

## ORIGINAL ARTICLE

# Application of a systems pharmacology model for translational prediction of hERG-mediated QTc prolongation

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## Keywords

Drug safety biomarkers, in vitro–in vivo correlation, mechanistic pharmacodynamic modeling, QTc interval, translational research

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## Funding Information

This project was supported by the Dutch Top Institute Pharma (TIPharma) PK-PD platform 2.0.

Received: 1 September 2016; Accepted: 14 September 2016

*Pharma Res Per*, 4(6), 2016, e00270, doi: 10.1002/prp2.270

doi: 10.1002/prp2.270

Part of the results have been published only in short abstract form at the 15<sup>th</sup> congress of the European Society for Developmental, Perinatal and Paediatric Pharmacology (ESDPPP), Belgrade, June 2015, and at the 15<sup>th</sup> Annual Meeting of the Safety Pharmacology Society (SPS), Prague, September 2015.

## Abstract

Drug-induced QTc interval prolongation ( $\Delta$ QTc) is a main surrogate for proarrhythmic risk assessment. A higher in vivo than in vitro potency for hERG-mediated QTc prolongation has been suggested. Also, in vivo between-species and patient populations' sensitivity to drug-induced QTc prolongation seems to differ. Here, a systems pharmacology model integrating preclinical in vitro (hERG binding) and in vivo (conscious dog  $\Delta$ QTc) data of three hERG blockers (dofetilide, sotalolol, moxifloxacin) was applied (1) to compare the operational efficacy of the three drugs in vivo and (2) to quantify dog–human differences in sensitivity to drug-induced QTc prolongation (for dofetilide only). Scaling parameters for translational in vivo extrapolation of drug effects were derived based on the assumption of system-specific myocardial ion channel densities and transduction of ion channel block: the operational efficacy (transduction of hERG block) in dogs was drug specific (1–19% hERG block corresponded to  $\geq 10$  msec  $\Delta$ QTc). System-specific maximal achievable  $\Delta$ QTc was estimated to 28% from baseline in both dog and human, while %hERG block leading to half-maximal effects was 58% lower in human, suggesting a higher contribution of hERG-mediated potassium current to cardiac repolarization. These results suggest that differences in sensitivity to drug-induced QTc prolongation may be well explained by drug- and system-specific differences in operational efficacy (transduction of hERG block), consistent with experimental reports. The proposed scaling approach may thus assist the translational risk assessment of QTc prolongation in different species and patient populations, if mediated by the hERG channel.

## Abbreviations

$C_{u,plasma}$ , unbound plasma concentration;  $E_m$ , maximal hERG-mediated QT prolongation;  $f_u$ , fraction unbound; hERG, human ether-à-go-go-related gene;  $I_{kr}$ , repolarizing potassium current mediated by the delayed rectifier potassium channel;  $K_i$ , receptor affinity; L, ligand; LR, ligand–receptor complex (receptor occupancy);  $LR_{50}$ , ligand–receptor complex concentration leading to half-maximal system effect; NONMEM, nonlinear mixed effect modeling; QTc, QT interval corrected for heart rate and circadian variation;  $\Delta$ QTc, drug-induced QTc prolongation;  $R_0$ , receptor density;  $\gamma$ , slope (sigmoidicity) of transducer function;  $\Delta$ OFV, difference in NONMEM objective function value;  $\tau$ , transducer ratio;  $f_r$ , relative transducer ratio.

## Introduction

The heart-rate-corrected QT (QTc) interval of the electrocardiogram is indicative of the duration of ventricular repolarization. Delayed ventricular repolarization can lead to potentially life-threatening ventricular arrhythmias. Therefore, drug-induced QTc interval prolongation ( $\Delta$ QTc) is a main surrogate biomarker for proarrhythmic risk assessment and is extensively studied in early preclinical (ICH S7B Guideline, 2005) and clinical (ICH E14 Guideline, 2005; Darpo et al. 2015) phases of drug development.

The most common mechanism is a blockade of the human ether-à-go-go-related gene (*hERG*)-encoded channel, also referred to as  $K_{v}11.1$  channel, and its repolarizing potassium current ( $I_{Kr}$ ). Pharmacodynamic in vitro studies therefore systematically evaluate drug interactions with this ion channel. The mechanistic translation of respective in vitro potency estimates (affinity  $K_i$ ) to in vivo potency ( $EC_{50}$ ) is not completely understood. In vitro hERG potency may be used to generate in silico action potential duration (APD) predictions (O'Hara et al. 2011; Mirams et al. 2014), or to determine a rough safety margin for therapeutic exposure (Redfern et al. 2003). It is, however, usually ignored in in vivo QTc pharmacodynamic data analysis.

Jonker et al. (2005) previously proposed to integrate in vitro hERG potency data of dofetilide in a pharmacodynamic in vivo analysis to characterize the dynamic effects of hERG block on clinical  $\Delta$ QTc ("transduction"). To this purpose, the authors applied a simple systems pharmacology model ("operational model") proposed by Black and Leff (1983) (Fig. 1).

They showed that for dofetilide a 10% hERG block translates clinically already into a 20 msec  $\Delta$ QTc (Jonker et al. 2005). It is, however, not clear if this relationship holds true also for other hERG-blocking drugs. The operational efficacy of a drug-receptor interaction can actually differ between drugs (explaining, e.g., full and partial agonism), and differ between systems (Black and Leff 1983). Species- (Chain et al. 2013), gender- (Darpo et al. 2014), and age-related (Läer et al. 2005) differences in sensitivity to drug-induced QTc prolongation (" $\Delta$ QTc sensitivity") have indeed been reported, and have physiologically been explained in a qualitative manner by differences in myocardial ion channel densities (Ebert et al. 1998; Wang et al. 2000; Obrezhtchikova et al. 2003; Szabó et al. 2005; Szentadrassy et al. 2005; Xiao et al. 2006). These observations make the application of the discussed systems pharmacology model (Black and Leff 1983) even more interesting for quantitative scaling of  $\Delta$ QTc effects, since it explains in vivo differences in  $\Delta$ QTc sensitivity by system-specific transduction processes, that is:

- 1 maximal QTc prolongation that can be achieved in vivo via hERG block ( $E_m$ )
- 2 operational efficacy of fractional hERG block ( $\tau$ )  
 $\tau$  is also named the transducer ratio and can be interpreted as the inverse of fractional hERG block leading to half-maximal effect, since:

$$1/\tau = LR_{50}/R_0$$

where  $R_0$  is the in vivo receptor ( $hERG_r$ ) density, and  $LR_{50}$  is the receptor occupancy leading to a half-maximal effect.  $\tau$  is introduced since the absolute concentrations of  $R_0$  and  $LR_{50}$  are usually not measurable in vivo. The higher  $\tau$ , the higher the drug-specific operational efficacy (lower  $LR_{50}$ , i.e., lower hERG block associated with half-maximal QTc prolongation) and/or the higher the system-specific  $\Delta$ QTc sensitivity (higher  $R_0$ ).

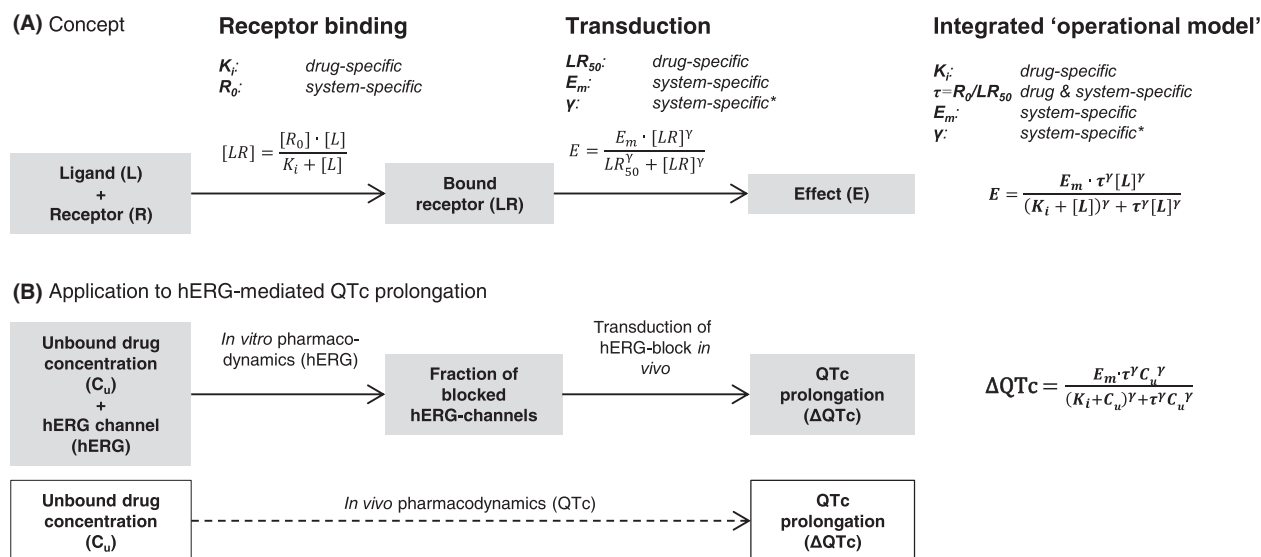
Here, we present an extension of the work of Jonker et al. (2005), with the objective to dissociate drug- and system-specific characteristics of hERG block transduction, and to derive scaling parameters allowing translational clinical  $\Delta$ QTc prediction from integrated preclinical (in vitro and in vivo conscious dog) data. Furthermore, we explore (using literature data) the possible application of this system-specific scaling approach for predicting QTc effects in patient populations not regularly included in early clinical QTc studies (women and pediatrics), and compare predictions with translational methods integrating either in vitro or preclinical in vivo data only.

## Materials and Methods

### Outline

Figure 2 shows an outline of this work:

- 1 *Estimation of scaling parameters:* Using in vitro (hERG) and preclinical in vivo dog (QTc) pharmacodynamic metadata of three hERG blockers (dofetilide, sotalol, moxifloxacin) and clinical pharmacodynamic (QTc) metastudy data of dofetilide, drug- and system-specific parameters were estimated for translational prediction of hERG block transduction.
- 2 *External evaluation:* The derived scaling parameters were applied to predict clinical dofetilide, sotalol, and moxifloxacin  $\Delta$ QTc. Those "system- and drug-specific" translational predictions were externally evaluated using literature data, and compared with "empirical" predictions (from in vivo data alone) (Gotta et al. 2015) and in silico predictions (from in vitro data alone) (O'Hara et al. 2011; Mirams et al. 2014).
- 3 *Refinement:* Besides using literature data, differences in  $\Delta$ QTc sensitivity between men and women (Darpo



**Figure 1.** (A) General concept of the systems pharmacology model (operational model proposed by Black and Leff 1983). (B) Application to hERG-mediated QTc prolongation: pharmacodynamic effects on the QTc interval *in vivo* are a result of receptor binding and transduction.  $K_i$ : receptor affinity,  $R_0$ : receptor density,  $LR_{50}$ : receptor occupancy leading to a half-maximal effect,  $E_m$ : maximal effect,  $\gamma$ : steepness of transducer function (\*the assumption of system specificity was investigated),  $\tau$ : transducer ratio (“operational efficacy”).

et al. 2014) and children and neonates (Läer et al. 2005) were quantitatively investigated by reestimating and comparing  $\tau$  ratios in those populations.

