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Research Article

Towards Bridging Translational Gap in Cardiotoxicity Prediction: an Application of Progressive Cardiac Risk Assessment Strategy in TdP Risk Assessment of Moxifloxacin

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Abstract. Drug-induced cardiac arrhythmia, especially occurrence of torsade de pointes (TdP), has been a leading cause of attrition and post-approval re-labeling and withdrawal of many drugs. TdP is a multifactorial event, reflecting more than just drug-induced cardiac ion channel inhibition and QT interval prolongation. This presents a translational gap in extrapolating pre-clinical and clinical cardiac safety assessment to estimate TdP risk reliably, especially when the drug of interest is used in combination with other QT-prolonging drugs for treatment of diseases such as tuberculosis. A multi-scale mechanistic modeling framework consisting of physiologically based pharmacokinetics (PBPK) simulations of clinically relevant drug exposures combined with Quantitative Systems Toxicology (QST) models of cardiac electro-physiology could bridge this gap. We illustrate this PBPK-QST approach in cardiac risk assessment as exemplified by moxifloxacin, an anti-tuberculosis drug with abundant clinical cardiac safety data. PBPK simulations of moxifloxacin concentrations (systemic circulation and estimated in heart tissue) were linked with *in vitro* measurements of cardiac ion channel inhibition to predict the magnitude of QT prolongation in healthy individuals. Predictions closely reproduced the clinically observed QT interval prolongation, but no arrhythmia was observed, even at ×10 exposure. However, the same exposure levels in presence of physiological risk factors, e.g., hypokalemia and tachycardia, led to arrhythmic event in simulations, consistent with reported moxifloxacin-related TdP events. Application of a progressive PBPK-QST cardiac risk assessment paradigm starting in early development could guide drug development decisions and later define a clinical "safe space" for post-approval risk management to identify high-risk clinical scenarios.

KEY WORDS: hERG; moxifloxacin; torsade de pointes; quantitative systems toxicology; QT prolongation.

INTRODUCTION

Cardiac toxicity is one of the leading causes of high attrition rate at various stages of drug development, withdrawal of several marketed drugs, and re-labelling of many drugs ([1](#)–[4](#)). Among various types of cardiotoxic effects, arrhythmogenicity is the most common post-approval adverse event ([3](#)). Of particular concern is torsade de pointes (TdP), a life-threatening arrhythmia that can degenerate into ventricular fibrillation.

Due to the potentially significant safety impact and corresponding regulatory concern of drug-induced cardiotoxicity, careful assessment of pro-arrhythmic potential is an integral part of pre-clinical and clinical safety evaluation of an investigational new drug (IND). There are several methods available for cardiac risk assessment, including *in silico*, *in vitro*, and *ex vivo* models as well as *in vivo* studies in animals and humans. Selection of the appropriate model is dependent upon the stage of development and the cardiac safety questions relevant to each development program. The most commonly utilized models during pre-clinical development include *in vitro* ion current inhibition models, where the human ion channels involved in cardiac electrophysiology are

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heterologously expressed in non-human or human cell lines (5). These approaches continue to evolve, with newer approaches such as the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative, assessing the role of stem cell-derived cardiomyocytes in cardiac safety assessment (6–10).

Despite the availability of multiple models and the continued advances in cardiac risk assessment, the number of reports of TdP remains significant (over 100 cases of the TdP reported to the FDA Adverse Event Reporting System (FAERS) every year) and almost half of them are related to non-cardiovascular drugs (4,11). This reflects a translational gap in the quantitative clinical extrapolation from the typical cardiac safety data collected, such as early non-clinical drug screens for cardiac ion channel inhibition, particularly hERG (human ether-a-go-go gene) inhibition, or the propensity for QT interval prolongation as assessed in early phase and thorough QT trials (TQT) clinical trials (12). Consequently, to minimize risk under an optimized drug development paradigm, early stage gates are often employed which only progress compounds predicted or observed to have little to no increase in QT interval, raising concerns that therapeutically promising agents may be triaged inappropriately. These concerns represent findings that the occurrence of TdP is multifactorial, reflecting more than just a drug's ability to inhibit hERG or increase the QT interval. These additional factors, such as gender, co-medications, comorbidities, and genetic polymorphisms, make a drug relatively safe for most people potentially cardiotoxic for a few individuals under specific circumstances (13).

