the therapeutic levels at the time of drug screening and consequently the importance of characterising drug exposure. Clearly, between- and within-species differences in drug exposure can lead to variations in pharmacodynamics (PD), the magnitude of which varies from drug to drug and physiological conditions. Such differences cannot be ignored when scaling or extrapolating *in vitro* and *in vivo* results to humans [31–33].

Therefore, a natural extension of the work presented here will involve better prediction of the pharmacokinetic profiles of the parent drug and relevant metabolites a therapeutic and supratherapeutic levels in humans. The predictive performance of a model-based framework will certainly benefit from initiatives such as the CiPA (Comprehensive *In vitro* Proarrhythmia Assay), in that *in vitro* findings may better guide experimental protocol conditions *in vivo.*
For instance, one could envisage the concurrent evaluation of drug effects on multiple ion channels to ensure a more accurate assessment of pro-arrhythmic risk [34,35]. The concept has been partly introduced by Mirams and collaborations, who have proposed the use of simulations based on mathematical models for the electrophysiology of cardiac myocytes to integrate information on how a compound affects different ion channels. The approach seems promising if appropriate details on pharmacokinetics of the compound can be integrated into simulation scenarios (Figure 9.6).

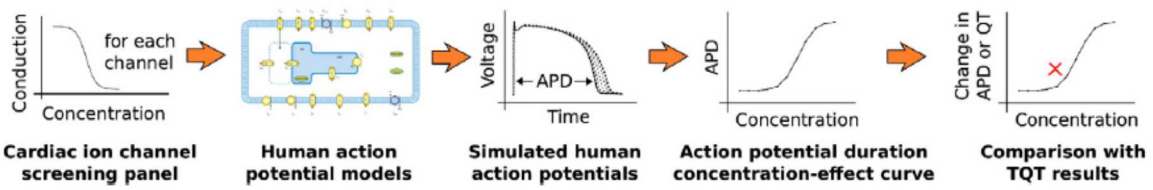

Figure 9.6. Simulations to assess QT prolongation. *An overview of the steps involved in the characterisation of ion channel concentration-effect and subsequent translation into a human in silico action potential model, in which QT prolongation can be assessed (modified from Mirams et al., 2014* [25]*).*

In addition, the inclusion of new candidate molecules into the library of compounds evaluated so far will allow further integration of predicted parameter estimates from *in silico* models of QT prolongation in dogs. Of interest will be the performance of physiologically-based pharmacokinetic models as basis for predicting the impact of interspecies differences as well as incorporating the contribution of covariate factors as non-random sources of variability in pharmacokinetics in human. The contribution of genetic variability, age, disease and drug-drug interactions can be decisive for the progression or discontinuation of a candidate molecule (Figure 9.7).