## Data

Details of the extensive pharmacodynamic metastudy data of the three hERG channel blockers used for this work have been published before (Table 1). Total drug concentrations were converted to unbound plasma concentrations ( $C_{u,plasma}$ ) using the following unbound fractions ( $f_u$ ); dofetilide:  $f_{u,dog} = 0.46$ ,  $f_{u,human} = 0.36$  (Smith et al. 1992); sotalolol:  $f_{u,dog} = 1$ ,  $f_{u,human} = 1$  (Campbell and Williams 1998); and moxifloxacin:  $f_{u,dog} = 0.71$ ,  $f_{u,human} = 0.55$  (Siefert et al. 1999).

QTc was both in preclinical (Gotta et al. 2015) and clinical (Jonker et al. 2005) studies centered to a heart rate of 60 bpm (RR interval of 1000 msec). Clinical QT intervals were corrected for heart rate using Fridericia’s formula ( $QT_{cF} = QT [1000/RR]^{1/3}$ ) (Jonker et al. 2005), and preclinically using an individual linear correction with heart rate (QT<sub>c</sub> = QT – individual slope<sub>i</sub> [HR – 60]) (Gotta et al. 2015).

## Modeling and statistical analysis

Pharmacodynamic modeling was performed using nonlinear mixed effect modeling (NONMEM software, version 7.3.0; Icon Development Solutions, Ellicott City, MD)

and Perl-speaks-NONMEM scripts (PsN version 3.7.6, <http://psn.sourceforge.net>; Keizer et al. 2013). Parameters were estimated using the first-order conditional estimation (FOCE) method, as an additive error model was used to describe residual variability. Between-subject variability (estimated on all parameters) and interoccasion variability (estimated only on baseline QTc) were assumed to be log-normally distributed as described previously (Jonker et al. 2005; Gotta et al. 2015). Statistics and figures were created using R (version 2.10.1; R Development Core Team, Vienna, Austria, <http://www.r-project.org>).

## Model assumptions

The assumptions of the systems pharmacology model applied (Fig 1) can be briefly summarized as follows (Black and Leff 1983; Jonker et al. 2005):

**Receptor binding.** The concentration of bound hERG receptors at equilibrium at a given drug concentration can be derived from the law of mass action

$$[LR] = \frac{[R_0] \cdot [L]}{[K_i] + [L]} \quad (1 \text{ “binding model”})$$

where [LR] is the concentration of the ligand–receptor complex (bound or blocked hERG channels),  $[R_0]$  is the receptor concentration (hERG channel density), [L] is the

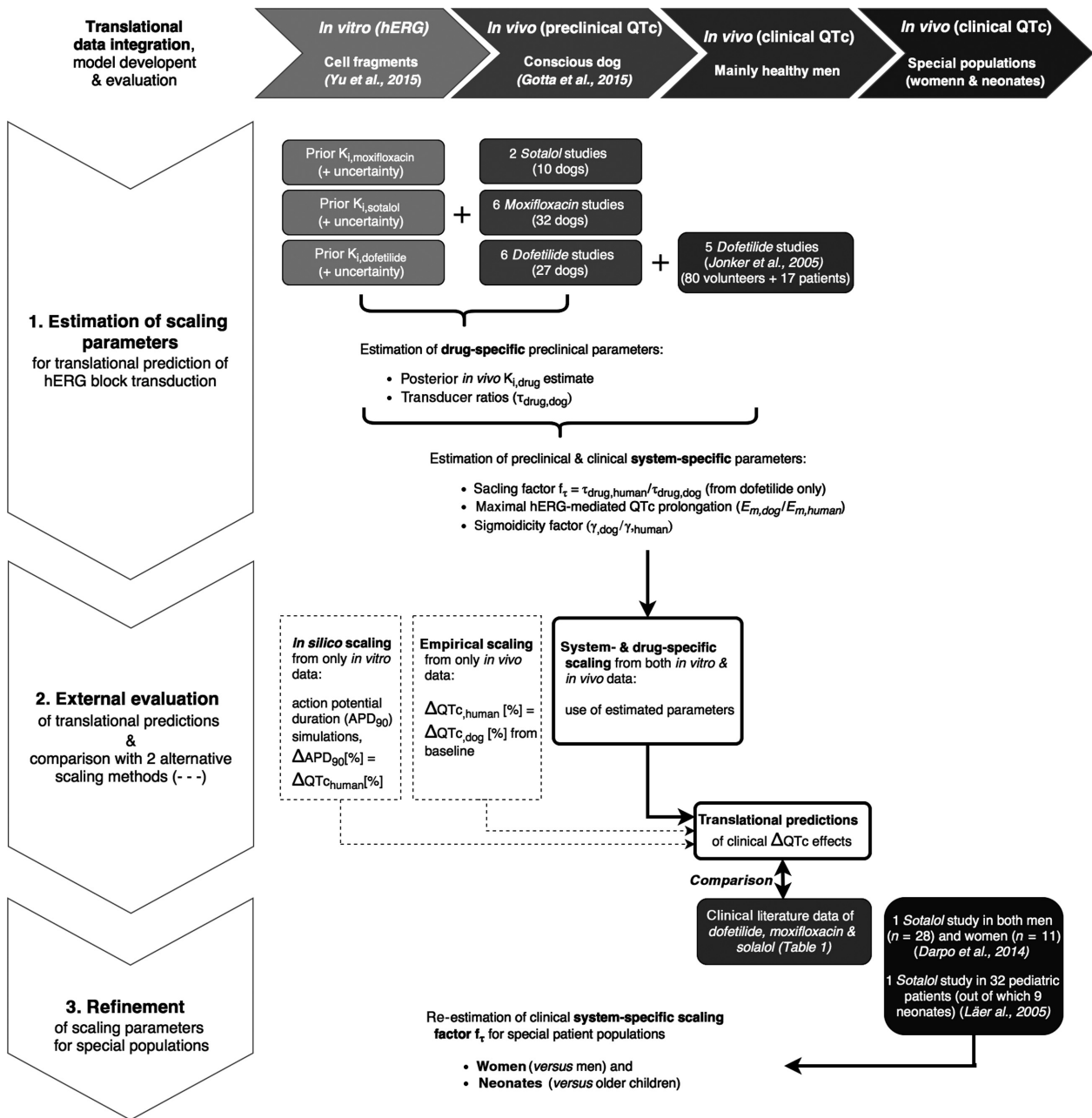


Figure 2. Outline of workflow (for details, see Materials and Methods section).

unbound ligand (drug) concentration, and  $K_i$  is the affinity of the drug (inhibitory binding constant, measured in vitro).

**Transduction.** The extent of QTc prolongation is then assumed to be mediated by the concentration of bound or blocked hERG channels:

$$E = \frac{E_m \cdot [LR]^\gamma}{LR_{50}^\gamma + [LR]^\gamma} \quad (2 \text{ "transducer function"}),$$

where  $E$  is the effect ( $\Delta$ QTc),  $E_m$  is the maximal effect that can be achieved in vivo by an hERG-blocking drug with high “operational efficacy”,  $LR_{50}$  is the LR concentration leading to half-maximal effect, and  $\gamma$  is the sigmoidicity parameter of the transducer function. For  $\gamma = 1$ , this function describes a hyperbolic curve, for  $LR \ll LR_{50}$  a linear transducer function and linear in vivo pharmacodynamic (drug concentration– $\Delta$ QTc effect) relationship would be observed (Black and Leff 1983).

**Table 1.** Pharmacodynamic data (overview).

Pharmacodynamic data used to establish scaling factors for translational predictions	
Data type and reference	Data details
In vitro hERG pharmacodynamics* (Yu et al. 2015)	<ul style="list-style-type: none"> <li>Affinity estimates (<math>K_i</math>) from hERG equilibrium binding assay of dofetilide, sotalol, and moxifloxacin</li> </ul>
Preclinical in vivo QTc pharmacodynamics (Gotta et al. 2015)	<ul style="list-style-type: none"> <li>14 pooled preclinical cardiovascular safety studies of dofetilide, sotalol, and moxifloxacin</li> <li>QT interval corrected for individual heart rate and circadian variation (QTc) along with unbound plasma concentration (or effect side concentration, if applicable)</li> <li>10–32 freely moving telemetered beagle dogs per compound</li> </ul>
Clinical in vivo QTc pharmacodynamics (Jonker et al. 2005)	<ul style="list-style-type: none"> <li>5 pooled clinical studies of dofetilide</li> <li>Friederica-corrected QTc interval along with unbound effect side concentration</li> <li>97 subjects from 5 clinical studies (3% women) <ul style="list-style-type: none"> <li>80 healthy volunteers</li> <li>17 patients with ischemic heart disease</li> </ul> </li> </ul>
Clinical pharmacodynamic data used for external evaluation of translational predictions	
Drug and reference	Study details
Dofetilide <sup>†</sup> (Allen et al. 2000)	25 healthy volunteers (all men)
Dofetilide <sup>†</sup> (Allen et al. 2002)	18 healthy volunteers (all men)
Dofetilide (Le Coz et al. 1995)	10 healthy volunteers (all men)
Dofetilide (Abel et al. 2000)	16 healthy volunteers (all men)
Sotalol (Barbey et al. 1999)	34 healthy volunteers (24 men/10 women)
Sotalol (Somberg et al. 2010)	15 healthy volunteers (gender not reported)
Sotalol (Kimura et al. 1996)	18 healthy volunteers (all male)
Sotalol <sup>‡</sup> (Läer et al. 2005)	32 pediatric patients with incessant or periodic supraventricular tachycardia 9 neonates 8 infants/toddlers, 12 children, 3 adolescents
Sotalol (Darpo et al. 2014) <sup>‡</sup>	34 healthy volunteers (28 men/11 women)
Moxifloxacin (Bloomfield et al. 2008)	20 healthy volunteers (10 men/10 women)
Moxifloxacin (Dixon et al. 2008)	152 healthy volunteers (97 men/55 women)
Moxifloxacin (Hulhoven et al. 2007)	54 healthy volunteers (52 men/2 women)
Moxifloxacin (Malik et al. 2009)	44 healthy volunteers (24 men/20 women)
Moxifloxacin (Florian et al. 2011)	1045 healthy volunteers from 20 thorough QT studies (0–100% women)

\* $K_i$  was assumed to be equivalent to the drug concentration at which half of hERG channels are occupied ( $IC_{50}$ ), and to be the same in dogs and in human, since the amino sequences of the canine and human ERG channel are 100% homolog (Zehelein et al. 2001).