Translational tools with improved predictive performance that account for the multifactorial determinants of cardiotoxicity, particularly TdP, are needed to improve both the cardiac risk assessment and attrition rates of compounds in development. One such set of tools are physiologically based, biophysically detailed models of cardiac physiology that can characterize the contributions of multiple ion channel inhibition on the electrophysiology of human cardiomyocytes. These models can bridge the translational gap in cardiac safety assessment during the early drug development stage by faithfully reproducing the mechanistic exposure–response relationships from the molecular level to the full organism level. Specifically, simulations using this framework can link predicted or observed drug pharmacokinetics (PK) via physiologically based PK (PBPK) modeling with dynamic ion channel inhibition and subsequent effects on electrocardiography (ECG) parameters in virtual patients, which represent an array of physiologies. In addition to ECG metrics of interest (i.e., change in QTc interval) across a range of drug exposures, the derived ECG outputs can be directly utilized in the cardiac risk algorithm recently developed by Polak *et al.* (14). This algorithm, which can operate without well-characterized human PK and pharmacodynamics (PD) aspects, predicts the probability of a compound causing TdP at early stage of development when used as monotherapy in the context of the physiology of a generally healthy average human and improves upon earlier algorithms in that it considers potential supra-therapeutic concentrations as well as the impact of inhibition of multiple ion channels other than hERG in risk stratification.

The current work exemplifies a model-based quantitative systems toxicology (QST) strategy for progressive cardiac risk assessment, where the risk assessment for a novel compound is refined progressively as clinical knowledge is enriched through predict-learn-confirm cycles. The individual model components are iteratively verified/refined, to reduce uncertainty and increase confidence, making them adaptable to more adequately address questions around therapeutic performance, as they arise during clinical development. The Cardiac Risk Algorithm as proposed by Polak *et al.* is one component model that can be particularly informative during early phase development to estimate potential cardiac safety risk even in the absence of detailed information about human PK and PD, as sensitivity analyses may be performed to simulate a range of possible clinical scenarios. As more PK and PD information about the drug becomes available with further drug development, these initial predictions can be verified and refined, and ultimately may be superseded by a more detailed PBPK-QST model platform. Once sufficiently verified, the established and enriched model can be used to simulate clinical scenarios to evaluate effects of intrinsic and extrinsic factors (i.e., special populations, drug–drug interactions, co-morbidities) to establish the clinical cardiac risk scenarios (Fig. 1).

To illustrate this approach, a series of simulations were performed to reflect virtual administration of the anti-infective, moxifloxacin (MOXI). MOXI was chosen as an exemplar compound as it is the most commonly used positive control in TQT trials and thus has well-established human PK and PK-ECG profiles. Furthermore, MOXI is active against *Mycobacterium tuberculosis* (Mtb), the causative pathogen of tuberculosis (TB), and thus is administered as a component of anti-TB drug regimens. Cardiac risk assessment in TB is of specific importance, as many existing and emerging anti-TB agents have a known or suspected propensity for QT prolongation. As the application of a model-based QST strategy for progressive cardiac risk assessment could be advantageous in the prioritization of compounds for inclusion in anti-TB regimens, MOXI serves as an illustrative example of how this approach could be applied during pre-clinical and early clinical development. Here, we report a utilization of the cardiac QST models implemented within Cardiac Safety Simulator (CSS) platform (Certara, Sheffield, UK), previously known as Tox-Comp platform (15) for the quantitative prediction of QT prolongation in humans at therapeutic dose and the risk of causing TdP. We also assessed the impact of potential supra-therapeutic concentration of MOXI in causing TdP *in silico* as well as existing conditions such as hypokalemia which was not part of early-stage risk stratification algorithm.