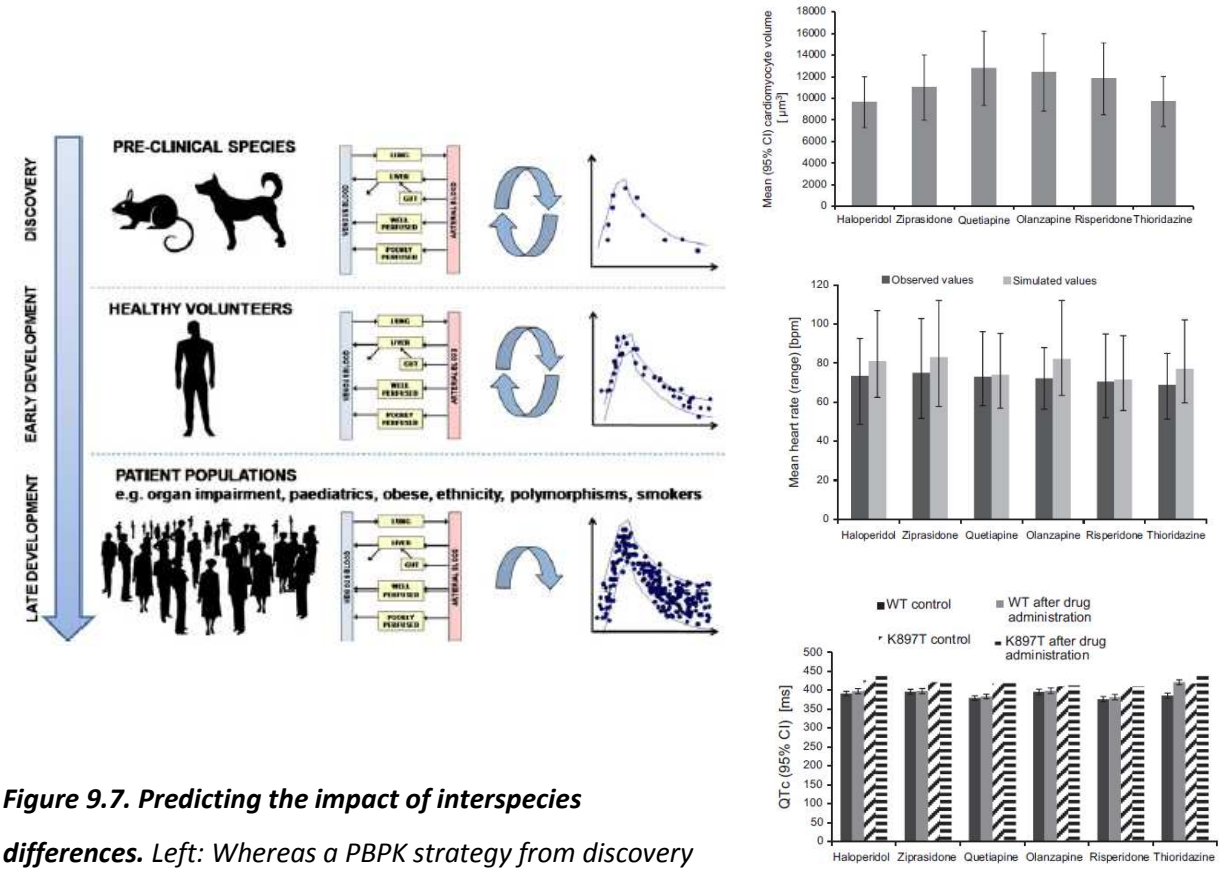

Figure 9.7. Predicting the impact of interspecies differences. *Left: Whereas a PBPK strategy from discovery to early- and late-stage development may allow a more detailed description of pharmacokinetics and overall drug exposure in patients, there is no comprehensive framework to account for variability in absorption, distribution and metabolisms that arise from species-specific differences other than size and ontogeny of enzymatic systems. Upper right panel shows mean (95%CI) ventricular cardiomyocytes volume in each simulated population. Mid right panel depicts mean heart rate and its minimal and maximal values observed in publication (baseline HR) and predicted. Lower right panel summarises the absolute mean QTc values with their 95% confidence intervals obtained in the simulations for wild type (WT) individuals and polymorphism carriers before and after drug administration [Adapted from Jones et al, 2013; Glinka et al, 2014* [36,37]*]*

In summary, translation of cardiovascular safety findings will require a stronger rationale for dose selection and better characterisation of differences in drug metabolism between preclinical species and humans. We anticipate that careful evaluation of concentration-effect relationships will remain pivotal for the characterisation of pro-arrhythmic risk. Irrespective of the degree of sophistication of

in silico models, clinically relevant factors will have to be incorporated into the analysis to ensure drug effects are evaluated accordingly, minimising the risk of discarding safe molecules and/or progressing with unsuitable ones.

5. References

1. Monahan BP, Ferguson CL, Killeavy ES, Lloyd BK, Troy J, Cantilena LR. Torsades de pointes occurring in association with terfenadine use. JAMA. 1990 Dec 5;264(21):2788–90.

2. Cavero I, Holzgrefe H. Comprehensive in vitro Proarrhythmia Assay, a novel in vitro/in silico paradigm to detect ventricular proarrhythmic liability: a visionary 21st century initiative. Expert Opin Drug Saf. 2014;13(6):745–58.