<sup>†</sup>These studies were also included in the meta-analysis of Jonker et al. (2005).

<sup>‡</sup>Studies used to investigate gender- and age-dependent  $\Delta$ QTc sensitivity.

Combining equations (1) and (2) will yield the following general expression:

$$E = \frac{E_m \cdot (\tau \cdot [L])^\gamma}{(K_i + [L])^\gamma + (\tau \cdot [L])^\gamma} \quad (3 \text{ "operational model"})$$

where  $\tau$  is the transducer ratio, the “operational efficacy” of a drug in a given system, which is proportional to the system-specific receptor density  $R_0$ , and inverse proportional to  $LR_{50}$ , a parameter that can be interpreted equivalent, or at least proportional, to the drug-specific intrinsic efficacy of a drug (Black and Leff 1983):

$$\tau = \frac{R_0}{LR_{50}} \quad (4)$$

### Estimation of drug- and system-specific model parameters

The system- and drug-specific parameters were estimated by fitting the integrated “operational model” (eq. 3) (Black et al. 1985) to preclinical dog (eq. 5) and clinical (eq. 6) pharmacodynamic data:

$$\Delta QTc_{dog} = \frac{E_{m,dog} \cdot (\tau_{drug,dog} \cdot C_u)^{\gamma,dog}}{(K_{i,drug} + C_u)^{\gamma,dog} + (\tau_{drug,dog} \cdot C_u)^{\gamma,dog}} \quad (5)$$

$$\Delta QTc_{human} = \frac{E_{m,human} \cdot (\tau_{drug,dog} \cdot f_\tau \cdot C_u)^{\gamma,human}}{(K_{i,drug} + C_u)^{\gamma,human} + (\tau_{drug,dog} \cdot f_\tau \cdot C_u)^{\gamma,human}} \quad (6)$$

Equation (6) thus predicts the drug-induced clinical QTc prolongation ( $\Delta$ QTc) as a function of:

- 1 unbound drug plasma concentration ( $C_u$ ),
- 2 in vitro potency (affinity  $K_{i,drug}$ ) and preclinically estimated reference transducer ratios  $\tau_{drug,dog}$  (drug-specific parameters), and
- 3 maximal hERG-mediated QTc prolongation ( $E_{m,human}$ ) sigmoidicity of transducer function ( $\gamma, human$ ), and relative in vivo  $\Delta$ QTc sensitivity ( $f_\tau$ ) in human (system-specific parameters).

The latter was estimated as a fraction from dofetilide, assuming that this cancels out all drug-specific components and allows to conclude about relative tissue-specific properties (see Black and Leff 1983):

$$f_\tau = \frac{\tau_{dofetilide,human}}{\tau_{dofetilide,dog}} = \frac{\frac{R_{0,human}}{LR_{50,dofetilide,human}}}{\frac{R_{0,dog}}{LR_{50,dofetilide,dog}}} \approx \frac{R_{0,human}}{R_{0,dog}} \quad (7)$$

It can be seen that  $f_\tau > 1$  reflects increased  $\Delta$ QTc sensitivity conceptually because of higher hERG channel density in human ( $R_{0,human} \geq R_{0,dog}$ ). As it cannot be proven that  $LR_{50,dofetilide}$  is equal in dog and human, however, only its relative consistency across drugs can be assumed (Black and Leff 1983); it could also generally be interpreted as a higher net contribution of hERG-mediated  $I_{Kr}$  current to cardiac repolarization.

The feasibility of assuming a single system-specific  $\gamma$  value was evaluated in dogs. To the QTc baseline ( $QT_{c,BL}$ ), additive (eq. 8) and proportional (eq. 9) drug effect models were tested:

$$QTc = QT_{c,BL} + \Delta QTc_{[ms]} \quad (8)$$

$$QTc = QT_{c,BL}(1 + \Delta QTc_{[\%]})/100, \quad (9)$$

The pharmacodynamic parameters describing simply the pharmacodynamic concentration– $\Delta$ QTc relationship in vivo (drug-specific maximal  $\Delta$ QTc  $E_{max}$ ,  $EC_{50}$ , hill coefficient  $n$ )

$$\Delta QTc = \frac{E_{max} \cdot C_u^n}{EC_{50}^n + C_u^n} \quad (10)$$

were derived as follows (Black et al. 1985):

$$E_{max} = \frac{E_m \cdot \tau^\gamma}{\tau^\gamma + 1} \quad (11)$$

$$EC_{50} = \frac{\kappa_i}{(2 + \tau^\gamma)^{\frac{1}{\gamma-1}}} \quad (12)$$

$$G = \frac{0.576 \cdot \gamma \cdot (2 + \tau^\gamma)((2 + \tau^\gamma)^{\frac{1}{\gamma}} - 1)}{(2 + \tau^\gamma)^{\frac{1}{\gamma}} \cdot (1 + \tau^\gamma)} \quad (13)$$

where  $G$  is the midpoint gradient of equation 10 on a semilogarithmic scale (base 10), which can be used to approximate the slope parameter  $n$ . For  $\tau > 1$ ,  $G \rightarrow 0.576 \cdot n$ , and  $n = G/0.576$ .

The average individual predose baseline QTc ( $QT_{c,BL}$ ) measurement was used in the clinical dofetilide meta-analysis to calculate drug-induced QT prolongation ( $\Delta$ QTc = measured QTc – average measured predose  $QT_{c,BL}$ ) (Jonker et al. 2005). In the preclinical studies, baseline was measured over 24 h (following vehicle administration), and before each drug administration.  $\Delta$ QTc was therefore calculated by subtracting the occasion-specific estimated baseline and individual circadian variation ( $\Delta$ QTc = measured QTc – estimated occasion-specific  $QT_{c,BL}$  – estimated individual baseline circadian variation) (Gotta et al. 2015). The latter, thus, corresponds to a model-based equivalent of the double-delta QTc ( $\Delta\Delta$ QTc), which is corrected for predose baseline and time-matched placebo variation.

*Preclinical model development.* The pharmacodynamics of the three hERG blockers in conscious dogs was analyzed simultaneously. In vitro  $K_i$  estimates (Yu et al. 2015) were used as priors during model estimation and standard errors were used as variance of these priors (Langdon et al. 2007).

*Model evaluation.* The preclinical model was evaluated internally using standard goodness-of-fit plots (residual diagnostics, observations vs. predictions) and visual predictive check (VPC) diagnostics. The objective function value (equal to  $-2 \cdot \log$ -likelihood) was used to compare nested models (likelihood ratio test for  $\Delta$ OFV, decrease by 3.84 corresponds to a significant improvement in the model fit for one additional parameter at the 5% significance level). 95% confidence intervals (95% CI) of the parameter estimates were additionally derived from the 2.5th and 97.5th percentiles of the parameter distribution from a nonparametric bootstrap of 200 resampled data sets (stratified by drug), along with the bootstrap mean to assess the potential bias in typical parameter estimates.

*Clinical model and derived scaling parameters.* A clinical transduction model has previously been developed for dofetilide (Jonker et al. 2005), and dofetilide was thus used as the model compound to establish the scaling factor  $f_\tau$ , and to obtain  $E_m$  and  $\gamma$  estimates in human. We refitted the operational model to these data by fixing  $K_i$  to the preclinically estimated (“posterior”)  $K_i$  value (instead of previously estimated  $K_i$  of 5.13 ng/mL = 11.6 nmol/L) (Jonker et al. 2005), because we wanted to use in vitro data from one laboratory only to make  $\tau$  values estimated for the three drugs more comparable. As can be seen from the relationship between  $E_{max}$  and  $E_m$  in equation (11), the resulting system-specific  $E_m$  estimate is expected to remain almost unchanged for a drug with strong operational efficacy ( $\tau > 2$ ) with  $E_{max}$  close to  $E_m$ .

The preclinical and clinical NONMEM model code files are provided for reference in the supplemental data (Data S1 and S2). To facilitate visual evaluation of the clinical model and comparison to single-dose preclinical studies, clinical observations after multiple dosing were corrected for tolerance development (Jonker et al. 2005) in the graphical display.

### External evaluation

**Comparison with clinical references.** Derived scaling factors were applied to predict clinical  $\Delta$ QTc after dofetilide, sotalol, and moxifloxacin administration (“system- & drug-specific scaling”). Those translational predictions were contrasted with actual reported clinical  $\Delta$ QTc from literature. If possible, literature data were digitized using WebPlotDigitizer, version 3.4, Ankit Rohatagi, <http://arohatgi.info/WebPlotDigitizer>.

**Comparison with empirical predictions.** The “system- & drug-specific” scaling was compared with simple “empirical” scaling that predicted equal  $\Delta$ QTc<sub>[%]</sub> in human and dog at matching  $C_{u,plasma}$ , using previously published pharmacodynamic models (Gotta et al. 2015). From both methods,  $\Delta$ QTc predictions at therapeutic exposure were calculated.

**Comparison with in silico predictions.** The predicted clinical hERG block transduction was contrasted with in silico-predicted action potential duration at 90% repolarization (endocardial human APD<sub>90</sub>; O’Hara et al. 2011), which integrate only in vitro data. The model was downloaded from the CellML repository (<https://www.cellml.org/electrophysiology>), and simulations were performed using Matlab (The MathWorks, Inc., Natick, MA, 2000). This model appeared to show the best predictive performance in a recent publication compared to two other electrophysiology models (Mirams et al. 2014). Still, a range of other models was also simulated online (Cardiac Electrophysiology Web Lab© University of Oxford 2013–2015) and predicted hERG block transduction is summarized for completeness in Data S3.

**Prediction discrepancies.** The prediction discrepancies (pds) between clinical literature and translational predictions were calculated using clinical literature  $\Delta$ QTc predictions (typical  $\Delta$ QTc<sub>clinical</sub>) as reference:

$$\text{prediction discrepancy} = \Delta\text{QTc}_{\text{clinical}} - \Delta\text{QTc}_{\text{translational}} \quad (14)$$

where  $\Delta$ QTc<sub>translational</sub> is the prediction from preclinical data only.

### Model refinement for special patient populations

Quantitative differences in hERG block transduction were explored between neonates and children (Lärer et al. 2005) and men and women (Darpo et al. 2014) respectively, by reestimating  $\tau$  for each subpopulation from digitized data (nonlinear fixed effect least square regression). Derived  $\tau$  ratios were used to predict the pharmacodynamics of moxifloxacin and dofetilide in neonates and women. Predictions for gender-related differences in moxifloxacin pharmacodynamics could be compared with literature (Malik et al. 2009; Florian et al. 2011)

## Results

### Estimation of scaling parameters

The pharmacodynamic in vivo data used to derive drug- and system-specific scaling parameters of hERG-mediated QTc prolongation (Table 1) are illustrated in Figure 3 along with model predictions. Final model parameter estimates and derived parameters are summarized in Table 2.