MATERIALS AND METHODS

Simcyp Simulator V16 (Certara, Sheffield, UK) was used for PBPK simulations. The Advanced Dissolution, Absorption and Metabolism (ADAM) model was used to mechanistically simulate the formulation effects on drug absorption, and 14 organ full body perfusion limited PBPK distribution model was used to model disposition after systemic absorption (16,17). The recently established PBPK model parameters (Supplement 1) were used to simulate the
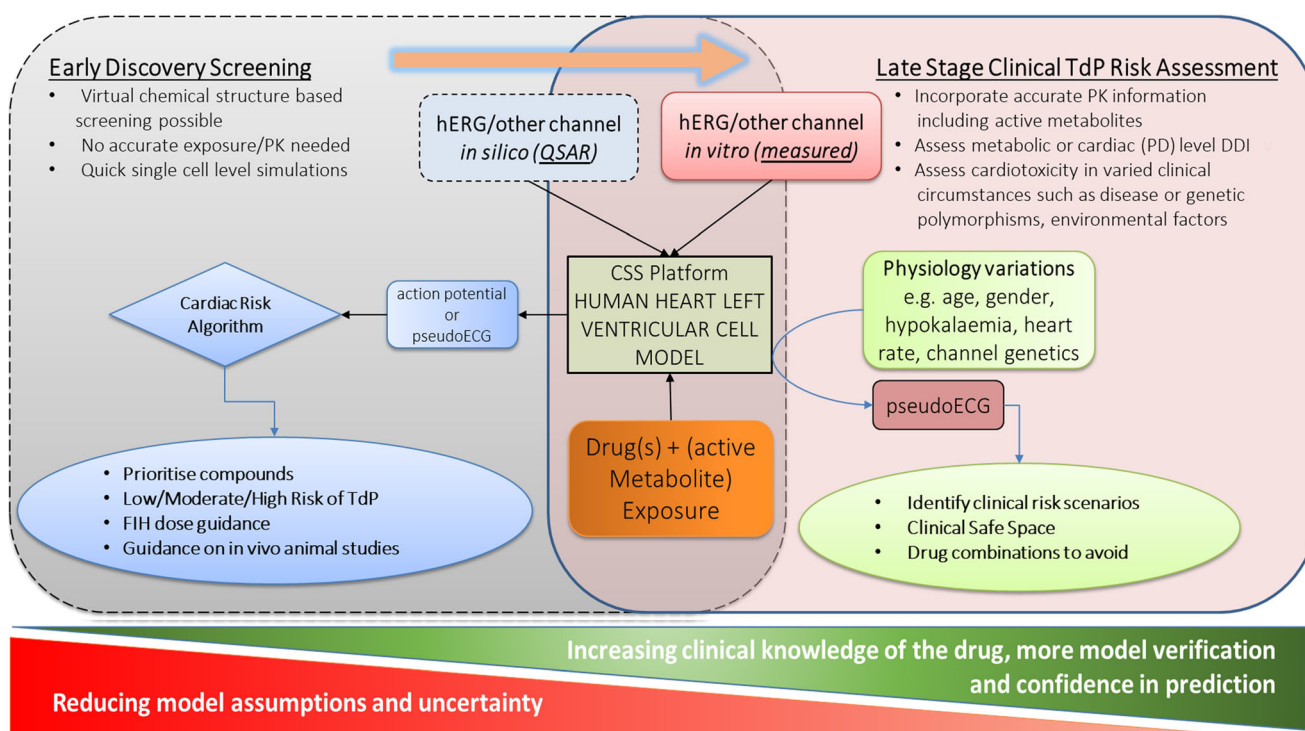
Fig. 1. Progressive cardiac risk assessment strategy in TdP risk assessment

pharmacokinetics of MOXI after oral administration and estimate the population variability in the lung tissue disposition (18), resulting in a mechanistic PK-PD model for translation of the *in vitro* cardiac safety assessment to clinical level. Poulin and Theil method (19) with Berezhkovskiy correction (20) was used to estimate the tissue:plasma partitioning of MOXI. This method underpredicted the volume of distribution in comparison to volume of distribution reported for MOXI after intravenous administration to human volunteers; hence, the model was calibrated with "Kp scalar" using Simcyp parameter estimation module. The PBPK model was used to derive the concentration–time profiles for various bio-phases used to drive the CSS module (described below). The PBPK model was linked with the mechanistic cardiac QST model for MOXI. As the purpose of the study was to show application of the PBPK-QST framework in early clinical development, a phase 1 healthy volunteer study was simulated. A virtual population of 20 healthy Caucasian individuals (50% female) aged 20–50 years old was used to simulate single-dose fasted-state administration of a 400-mg MOXI tablet formulation, similar in size to a positive control arm in a typical TQT trial. The resulting plasma concentration–time profiles were verified by comparison to the observed clinical PK data reported by Stass *et al.* (21).