3. FDA. S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals. 2005.

4. Cavero I, Holzgrefe H, Clements M. The prospective IQ-CSRC trial : A prototype early clinical proarrhythmia assessment investigation for replacing the ICH E14 thorough QTc (TQT) study. J Pharmacol Toxicol Methods. 2016;80:1–8.

5. Cavero I, Holzgrefe H. CiPA: Ongoing testing, future qualification procedures, and pending issues. J Pharmacol Toxicol Methods. 2015;76:27–37.

6. Vicente J, Stockbridge N, Strauss DG. ScienceDirect Evolving regulatory paradigm for proarrhythmic risk assessment for new drugs. J Electrocardiol. 2016;49(6):837–42.

7. Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc Res. 2003;58(1):32–45.

8. Jonker DM, Kenna LA, Leishman D, Wallis R, Milligan PA, Jonsson EN. A pharmacokinetic-pharmacodynamic model for the quantitative prediction of dofetilide clinical QT prolongation from human ether-a-go-go-related gene current inhibition data. Clin Pharmacol Ther. 2005;77(6):572–82.

9. Caruso A, Frances N, Meille C, Greiter-Wilke A, Hillebrecht A, Lave T. Translational PK/PD Modeling for Cardiovascular Safety Assessment of Drug Candidates. J Pharmacol Toxicol Methods [Internet]. 2014 May [cited 2014 May 29];70(1):73–85. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1056871914002160

10. Parkinson J, Visser SAGG, Jarvis P, Pollard C, Valentin J-P, Yates JWTT, et al. Translational pharmacokinetic–pharmacodynamic modeling of QTc effects in dog and human. J Pharmacol Toxicol Methods. 2013;68(3):357–66.

11. E14 Implementation Working Group . ICH E14 Guideline: The Clinical Evaluation of QT / QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs Questions & Answers (R3). 2015;R3(December).

12. Gotta V, Cools F, van Ammel K, Gallacher DJ, Visser S a G, Sannajust F, et al. Inter-study variability of preclinical in vivo safety studies and translational exposure-QTc relationships - a PKPD meta-analysis. Br J Pharmacol. 2015;172:4364–79.

13. Gotta V, Cools F, Ammel K Van, Gallacher DJ, Visser SAG, Sannajust F, et al. Sensitivity of pharmacokinetic-pharmacodynamic analysis for detecting small magnitudes of QTc prolongation in preclinical safety testing. J Pharmacol Toxicol Methods. 2014;

14. Hanton G, Rabemampianina Y. The electrocardiogram of the Beagle dog: reference values and effect of sex, genetic strain, body position and heart rate. Lab Anim [Internet]. 2006 Apr;40(2):123–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16600072

15. Arrigoni C, Crivori P. Assessment of QT liabilities in drug development. Cell Biol Toxicol. 2007;23(1):1–13.

16. Chiang AY, Mallinckrodt CH, Dmitrienko A a, Leishman DJ. Utility of positive controls in assessing assay sensitivity in ICH S7B and ICH E14 guidance for evaluation of QT/QTc interval prolongation. J Pharmacol Toxicol Methods. 2010;62(2):143–7.

17. Florian J, Garnett CE, Nallani SC, Rappaport BA, Throckmorton DC. A modeling and simulation approach to characterize methadone QT prolongation using pooled data from five clinical trials in MMT patients. Clin Pharmacol Ther. 2012;91(4):666–72.

18. CIPA Guiding Principles [Internet]. [cited 2015 Dec 27]. Available from: https://www.ilsiextra.org/hesi/science/cardiac/cipa/Project Documents/CIPA data sharing final.pdf

19. Fermini B, Hancox JC, Abi-Gerges N, Bridgland-Taylor M, Chaudhary KW, Colatsky T, et al. A New Perspective in the Field of Cardiac Safety Testing through the Comprehensive In Vitro Proarrhythmia Assay Paradigm. J Biomol Screen. 2016;21(1):1–11.

20. Chain ASY, Dieleman JP, Noord C Van, Hofman A, Stricker BHC, Danhof M, et al. Not-in-trial simulation I : Bridging cardiovascular risk from clinical trials to real-life conditions. Br J Clin Pharmacol. 2013;76(6):964–72.