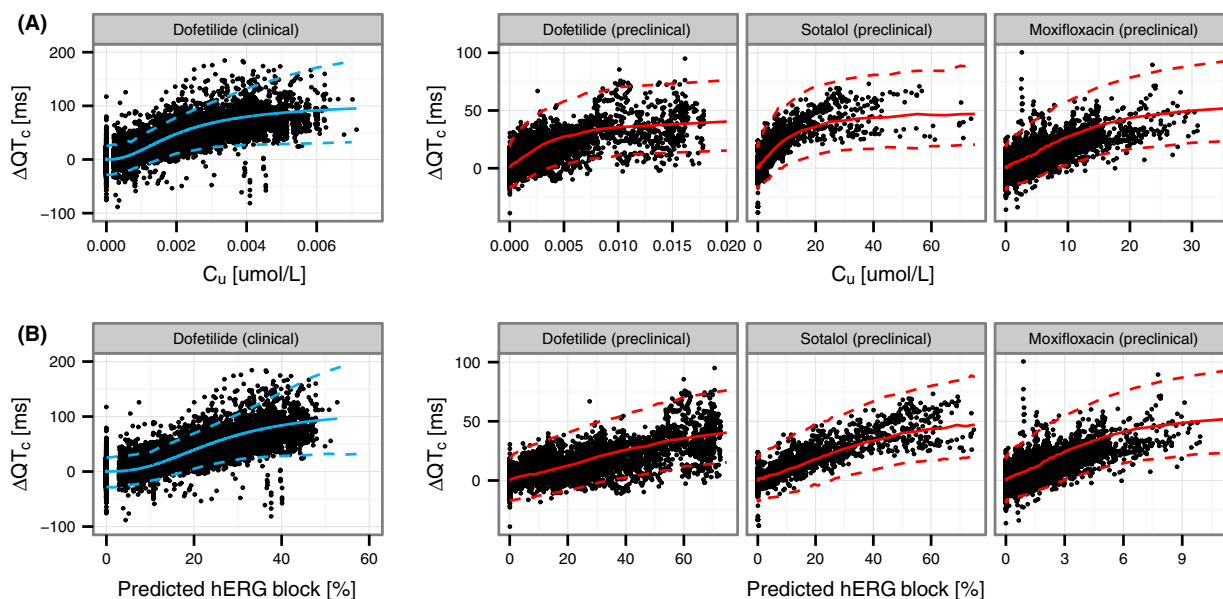
Figure 4 illustrates corresponding predicted in vitro (hERG) and in vivo (QTc) pharmacodynamic relationships, and system-specific hERG block transduction for all three drugs.

### Preclinical transduction model

A proportional drug effect model provided a slightly better model fit than an additive drug effect model ( $\Delta$ OFV –11) and decorrelated individual baseline (QTc<sub>BL</sub>) and  $E_m$  effects (decrease in correlation from 19% to 5%). The typical QTc<sub>BL</sub> was best characterized by a mixture distribution: QTc<sub>BL</sub> was particularly low in two moxifloxacin studies (26 msec lower, already previously observed; Gotta et al. 2015). This corrected for a bias in QTc<sub>BL</sub> prediction and better predicted overall variability (observed in visual predictive check diagnostics), since it decreased between-subject variability in QTc<sub>BL</sub> from 6.4% to 4.8%. In a sensitivity analysis, the sigmoidal operational model provided a better model fit than alternative transducer functions evaluated (linear or power relationships, showing a bias at higher hERG block and a higher OFV:  $\Delta$ OFV +1000 and +600 for 1 and 2 removed parameters).

### Preclinically estimated drug-specific parameters

Estimated (posterior)  $K_i$  values were close to prior in vitro estimates (Table 2). Transducer ratios were similar for dofetilide ( $\tau_{\text{dofetilide,dog}} = 1.6$ , bootstrap 95% CI: 1.1–1.9) and sotalol ( $\tau_{\text{sotalol,dog}} = 2.3$ , bootstrap 95%



**Figure 3.** Pharmacodynamic data used to derive system-specific scaling parameters (preclinical = conscious telemetered dog). (A) In vivo QTc pharmacodynamics. (B) In vivo transduction of hERG block. Dots: observed  $\Delta$ QTc from individual baseline. Solid lines: Typical model predictions (median). Dashed lines: 80% prediction intervals. 95% CI of the median and 80% prediction intervals comprised the observed median, 10th and 90th percentiles of the data and are not illustrated to improve clarity. Clinical QTc multiple dose observations were corrected for tolerance development to make them more comparable to single-dose preclinical data.

CI: 1.4–3.1), and were highest for moxifloxacin  $\tau_{\text{moxifloxacin,dog}} = 20.3$ , bootstrap 95% CI: 9–28).

### System-specific parameters (dog)

The maximal  $\Delta$ QTc that can be achieved in dogs via hERG inhibition ( $E_{m,\text{dog}}$ ) was estimated to 28% (bootstrap 95% CI: 22–46) from baseline (typical estimate: 249 msec). Estimating sigmoidicity coefficients of the transducer function for each drug separately indicated that a common dog-specific sigmoidicity coefficient ( $\gamma_{\text{dog}}$ ) could be assumed (range of drug-specific  $\gamma_{\text{drug,dog}}$  estimates: 1.43–1.74). Accordingly, estimating only one common  $\gamma_{\text{dog}}$  did not worsen the model fit (OFV +4.8 for 2 removed parameters,  $P > 0.05$ ).

### System-specific parameters (clinical)

Using the preclinical posterior  $K_i$  estimate of 6.8 nmol/L (Table 2), instead of previously estimated  $K_i$  of 11.6 nmol/L (Jonker et al. 2005), did not change the previous main model predictions: The  $E_{m[\%],\text{human}}$  estimate (29%, corresponding to 113 msec at a typical baseline of 390 msec) was very similar to its previous estimate (Jonker et al. 2005) (107 msec, 95% CI: 80–134 msec). Also, while the  $\tau_{\text{dofetilide,human}}$  estimate was expectedly lower, the model still predicted that a %hERG block close to

10% (=previous estimate [Jonker et al. 2005]; here: 13%) leads to 20 msec  $\Delta$ QTc. Estimated as a fraction ( $f_\tau$ ),  $\tau_{\text{dofetilide,human}}$  was 2.4 times (95% CI: 2.06–2.72) higher than  $\tau_{\text{dofetilide,dog}}$ .

### Derived system- and drug-specific scaling approach

The following relationships were used to predict hERG block transduction in human for sotalol and moxifloxacin from integrated (in vitro and in vivo) preclinical data:

$$K_{i,\text{human}} = \text{posterior } K_{i,\text{dog}} (\approx \text{in vitro hERG-potency})$$

$$\tau_{\text{drug,human}} = f_\tau \cdot \tau_{\text{drug,dog}} (\approx R_{0,\text{human}}/R_{0,\text{dog}} \cdot \tau_{\text{drug,dog}})$$

$$E_{m[\%],\text{human}} = 29\% (\approx E_{m[\%],\text{dog}} \text{ from baseline})$$

$$\gamma_{\text{human}} = 2.4 (\text{vs. } \gamma_{\text{dog}} = 1.6)$$

### External evaluation

The agreement between translational predictions and clinical  $\Delta$ QTc (observed and/or predicted from different clinical studies) is illustrated in Figure 5A. Corresponding prediction discrepancies (pds) between clinical and translational predictions are shown in Figure 5B and summarized in the following.



**Table 2.** Parameter estimates of the systems pharmacology model.

Parameter	Dog estimate (RSE%)	Human estimate (RSE%)
<b>Typical values</b>		
Baseline QT <sub>CBL</sub> (msec)	249 (0.6%) 222 (2%) in 2 moxifloxacin studies	390 (–)
$E_m$ [%] from baseline	0.274 (10%) → $E_{m,dog}$	0.29 (10%) → $E_{m,human}$
$\gamma$ (sigmoidicity)	1.64 (11) → $\gamma_{dog}$	2.4 (4%) → $\gamma_{human}$
Transducer ratios (–) $\tau_{drug}$		
$\tau_{dofetilide}$	1.61 (19%)	3.85 (predicted)
$f_\tau$		2.39 (7)
$\tau_{sotalol}$	2.26 (18%)	5.4 (predicted)
$\tau_{moxifloxacin}$	20.3 (33%)	48.4 (predicted)
$K_i$ (posterior*) ( $\mu$ mol/L free)		
$K_{i,dofetilide}$	0.0068 (11%)	Fixed to 0.0068
$K_{i,sotalol}$	24.8 (5%)	Fixed to 24.8
$K_{i,moxifloxacin}$	281 (31%)	Fixed to 280
<b>Variability</b>		
IOV QT <sub>CBL</sub>	1.8% (6%)	–
BSV QT <sub>CBL</sub>	4.8% (9%)	–
BSV $E_m$	53% (14%)	85% (10)
BSV $\gamma$	83% (9%)	56% <sup>†</sup>
BSV $\tau$	57% (15%)	44% (12)
Correlation $E_m$ – $\gamma$	–0.42 (37%) <sup>‡</sup>	–0.28 <sup>†</sup>
Correlation $E_m$ – $\tau$	–0.53 (47%) <sup>‡</sup>	–0.93 (24) <sup>‡</sup>
Correlation $\tau$ – $\gamma$	0.49 (40%) <sup>‡</sup>	0.57 <sup>†</sup>
<b>Residual error</b>		
Additive residual (msec)	6.3 (1%)	15.2 (1%)
<b>Derived parameters</b>		
hERG–block (%) leading to half-maximal $E_m$ ( $=1/\tau = LR_{50}/R_0$ )		
Dofetilide	62%	26%
Sotalol	44%	18%
Moxifloxacin	5%	2%
hERG block (%) leading to 2.5% QTc prolongation	=6 msec	=10 msec
Dofetilide	19%	12%
Sotalol	13%	8%
Moxifloxacin	1.3%	0.8%
Pharmacodynamic parameters of concentration– $\Delta$ QTc relationship		
$E_{max}$ (%) / $EC_{50}$ (mol/L free) / $n$ (–)		
Dofetilide	19%/0.0048/1.3	28%/0.0023/1.9
Sotalol	22%/12.9/1.3	29%/5.5/2.0
Moxifloxacin	27%/14.4/1.6	29%/5.9/2.4

BSV, between-subject variability; reported as  $CV\% = \sqrt{e^{\omega^2} - 1}$ ; IOV, interoccasion variability.

\*Prior  $K_i$  values (relative standard errors) from in vitro experiments – dofenilide: 5.4 nmol/L (15%), sotalol: 24.6  $\mu$ mol/L (5.6%), moxifloxacin: 252  $\mu$ mol/L (48%).