The CSS version 2.1 was used to perform all cardiac electrophysiology simulations. The CSS platform combines electro-physiologically based models of the human left ventricular cardiomyocytes and a database of human physiological, genotypic, and demographic data thus allowing generation of a virtual realistic population variability in cardiac physiology during simulations (13,22). Specifically, the CSS platform accounts for circadian variability in heart rate as well as plasma electrolyte (Na$^+$, K$^+$ and Ca^{2+}) concentrations using covariate models derived from actual clinical data (23). Epidemiological and covariate models which

take into account the effect of gender and age on ventricular heart wall thickness, cardiomyocyte volume and capacitance, and sarcoplasmic reticulum volume are also built into the CSS platform for the north European Caucasian population with healthy heart physiology (15). The output from the CSS is in the form of a pseudo-electrocardiogram (i.e., a virtual ECG), including measures of interest (e.g., QRS, QT). It is termed pseudoECG as it is based only on a string of ventricular cells and hence is missing atrial activity characterized by P wave on a clinical ECG signal.

For this study, the CSS implementation of the ten Tusscher 2006 (TT2006) ventricular cardiomyocyte cell model (24) was used primarily to form a one-dimensional cardiomyocyte string mimicking the ventricular wall cross-section (thickness). The use of another widely used cardiomyocyte model, O'Hara and Rudy (ORd) model (25), was also assessed on the predicted pseudoECG. The cells were paced at the endocardial side and used a 50:30:20 distribution of endocardium, midcardium, and epicardium cells with an average diffusion coefficient of 0.0016 cm^2/ms. The forward Euler method was used to integrate the model equations with a space step and a time step of 0.01 mm and 0.01 ms, respectively, and a total simulation time of 10,000 ms.

For simulation of the effects of a compound on the pseudoECG outputs, the required model inputs are the *in vitro* measured IC$_{50}$ values for relevant cardiac ion currents, operating drug exposure concentrations, time of day (clock time for circadian model), and age and gender of each simulated subject which can be directly read into the CSS platform from the Simcyp PBPK model output. MOXI's cardiac effects are primarily linked to rapid rectifier potassium current (I$_{Kr}$) inhibition (encoded by hERG gene), as effects on other currents, namely the late calcium (I$_{CaL}$) and sodium currents (I$_{Na}$), show IC$_{50}$ values many-fold higher than the operating physiological tissue concentrations of MOXI after 400 mg single-dose administration and were obtained

from non-human cell systems. Hence, we considered only the inhibition of I_{Kr} by MOXI in our simulations, with corresponding IC_{50} values obtained from the freely available tox-database.net (Table I) (43). Consistent with other experiments in biological systems, there is considerable inter-laboratory variability in reported IC_{50} values for I_{Kr} inhibition by MOXI, which range from 0.93 to 398 μM and are dependent upon *in vitro* cell line and experimental conditions. Hence, the presumably "bio-relevant" IC_{50} value of 29 μM for I_{Kr} inhibition by MOXI as per Alexandrou *et al.* (26) was chosen an input to the model and a corresponding sensitivity analysis conducted (see Discussion section for further details).