21. France NP, Della Pasqua O. The role of concentration − effect relationships in the assessment

of QT c interval prolongation. Br J Clin Pharmacol. 2015;79(1):117–31.

22. Kitaguchi T, Moriyama Y, Taniguchi T, Ojima A, Ando H, Uda T, et al. CSAHi study: Evaluation of multi-electrode array in combination with human iPS cell-derived cardiomyocytes to predict drug-induced QT prolongation and arrhythmia — Effects of 7 reference compounds at 10 facilities. J Pharmacol Toxicol Methods. 2015;

23. Sahota T, Sanderson I, Danhof M, Della Pasqua O. Model-based analysis of thromboxane B2 and prostaglandin E2 as biomarkers in the safety evaluation of naproxen. Toxicol Appl Pharmacol. 2014;278:209–19.

24. Sager PT, Gintant G, Turner JR, Pettit S, Stockbridge N. Rechanneling the cardiac proarrhythmia safety paradigm: A meeting report from the Cardiac Safety Research Consortium. Am Heart J. 2014;167(3):292–300.

25. Mirams GR, Davies MR, Brough SJ, Bridgland-taylor MH, Cui Y, Gavaghan DJ, et al. Prediction of Thorough QT study results using action potential simulations based on ion channel screens. J Pharmacol Toxicol Methods. 2014;70(3):246–54.

26. ten Tusscher KHWJ, Noble D, Noble PJ, Panfilov A V. A model for human ventricular tissue. Am J Physiol Heart Circ Physiol. 2004;286:H1573–89.

27. O'Hara T, Virág L, Varró A, Rudy Y. Simulation of the Undiseased Human Cardiac Ventricular Action Potential: Model Formulation and Experimental Validation. PLoS Comput Biol. 2011;7(5):e1002061.

28. Grandi E, Pasqualini FS, Bers DM. A novel computational model of the human ventricular action potential and Ca transient. J Mol Cell Cardiol. 2010;48(1):112–21.

29. Marostica E, Ammel K Van. Modelling of drug-induced QT-interval prolongation : estimation approaches and translational opportunities. J Pharmacokinet Pharmacodyn. 2015;42(6):659–79.

30. Livshitz L, Rudy Y. Uniqueness and stability of action potential models during rest, pacing, and conduction using problem-solving environment. Biophys J. 2009;97(5):1265–76.

31. Dalvie D, Zhang C, Chen W, Smolarek T, Obach RS, Loi CM. Cross-species comparison of the metabolism and excretion of zoniporide: Contribution of aldehyde oxidase to interspecies differences. Drug Metab Dispos. 2010;38(4):641–54.

32. Vuppugalla R, Marathe P, He H, Jones RDO, Yates JWT, Jones HM, et al. PHRMA CPCDC

Initiative on Predictive Models of Human Pharmacokinetics, Part 4: Prediction of Plasma Concentration–Time Profiles in Human from In Vivo Preclinical Data by Using theWajima Approach. J Pharm Sci. 2011;100(10):4111–26.

33. Uusitalo J, Turpeinen M, Tolonen A, Koskimies P, Lammintausta R, Pelkonen O. Metabolism and metabolite profiles in vitro and in vivo of ospemifene in humans and preclinical species. Drug Metab Pers Ther. 2015;ePub.

34. Kramer J, Obejero-paz CA, Myatt G, Kuryshev YA, Bruening-wright A, Verducci JS, et al. MICE Models : Superior to the HERG Model in Predicting Torsade de Pointes. Sci Rep. 2013;3(2100):1–7.

35. Mirams GR, Davies MR, Cui Y, Kohl P, Noble D. Application of cardiac electrophysiology simulations to pro- arrhythmic safety testing. 2012;

36. Jones HM, Mayawala K, Poulin P. Dose Selection Based on Physiologically Based Pharmacokinetic (PBPK) Approaches. AAPS J. 2013;15(2):377–87.

37. Glinka A, Polak S. The effects of six antipsychotic agents on QTc-An attempt to mimic clinical trial through simulation including variability in the population. Comput Biol Med. 2014;47(1):20–6.