<sup>†</sup>BSV/correlation terms were estimated from a full covariance block for individual random effects. Since the uncertainty could, however, not be estimated, these values are only reported to make the comparison between dog and human estimates more complete.

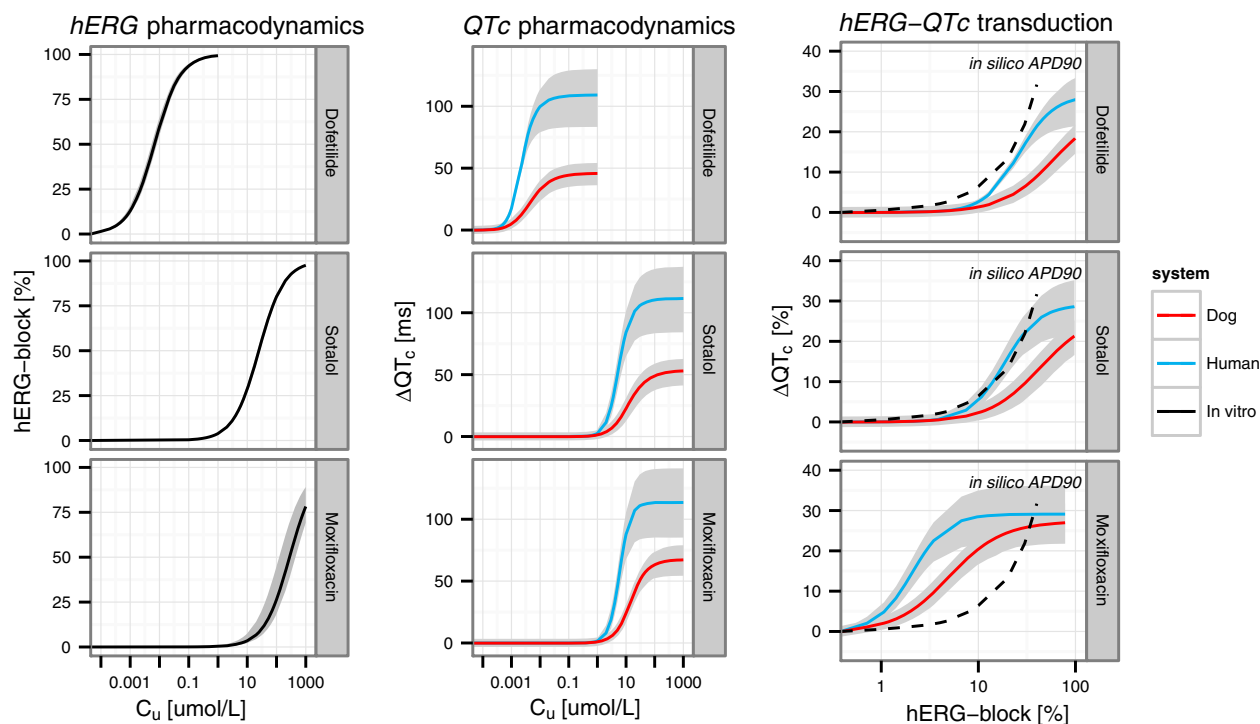
<sup>‡</sup>Relative standard error of respective covariance reported.

## Comparison with “empirical” predictions

Both the “empirical” scaling (based on preclinical in vivo data only, assuming equal  $\Delta$ QTc<sub>[%]</sub> from baseline in dog and human) and the “system- and drug-specific” scaling (integrating both in vitro and in vivo data) predicted  $\Delta$ QTc of >10 msec within therapeutic exposure (*dofetilide* [0.4–2 nmol/L]: 3–20 msec vs. 5–

49 msec, respectively; *sotalol* [3.7–11  $\mu$ mol/L]: 22–45 msec vs. 37–83 msec; *moxifloxacin* [2.9–5.6  $\mu$ mol/L]: 7–19 msec vs. 22–53 msec). Predictions and pds are compared in Figure 5.

*Dofetilide*. A systematic underprediction of clinical data was observed from the empirical method for predicted  $\Delta$ QTc of >10 msec, whereas pds of the estimated



**Figure 4.** Predicted typical pharmacodynamic relationships and system-specific hERG block transduction.  $C_u$ : unbound plasma/effect side concentration.  $\Delta$ QTc: QTc prolongation (in [msec] and [%]) from baseline. half-maximal  $\Delta$ QTc<sub>1%</sub> is achieved in human at lower hERG block than in dog, maximal possible effects are similar ( $E_m = 27$ – $29\%$  from baseline, which equals approximately  $E_{max}$  in case of a high transducer ratio  $\gg 1$ , right panel). Shaded areas: 95% confidence intervals. Dashed line: predicted action potential duration at 90% repolarization ( $\Delta$ APD<sub>90</sub> in [%]) from in silico simulations (O’Hara et al. 2011).

operational model were  $<5$ – $10$  msec over the whole dynamic range of plasma concentration, and until a predicted  $\Delta$ QTc of 30 msec compared to one small study (Le Coz et al. 1992).

**Sotalol.** Pds were small ( $<5$ – $10$  msec) until a predicted  $\Delta$ QTc of 35 msec using both methods, with one exception (neonatal  $\Delta$ QTc data [Läer et al. 2005]: pds  $>20$  msec /  $>100\%$ ). Above predictions of 35 msec, empirical scaling tended to underpredict clinical QTc more than system-specific scaling tended to overpredict clinical effects (pds up to  $+40$  msec vs.  $-20$  msec).

**Moxifloxacin.** Pds were small ( $<5$ – $10$  msec) using both methods in the range of typically observed clinical  $\Delta$ QTc (up to 20 msec). Above predictions of 25 msec, the system-specific method tended to overpredict clinical  $\Delta$ QTc, whereas no predictions  $>20$  msec (and thus pds) were obtained from the empirical method.

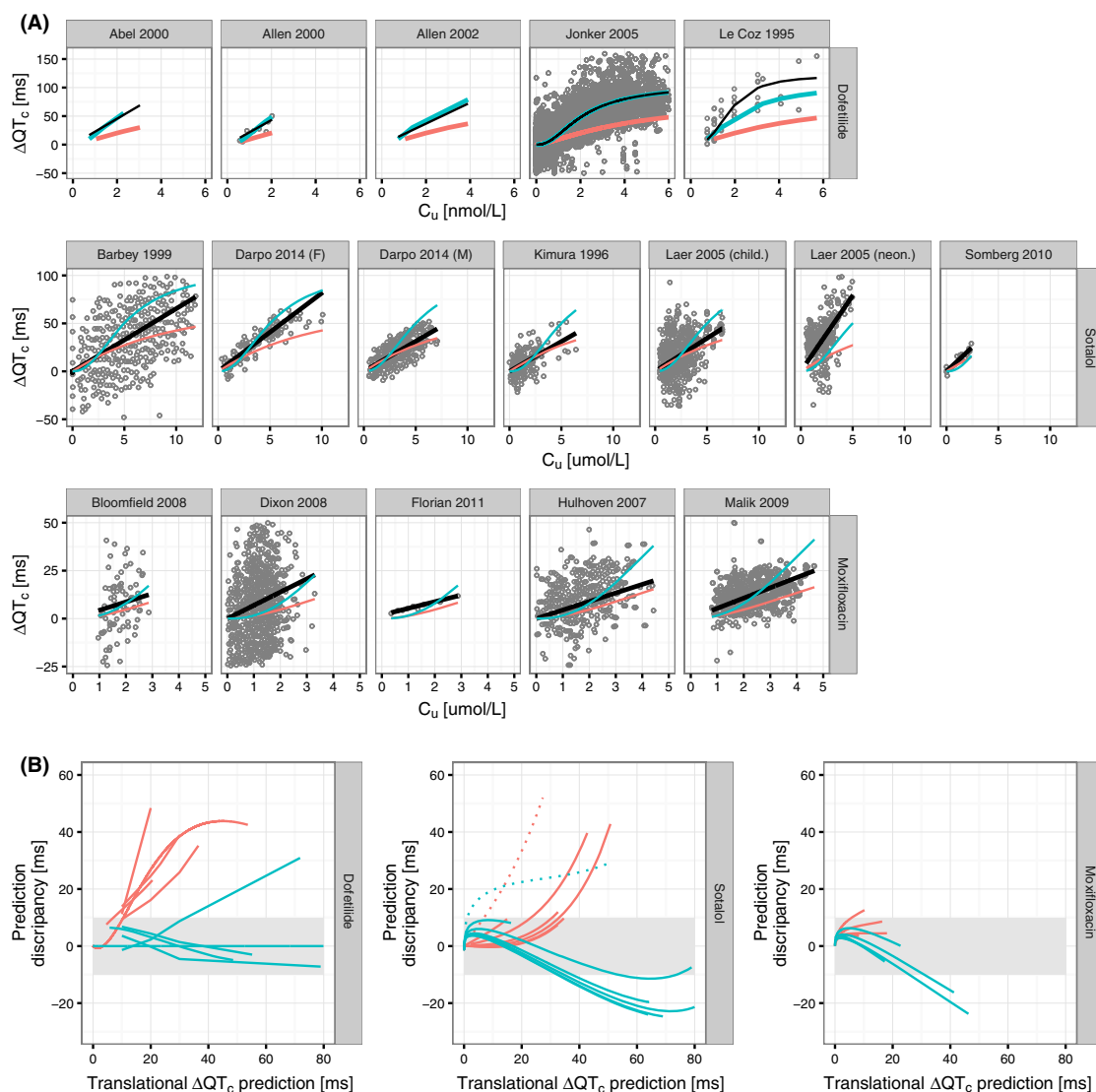
### Comparison with “in silico” predictions

“In silico”  $\Delta$ APD<sub>90</sub> simulations (using only in vitro data and the O’Hara model) predicted for sotalol similar

$\Delta$ QTc as the “system- and drug-specific” scaling approach (integrating both in vitro and in vivo data), but clearly underpredicted moxifloxacin  $\Delta$ QTc, and slightly overpredicted dofetilide data (Fig. 3). A comparison of the predictions of other electrophysiology models is given in Data S3. All other models predict a less steep increase in APD<sub>90</sub> than the O’Hara model and would thus mostly underpredict the observed in vivo transduction.