Drug concentration-dependent inhibition of ion channel activity was modeled by multiplying the specified ionic current of interest by an inhibition factor as described in Eq. 1:

$$\text{Inhibition Factor} = \frac{1}{1 + (IC_{50}/[D])^n} \qquad (1)$$

where IC_{50} is the drug concentration responsible for the 50% inhibition, n is the shape parameter (Hill coefficient), and $[D]$ indicates the drug concentration in micromolar. The inhibition factor was calculated using MOXI concentrations simulated by the PBPK model for the following bio-phases: (i) unbound

plasma concentration, (ii) total plasma concentration, (iii) unbound heart tissue concentration, and (iv) total heart tissue concentration. Each bio-phase concentration was sampled at 13 time points similar to those used in a typical phase 1 study (i.e., 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 12, 18, and 24 h post-dose) and used as input to the CSS. The start of the treatment was assumed to be 09:00 o'clock time on day 1 and continues till 09:00 on day 2 (24 h). The simulated QT interval was corrected for heart rate effect by Fridericia formula (44) and by placebo (QT interval simulated at same time of the day with zero drug concentration) for each individual and each time point. Each of the result sets (corresponding to the four bio-phase concentrations) was then compared with clinically observed QT prolongation reported after single-dose administration of 400 mg orally.

RESULTS

Performance Verification of PBPK Model

The simulated plasma concentration-time profile obtained after 400 mg MOXI oral dose in fasted state overlaid with clinically observed data is reported in Fig. 2a. The comparative average unbound plasma, total plasma, unbound heart tissue, and total heart tissue concentrations are shown in Fig. 2b. The concentrations in heart tissue are almost fourfold higher than the corresponding plasma

Table I. MOXI IC_{50} values for I_{Kr} current (marked raw indicates values used in simulations)

In vitro cell model	Temp (°C)	K⁺ bath (mM)	Holding potential (mV)	Depolarization potential (mV)	Repolarization potential (mV)	Measurement potential (mV)	Data source	IC_{50} (μM) (n)
HEK	22	4	−80	20	−80	−40	(26)	61 (0.96)
HEK	22	4	−80	20	−80	−80	(26)	65 (0.97)
HEK	22	4	−80	–	–	–	(26)	114 (0.94)
HEK	**35–37**	**4**	**−80**	**20**	**−80**	**−80**	(26)	**29 (1.14)**
AT-1	22–23	4	−40	20	−40	−40	(27)	0.93
CHO	22–24	5.4	−80	20	−40	−40	(28)	102.63 (1.1)
HEK	35–36	4	−80	0	−50	−50	(29)	>100
HEK	34–36	4	−80	20	−80	−80	(30)	35.7 (1)
HEK	–	–	–	–	–	–	(31)	122
CHO	Room	5	−80	20	−40	−40	(32)	129
HEK	Room	4	−80	40	−40	−40	(33)	86.2 (0.94)
HEK	36–38	4	−75	10	−40	−40	(34)	74.7
HEK	Room	4	−80	60	−40	−40	(35)	99
CHO	21	3.2?	−70	40	−30	−30	(36)	80.5
HEK	36.5–37	5	−80	0	−50	−50	(37)	58.5 (0.92)
CHO	–	–	−70	40	−30	−30	(38)	398
CHO	Room	4	−80	20	−50	−50	(39)	135.8 (0.91)
CHO	35–37	4	−80	20	−40	−40	(40)	34
HEK	–	–	–	–	–	–	(41)	102 (0.66)
CHO	24.5–25.5	4	−80	20	−40	−40	(42)	235