### Refinement of scaling parameter $\tau$ for special patient populations

A 77% higher  $\tau$  was estimated in neonates receiving sotalol compared to children ( $f_{\tau,neonates} = 1.77$ , 95% CI: 1.62–1.92) resulting in 38 msec higher  $\Delta$ QTc prediction in neonates ( $\Delta$ QTc<sub>children</sub> = 39 msec) at a drug exposure of  $5 \mu\text{mol/L}$ . The estimated  $\tau$  in children ( $\tau_{children} = 4.6$ , 95% CI: 4.3–4.9) and resulting predicted pharmacodynamic profile were very similar to the one predicted for adults (black line in Fig. 6A). In women receiving sotalol, a 9% higher  $\tau$  was estimated ( $f_{\tau,women} = 1.09$ , 95% CI: 1.03–1.15) compared to men ( $\tau_{men} = 4.1$ , 95% CI: 4.0–4.3) (Fig. 6B), resulting in a 15 msec higher  $\Delta$ QTc



**Figure 5.** External evaluation of translational  $\Delta$ QTc predictions. Blue lines: translational predictions from integrated preclinical in vivo and in vitro data (“system-specific” scaling). Red lines: translational predictions from preclinical in vivo data only (“empirical” scaling). (A) Agreement between model predictions and literature data. Gray dots: clinical observations (digitized data from references detailed in Table 1). Black lines: typical clinical QTc predictions (taken from indicated references). (B) Calculated prediction discrepancies between published clinical and translational  $\Delta$ QTc predictions. Gray band:  $\pm 10$  msec prediction discrepancy. Dotted lines: neonatal sotalol study (Läer et al. 2005).

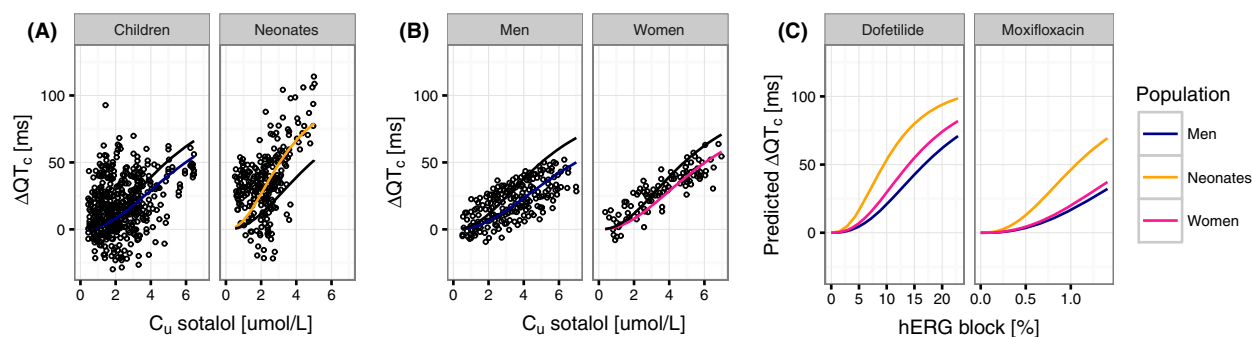
prediction at a drug exposure of  $5 \mu\text{mol/L}$  ( $\Delta\text{QTc}_{\text{men}} = 33$  msec).

Simulation of predicted dofetilide and moxifloxacin pharmacodynamic profiles within therapeutic concentrations (Fig. 6C) showed that an observable gender difference in  $\Delta$ QTc is expected for dofetilide (predicted  $\Delta\text{QTc}_{\text{men}}$  at  $2 \text{ nmol/L}$  free, corresponding to a hERG block of 23%: 71 msec,  $\Delta\text{QTc}_{\text{women}}$ : 11 msec) but not for moxifloxacin (predicted  $\Delta\text{QTc}_{\text{men}}$  at  $3 \mu\text{mol/L}$  free, corresponding to a hERG block of 1.1%: 18.8 msec;  $\Delta\text{QTc}_{\text{women}}$ : 3.6 msec). An observable developmental difference, that is a clearly

higher  $\Delta$ QTc (+30 msec) in neonates, was predicted in contrast for both drugs at this exposure.

## Discussion

In this work, we extended the use of a previously proposed integrated pharmacodynamic model (Black et al. 1985; Jonker et al. 2005) to dissociate and quantify drug- and system-specific characteristics in the transduction of hERG block to QTc prolongation ( $\Delta$ QTc). Three hERG blockers were used as model compounds (antiarrhythmic



**Figure 6.** Estimated difference in hERG block transduction between patient populations. (A) Children (blue line) and neonates (orange line): estimated  $\tau_{\text{neonates}} = 1.8 \cdot \tau_{\text{children}}$ ; sotalol data digitized from Laer et al. (2005). (B) Men (blue line) and women (pink line): estimated  $\tau_{\text{women}} = 1.1 \cdot \tau_{\text{men}}$  (pink line); sotalol data digitized from Darpo et al. (2014). Black line: sotalol pharmacodynamics predicted from the estimated general “ $\tau_{\text{human}}$ ” (see Table 2). (C) Predicted population differences in dofetilide and moxifloxacin over fractional hERG block, assuming  $\tau_{\text{men}} = \tau_{\text{human}}$  (from Table 2).

compounds dofetilide and sotalol, and fluoroquinolone antibiotic moxifloxacin), and in vivo hERG block was predicted from in vitro potency ( $K_i$ ) and in vivo unbound plasma concentration ( $C_{u,\text{plasma}}$ ). System-specific scaling parameters were derived for translating the relationship between %hERG block and in vivo  $\Delta$ QTc effects (“hERG block transduction”) from preclinical species (conscious dog) to clinical populations.

### In vitro–in vivo translation

An in vitro hERG block as low as <1–12% (human) and 1–19% (dogs) was already associated with a 2.5%  $\Delta$ QTc, corresponding to 10 msec in human and 6 msec in dogs. This is consistent with previous observations (Redfern et al. 2003; Jonker et al. 2005), and in silico  $\text{APD}_{90}$  (O’Hara et al. 2011), which predict a 2.5%  $\Delta\text{APD}_{90}$  at  $\geq 4$ –5%  $I_{\text{Kr}}$  inhibition (Fig. 4, Data S3). While this stresses the importance of characterizing this low pharmacodynamic range in vitro, it also indicates that predictions from in vitro data alone can be difficult because of drug-specific intrinsic efficacy: almost identical transducer ratios were estimated for the structurally similar compounds dofetilide and sotalol in dog, while it was 10 times higher for moxifloxacin, consistent with a recent publication (Marostica et al. 2015). The transducer ratio differences between drugs are supported by experimental data suggesting that the mechanism of moxifloxacin binding in the inner cavity of the hERG channel differs from antiarrhythmic drugs regarding, for example, interaction with specific amino acid residues, state and voltage dependency of hERG block (Thomas et al. 2004; Alexandrou et al. 2006; Witchel 2007). Interestingly, the larger  $\tau_{\text{moxifloxacin}}$  value also explained the steeper sigmoidal concentration– $\Delta$ QTc relationship estimated previously (compared to dofetilide and sotalol; Gotta et al. 2015), since both  $\tau$  and the curve-shape parameter  $\gamma$  influence the

steepness (i.e., Hill coefficient) of this pharmacodynamic relationship (eq. 13) (Black et al. 1985).

We hypothesized that drug tissue distribution could explain such differences in transducer ratios between drugs, and/or observed higher in vivo than in vitro potency (Redfern et al. 2003; Mirams et al. 2014). Drugs mainly bind from the inside of the cell to the hERG channel (Thomas et al. 2004; Witchel 2007), where basic drugs accumulate due to a lower intra- than extracellular pH. Additionally, the general distribution into heart tissue may be relevant. However, we found that  $C_{u,\text{plasma}}$  seems to be a better predictor of in vivo hERG binding and QTc prolongation than intracellular or heart tissue concentrations predicted from physiologically based pharmacokinetic approaches (Rodgers et al. 2005; Rodgers and Rowland 2006; Yun and Edginton 2013) (data not shown). This is consistent with the observation that hERG potency estimates from equilibrium binding (cell fragment) assays correlate well with those from patch-clamp (whole cell) experiments (Pollard et al. 2010), in which different drug concentrations are extracellularly added. For translational predictions from in vitro data, both the use of  $C_u$  (Polak 2013; Mirams et al. 2014) and tissue concentration (Chetty et al. 2014) have been proposed.

Interestingly, the hyperbolic (at higher concentrations or hERG block saturating) transducer function could best describe the hERG block– $\Delta$ QTc relationship that is consistent with the previously observed exposure– $\Delta$ QTc relationship in vivo (Gotta et al. 2015). It should be noted that based on electrophysiology principles, a saturating QTc prolongation following increasing hERG block would, however, not be expected as can be seen from the  $\text{APD}_{90}$  simulations (Fig. 4 and Data S3). This discrepancy between mechanistic electrophysiological expectations and empirical observations could indicate that other processes than hERG block are involved (such as block of

other ion channels at increasing concentrations), or that although QTc is supposed to reflect the APD, both are not proportional because the surrogate QTc interval may be affected by additional factors.

### Dog–human translation

Three typical system-specific parameters ( $E_m$ ,  $\gamma$ ,  $f_\tau$ ) characterizing the transduction of hERG block in vivo were estimated from preclinical and clinical dofetilide metastudy data, and provided good translational  $\Delta$ QTc predictions also for sotalol and moxifloxacin. Resulting predicted human  $\Delta$ QTc<sub>[ms]</sub> was up to 4.4 times higher than in dog (overall range: 1.7–4.4, Fig. 4), with the largest difference observed around EC<sub>50</sub>. Interestingly, the absolute maximal  $\Delta$ QTc<sub>[ms]</sub> that can be achieved through hERG block ( $E_{m[ms]}$ ) by a drug with high operational efficacy in human was  $\approx$ 1.55 times higher than in dog (110 msec vs. 70 msec), corresponding to an allometric relationship using an exponent of 0.25 as suggested for time scales (Boxenbaum 1982; Mager et al. 2009). An allometric relationship would also be applicable to scale baseline QTc. Accordingly, the maximal relative prolongation from baseline ( $E_{m[\%]}$ ) was almost identical both in dog and human (27% vs. 29%), and this good proportional agreement, already found previously (Gotta et al. 2015), was used as comparative “empirical” scaling method over the whole pharmacodynamic range (instead of the up to 2.7 times higher  $\Delta$ QTc<sub>[%]</sub> in human predicted by the operational model, Fig. 4).

Higher sensitivity to drug-induced QTc prolongation was, however, estimated in human: the transducer ratio ( $\tau_{\text{dofetilide}}$ ) was estimated to be 2.4-fold higher than in dog, resulting in higher  $\Delta$ QTc at same %hERG block. Also, along with a higher sigmoidicity parameter ( $\gamma_{\text{human}}$ ), the  $\Delta$ QTc increase around EC<sub>50</sub> was predicted to be clinically steeper than in preclinical dog studies.

The parameters estimated by the transduction model can be converted to the parameters of a sigmoidal  $E_{\text{max}}$  model relating simply  $C_u$  to  $\Delta$ QTc (Black et al. 1985). For these three compounds, the derived EC<sub>50</sub> in human were accordingly 41–47% of their estimates in dog, and hill coefficients were 1.5-fold higher (range in dog: 1.3–1.6 vs. human: 1.9–2.4).  $E_{\text{max}}$  and EC<sub>50</sub> can frequently not be estimated clinically but from preclinical studies, in which a higher concentration range is used (Gotta et al. 2015; Sparve et al. 2014).

### Empirical versus drug- and system-specific scaling

While both evaluated translational scaling methods predicted clinical  $\Delta$ QTc of >10–20 msec for all three drugs

at therapeutic exposure, clinical predictions were clearly improved for dofetilide using the “system-specific” compared to the simple “empirical” scaling approach. This was of course partly expected, as transduction parameters were estimated from pharmacodynamic data of this drug. For sotalol and moxifloxacin, both methods yielded similarly good predictions until a  $\Delta$ QTc of  $\approx$ 25–35 msec. Above, a trend for underprediction was observed for the “empirical” method (relative to clinical relationships reported in literature, Fig. 4), and a smaller overprediction using the “system-specific” scaling. This suggests that application of the transduction model could improve translational  $\Delta$ QTc predictions for drugs with significant QTc prolongation of >5–8% (corresponding to a  $\Delta$ QTc of >20–30 msec in human and >12.5–20 msec in dog). For the prediction of a thorough QT (TQT) study, which aims to detect small effects of 10 msec only, the simple empirical method may be sufficient in a first step. However, in contrast to the transduction model, this method does not facilitate a mechanistic scaling of  $\Delta$ QTc effects to patient populations not included in early clinical studies.

### Clinical differences in sensitivity

Without adjusting for population-specific  $\Delta$ QTc sensitivity, both translational methods clearly underpredicted neonatal sotalol  $\Delta$ QTc effects (Läer et al. 2005). Reestimating  $\tau$  in this patient population suggested that neonates have 1.77 times higher transduction of hERG block than younger children, whereas the latter showed very similar  $\Delta$ QTc sensitivity than adults. Interestingly, the estimated 1.77 times higher  $\tau$  is in line with  $a \approx$  1.5–2 times higher  $I_{K_r}$  density reported in young (25 days old) canine myocytes than in adult (3–5 years) (Obreztkhikova et al. 2003). A lack of  $I_{K_s}$  channel expression in young myocytes probably also contributes to the higher effect of hERG block. For dofetilide and moxifloxacin, no neonatal pharmacodynamic data could be found to evaluate that prediction; the previously discussed study on canine myocytes (Obreztkhikova et al. 2003), however, also used dofetilide as model drug and showed higher neonatal  $\Delta$ QTc sensitivity. The use of moxifloxacin/fluroquinolones is restricted in pediatrics due to the concern about cartilage damage (Garazzino et al. 2014). Age-related differences in drug efficacy and safety are not yet well understood, but increasing evidence exists for differences in expression of target proteins in children that may affect drug response (Moita Santos 2014).

In women, only a small 10% higher  $\tau$  was estimated compared to men. Interestingly, simulations showed that this correctly predicted minimal gender differences in  $\Delta$ QTc after moxifloxacin administration (<5 msec)

(Malik et al. 2009; Florian et al. 2011). In contrast, for sotalol and dofetilide, a  $> 10$  msec difference was predicted at therapeutic exposure, probably because their therapeutic range is very close to  $EC_{50}$ , that is, the point where largest differences would be observed. This prediction could, however, not be further evaluated based on literature data as mainly men were included in the dofetilide studies.

In summary, interspecies difference in  $\Delta$ QTc sensitivity were, however, still larger than the difference between different patient populations (healthy men, women, and neonates).

### Limitations and perspectives

Unfortunately, not all translational predictions could be evaluated with actual clinical data. Also, while the system-specific scaling approach (derived from dofetilide only) showed good external predictive performance for both a drug with similar (methansulfonamid sotalol) and different structural properties (fluorochinolone moxifloxacin), it would be valuable to validate or refine the proposed in vivo preclinical–clinical differences transduction with more hERG blockers.

The up to 10-fold difference in  $\tau_{\text{drug}}$  estimates indicates that clinical predictions from only in vitro data are challenging. Methods to determine drug effects on the hERG channel and derived in vitro potency can additionally vary significantly between laboratories (Kirsch et al. 2004; Milnes et al. 2010). Our in vitro potency measures were therefore taken from one laboratory only. It should be acknowledged that measurements were performed at 25°C, and that some drugs may have a different (probably higher) potency at body temperature (Kirsch et al. 2004). While standardization of in vitro experiments is warranted to make potency estimates across laboratories more comparable, consistent pharmacodynamic predictions can be obtained from varying in vivo study designs when analyzed by systematic pharmacokinetic–pharmacodynamic modeling (Gotta et al. 2015). Further integration of drug-specific dynamic binding (Milnes et al. 2010) may also help to better predict the difference in drug-specific transducer ratios. Those may be explained by state-, time-, and voltage-dependent hERG binding and conductance changes (di Veroli et al. 2013). Integration of these dynamic aspects could also improve predictions of physiologic APD<sub>90</sub> simulations, which currently tend to underpredict clinical  $\Delta$ QTc (Mirams et al. 2014). In contrast, different tissue distribution does not seem to play a role (see above).

The transduction model could be extended to predict QTc–drug interactions from in vitro hERG/ $I_{Kr}$  inhibition experiments or to characterize the transduction of other

(single) ion channel blockers and ultimately predict  $\Delta$ QTc effects following mixed ion channel block. Scaling factors for other preclinical species could be also derived or for human-induced pluripotent stem cells which may further reduce animal studies (Braam et al. 2010).

### Conclusions

Our work not only illustrates the challenges of translational pharmacology (amplification of the signal was observed in each of the steps from in vitro  $\rightarrow$  preclinical in vivo  $\rightarrow$  clinical  $\rightarrow$  patient pediatric population), but also the utility of a quantitative systems pharmacology approach as a rational guide for drug discovery and development (Leishman 2014; Collins et al. 2015; Davies et al. 2016). We confirmed that a generally low but drug-dependent fractional hERG block (range:  $<1$ –20%) can result in clinically relevant QTc prolongation ( $\geq 10$  msec). In contrast, in vivo dog–human differences in  $\Delta$ QTc sensitivity derived from dofetilide appeared consistent (drug independent). As complementary pharmacodynamic analysis of phase I studies (Bloomfield 2015) for compounds showing preclinical  $\Delta$ QTc in the conscious dog, the scaling approach may also allow prediction of  $\Delta$ QTc effects in patient populations not regularly included in pharmacodynamic trials (women, pediatrics), and thus leverage the information gathered preclinically.

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#### Targets

Voltage-gated ion channels

K<sub>v</sub>11.1 (hERG)

<http://www.guidetopharmacology.org/GRAC/ObjectDisplayForward?objectId=572&familyId=81&familyType=IC>

#### Ligands

Dofetilide

<http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=2604>

Sotalol

<http://www.guidetopharmacology.org/GRAC/LigandDisplayForward?ligandId=7297>

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### Author Contributions

V. G., M. D., and P. H. G. designed the research; Z. Y., F. C., K. A., S. A. G. V., P. M., and F. S. contributed essentially to data acquisition; V. G. analyzed the data and wrote the manuscript; and all authors were involved in data interpretation, manuscript review, and approval.

### Acknowledgement

This project was supported by the Dutch Top Institute Pharma (TIPharma) PK-PD platform 2.0.

## Disclosure

D. J. G., F. C., and K. A. are employees of Janssen Pharmaceutica. S. A. G. V., P. M., and F. S. are employees of Merck. P. H. G. is an employee of Certara.

## References

- Abel S, Nichols DJ, Brearley CJ, Eve MD (2000). Effect of cimetidine and ranitidine on pharmacokinetics and pharmacodynamics of a single dose of dofetilide. *Br J Clin Pharmacol* 49: 64–71.
- Alexandrou AJ, Duncan RS, Sullivan A, Hancox JC, Leishman DJ, Witchel HJ, et al. (2006). Mechanism of hERG K<sup>+</sup> channel blockade by the fluoroquinolone antibiotic moxifloxacin. *Br J Pharmacol* 147: 905–916.
- Allen MJ, Nichols DJ, Oliver SD (2000). The pharmacokinetics and pharmacodynamics of oral dofetilide after twice daily and three times daily dosing. *Br J Clin Pharmacol* 50: 247–253.
- Allen MJ, Oliver SD, Newgreen MW, Nichols DJ (2002). Pharmacodynamic effect of continuous vs intermittent dosing of dofetilide on QT interval. *Br J Clin Pharmacol* 53: 59–65.
- Barbey JT, Sale ME, Woosley RL, Shi J, Melikian AP, Hinderling PH (1999). Pharmacokinetic, pharmacodynamic, and safety evaluation of an accelerated dose titration regimen of sotalol in healthy middle-aged subjects. *Clin Pharmacol Ther* 66: 91–99.
- Black JW, Leff P (1983). Operational Models of Pharmacological Agonism. *Proc R Soc B Biol Sci* 220: 141–162.
- Black JW, Leff P, Shankley NP, Wood J (1985). An operational model of pharmacological agonism: the effect of E/[A] curve shape on agonist dissociation constant estimation. *Br J Pharmacol* 84: 561–571.
- Bloomfield DM (2015). Incorporating exposure-response modeling into the assessment of QTc interval: a potential alternative to the thorough QT study. *Clin Pharmacol Ther* 97: 444–446.
- Bloomfield DM, Kost JT, Ghosh K, Hreniuk D, Hickey LA, Guitierrez MJ, et al. (2008). The effect of moxifloxacin on QTc and implications for the design of thorough QT studies. *Clin Pharmacol Ther* 84: 475–480.
- Boxenbaum H (1982). Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. *J Pharmacokinet Biopharm* 10: 201–227.
- Braam SR, Tertoolen L, van de Stolpe A, Meyer T, Passier R, Mummery CL (2010). Prediction of drug-induced cardiotoxicity using human embryonic stem cell-derived cardiomyocytes. *Stem Cell Res* 4: 107–116.
- Campbell TJ, Williams KM (1998). Therapeutic drug monitoring: antiarrhythmic drugs. *Br J Clin Pharmacol* 46: 307–319.
- Cardiac Electrophysiology Web Lab© University of Oxford. 2013–2015 IKr Block experiments: comparison of models, Available at <https://goo.gl/Of6MwR> (accessed 20 June 2016).
- Chain ASY, Dubois VFS, Danhof M, Sturkenboom MCJM, Pasqua O Della (2013). Identifying the translational gap in the evaluation of drug-induced QTc interval prolongation. *Br J Clin Pharmacol* 76: 708–724.
- Chetty M, Rose RH, Abduljalil K, Patel N, Lu G, Cain T, et al. (2014). Applications of linking PBPK and PD models to predict the impact of genotypic variability, formulation differences, differences in target binding capacity and target site drug concentrations on drug responses and variability. *Front Pharmacol* 5: 258.
- Collins T, Bergenholm L, Abdulla T, Yates J, Evans N, Chappell M, et al. (2015). Modeling and simulation approaches for cardiovascular function and their role in safety assessment. *CPT Pharmacometrics Syst Pharmacol* 4: 175–188.
- Coz F Le, Funck-Brentano C, Morell T, Ghadanfar MM, Jaillon P (1995). Pharmacokinetic and pharmacodynamic modeling of the effects of oral and intravenous administrations of dofetilide on ventricular repolarization. *Clin Pharmacol Ther* 57: 533–542.
- Darpo B, Karnad DR, Badilini F, Florian J, Garnett CE, Kothari S, et al. (2014). Are women more susceptible than men to drug-induced QT prolongation? concentration-QTc modelling in a phase 1 study with oral rac-sotalol. *Br J Clin Pharmacol* 77: 522–531.
- Darpo B, Benson C, Dota C, Ferber G, Garnett C, Green CL, et al. (2015). Results from the IQ-CSRC prospective study support replacement of the thorough QT study by QT assessment in the early clinical phase. *Clin Pharmacol Ther* 97: 326–335.
- Davies MR, Wang K, Mirams GR, Caruso A, Noble D, Walz A, et al. (2016). Recent developments in using mechanistic cardiac modelling for drug safety evaluation. *Drug Discov, Today*.
- Dixon R, Job S, Oliver R, Tompson D, Wright JG, Maltby K, et al. (2008). Lamotrigine does not prolong QTc in a thorough QT/QTc study in healthy subjects. *Br J Clin Pharmacol* 66: 396–404.
- Ebert SN, Liu XK, Woosley RL (1998). Female gender as a risk factor for drug-induced cardiac arrhythmias: evaluation of clinical and experimental evidence. *J Womens Health* 7: 547–557.
- Florian JA, Tornøe CW, Brundage R, Parekh A, Garnett CE (2011). Population pharmacokinetic and concentration-QTc models for moxifloxacin: pooled analysis of 20 thorough QT studies. *J Clin Pharmacol* 51: 1152–1162.
- Garazzino S, Scolfaro C, Raffaldi I, Barbui AM, Luccoli L, Tovo P-A (2014). Moxifloxacin for the treatment of

- pulmonary tuberculosis in children: a single center experience. *Pediatr Pulmonol* 49: 372–376.
- Gotta V, Cools F, van Ammel K, Gallacher DJ, Visser SAG, Sannajust F, et al. (2015). Inter-study variability of preclinical in vivo safety studies and translational exposure-QTc relationships - a PKPD meta-analysis. *Br J Pharmacol* 172: 4364–4379.
- Hulhoven R, Rosillon D, Letiexhe M, Meeus M-A, Daooust A, Stockis A (2007). Levocetirizine does not prolong the QT/QTc interval in healthy subjects: results from a thorough QT study. *Eur J Clin Pharmacol* 63: 1011–1017.
- ICH E14 Guideline. 2005. Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs.
- ICH S7B Guideline. 2005. The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals.
- Jonker DM, Kenna LA, Leishman D, Wallis R, Milligan PA, Jonsson EN (2005). 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* 77: 572–582.
- Keizer RJ, Karlsson MO, Hooker A (2013). Modeling and simulation workbench for NONMEM: tutorial on Pirana, PsN, and Xpose. *CPT Pharmacometrics Syst Pharmacol* 2: e50.
- Kimura M, Umemura K, Ikeda Y, Kosuge K, Mizuno A, Nakanomyo H, et al. (1996). Pharmacokinetics and pharmacodynamics of (+/-)-sotalol in healthy male volunteers. *Br J Clin Pharmacol* 42: 583–588.
- Kirsch GE, Trepakova ES, Brimecombe JC, Sidach SS, Erickson HD, Kochan MC, et al. (2004). Variability in the measurement of hERG potassium channel inhibition: effects of temperature and stimulus pattern. *J Pharmacol Toxicol Methods* 50: 93–101.
- Läer S, Elshoff J-P, Meibohm B, Weil J, Mir TS, Zhang W, et al. (2005). Development of a safe and effective pediatric dosing regimen for sotalol based on population pharmacokinetics and pharmacodynamics in children with supraventricular tachycardia. *J Am Coll Cardiol* 46: 1322–1330.
- Langdon G, Gueorguieva I, Aarons L, Karlsson M (2007). Linking preclinical and clinical whole-body physiologically based pharmacokinetic models with prior distributions in NONMEM. *Eur J Clin Pharmacol* 63: 485–498.
- Le Coz F, Funck-Brentano C, Poirier JM, Kibleur Y, Mazoit FX, Jaillon P (1992). Prediction of sotalol-induced maximum steady-state QTc prolongation from single-dose administration in healthy volunteers. *Clin Pharmacol Ther* 52: 417–426.
- Leishman DJ (2014). Predicting QTc Prolongation in Man From Only in vitro Data. *CPT Pharmacometrics Syst Pharmacol* 3: e131.
- Mager DE, Woo S, Jusko WJ (2009). Scaling Pharmacodynamics from in vitro and Preclinical Animal Studies to Humans. *Drug Metab Pharmacokinet* 24: 16–24.
- Malik M, Hnatkova K, Schmidt A, Smetana P (2009). Electrocardiographic QTc changes due to moxifloxacin infusion. *J Clin Pharmacol* 49: 674–683.
- Marostica E, Van Ammel K, Teisman A, Boussery K, Van Bocxlaer J, Ridder De F, et al. (2015). Modelling of drug-induced QT-interval prolongation: estimation approaches and translational opportunities. *J. Pharmacokinet, Pharmacodyn.*
- Milnes JT, Witchel HJ, Leaney JL, Leishman DJ, Hancox JC (2010). Investigating dynamic protocol-dependence of hERG potassium channel inhibition at 37 degrees C: Cisapride versus dofetilide. *J Pharmacol Toxicol Methods* 61: 178–191.
- Miramis GR, Davies MR, Brough SJ, Bridgland-Taylor MH, Cui Y, Gavaghan DJ, et al. (2014). Prediction of thorough QT study results using action potential simulations based on ion channel screens. *J Pharmacol Toxicol Methods* 70: 246–254.
- Moita Santos R. 2014. Differential drug response as a function of age (Thesis). University of Cambridge.
- Obreztkhikova MN, Sosunov EA, Plotnikov A, Anyukhovskiy EP, Gainullin RZ, Danilo P, et al. (2003). Developmental changes in IKr and IKs contribute to age-related expression of dofetilide effects on repolarization and proarrhythmia. *Cardiovasc Res* 59: 339–350.
- O'Hara T, Virág L, Varró A, Rudy Y (2011). Simulation of the undiseased human cardiac ventricular action potential: model formulation and experimental validation. *PLoS Comput Biol* 7: e1002061.
- Polak S (2013). In vitro to human in vivo translation - pharmacokinetics and pharmacodynamics of quinidine. *ALTEX* 30: 309–318.
- Pollard CE, Abi Gerges N, Bridgland-Taylor MH, Easter A, Hammond TG, Valentin JP (2010). An introduction to QT interval prolongation and non-clinical approaches to assessing and reducing risk. *Br J Pharmacol* 159: 12–21.
- Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, et al. (2003). 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* 58: 32–45.
- Rodgers T, Rowland M (2006). Physiologically based pharmacokinetic modelling 2: predicting the tissue distribution of acids, very weak bases, neutrals and zwitterions. *J Pharm Sci* 95: 1238–1257.
- Rodgers T, Leahy D, Rowland M (2005). Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. *J Pharm Sci* 94: 1259–1276.



- Siefert HM, Domdey-Bette A, Henninger K, Hucke F, Kohlsdorfer C, Stass HH (1999). Pharmacokinetics of the 8-methoxyquinolone, moxifloxacin: a comparison in humans and other mammalian species. *J Antimicrob Chemother* 43 (Suppl B): 69–76.
- Smith DA, Rasmussen HS, Stopher DA, Walker DK (1992). Pharmacokinetics and metabolism of dofetilide in mouse, rat, dog and man. *Xenobiotica* 22: 709–719.
- Somberg JC, Preston RA, Ranade V, Molnar J (2010). QT prolongation and serum sotalol concentration are highly correlated following intravenous and oral sotalol. *Cardiology* 116: 219–225.
- Sparve E, Quartino AL, Lüttgen M, Tunblad K, Gårdlund AT, Fåltling J, et al. (2014). Prediction and modeling of effects on the QTc interval for clinical safety margin assessment, based on single-ascending-dose study data with AZD3839. *J Pharmacol Exp Ther* 350: 469–478.
- Szabó G, Szentandrassy N, Bíró T, Tóth BI, Czifra G, Magyar J, et al. (2005). Asymmetrical distribution of ion channels in canine and human left-ventricular wall: epicardium versus midmyocardium. *Pflugers Arch* 450: 307–316.
- Szentadrassy N, Banyasz T, Biro T, Szabo G, Toth BI, Magyar J, et al. (2005). Apico-basal inhomogeneity in distribution of ion channels in canine and human ventricular myocardium. *Cardiovasc Res* 65: 851–860.
- Thomas D, Karle CA, Kiehn J (2004). Modulation of HERG potassium channel function by drug action. *Ann Med* 36 (Suppl 1): 41–46.
- Veroli GY Di, Davies MR, Zhang H, Abi-Gerges N, Boyett MR (2013). High-throughput screening of drug-binding dynamics to HERG improves early drug safety assessment. *Am J Physiol Heart Circ Physiol* 304: H1104–H1117.
- Wang L, Swirp S, Duff H (2000). Age-dependent response of the electrocardiogram to K(+) channel blockers in mice. *Am J Physiol Cell Physiol* 278: C73–C80.
- Witchel HJ (2007). The hERG potassium channel as a therapeutic target. *Expert Opin Ther Targets* 11: 321–336.
- Xiao L, Zhang L, Han W, Wang Z, Nattel S (2006). Sex-based transmural differences in cardiac repolarization and ionic-current properties in canine left ventricles. *Am J Physiol Heart Circ Physiol* 291: H570–H580.
- Yu Z, IJzerman A. P., and Heitman L. H.. (2015). K v 11.1 (hERG)-induced cardiotoxicity: a molecular insight from a binding kinetics study of prototypical K v 11.1 (hERG) inhibitors. *Br J Pharmacol* 172: 940–955.
- Yun YE, Edginton AN (2013). Correlation-based prediction of tissue-to-plasma partition coefficients using readily available input parameters. *Xenobiotica* 43: 839–852.
- Zehelein J, Zhang W, Koenen M, Graf M, Heinemann SH, Katus HA (2001). Molecular cloning and expression of cERG, the ether à go-go-related gene from canine myocardium. *Pflugers Arch* 442: 188–191.

## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Two NONMEM model code files:

**Data S1.** Dog model NONMEM.pdf.

**Data S2.** Clinical model NOMEM.pdf.

**Data S3.** Simulation of electrophysiology-based transducer functions.