The IC_{50} value in bold font were considered most bio-relevant and used during QST simulations, see discussion section for further details
HEK human embryonic kidney cells, *CHO* Chinese hamster ovary cells, *AT-1* atrial tumor myocytes derived from transgenic mice, IC_{50} concentration of drug required to inhibition 50% of ion channel current, n Hill coefficient
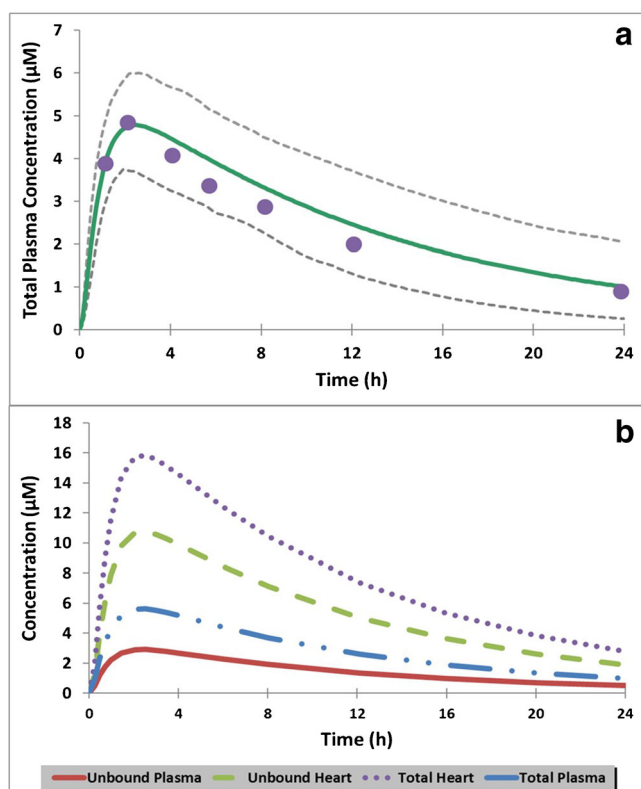
Fig. 2. a Simcyp simulated total plasma concentration time profile (thick green line is arithmetic mean, dotted gray lines are 5th and 95th percentile) from 20 virtual subjects overlaid with mean profile (filled blue circle markers) obtained from clinical study by Stass *et al.* (21) after 400-mg oral dose of MOXI in fasted state; **b** Simulated population mean of unbound plasma, total plasma, unbound heart tissue, and total heart tissue concentrations of MOXI

concentrations, and there is a slight delay in achieving T_{max} in heart tissue (2.6 h) as compared to plasma (2.4 h) due to time taken to perfuse and partition in the heart tissue from systemic circulation.

Performance Verification of the Cardiac QST Model

Considerable variability in clinical studies measuring QT prolongation is reported in literature mainly due to the differences in study protocols, baseline and heart rate correction approaches, demographic differences, and drug formulations. For example, over encapsulation of MOXI (and the investigational product) is sometimes used for blinding purposes in TQT trials, which may contribute to inter-study variability in MOXI pharmacokinetics, specifically mean transit time and time of peak plasma MOXI concentration as shown by Florian *et al.* (45). Due to this and other such variations in clinical trial design, before embarking on QST model verification, we summarized the reported QT prolongation from clinical study reports where placebo, baseline and heart rate-corrected QT prolongation, known as $\Delta\Delta$QTc, was reported. We identified six clinical study reports (46–51) that provide the $\Delta\Delta$QTc profiles post-MOXI 400-mg oral dose where matching PK and $\Delta\Delta$QTc—time profiles were reported; these are depicted in Fig. 3 along with a pooled average. The profiles were digitized from the graphs reported

in study reports using GetData Graph Digitizer software version 2.26.

The simulated $\Delta\Delta$QTcF prolongation derived using simulated MOXI concentrations in the four bio-phases are shown in Fig. 4 and are overlaid with the pooled average $\Delta\Delta$QTc as shown in Fig. 3. Figure 4a shows the results when unbound plasma MOXI concentration was used as the operating concentration to drive the cardiac QST model, with Fig. 4b–d showing the equivalent for total plasma, unbound heart tissue, and total heart tissue MOXI concentrations, respectively. Comparison of the simulated and observed results clearly shows that the unbound heart tissue concentrations (Fig. 4c) most closely predicted the clinically observed $\Delta\Delta$QTc values at all the time points.

TdP Risk Prediction at Supra-Therapeutic Exposure and with Other Physiological Risk Factors

MOXI is considered a potentially torsadogenic drug (52) which is attributed mainly due to its ability to inhibit the hERG channel current. However, the actual reported cases of TdP after MOXI treatment are very rare, which is consistent with the lack of pro-arrhythmic pseudoECG waveforms in any of the virtual patients simulated in Fig. 4 as well as the mid-range probability (54%) of being TdP positive as estimated by the Cardiac Risk Algorithm of Polak *et al.* (14). Most of the reported TdP cases occurred when MOXI was administered with other cardiotoxic medications potentiating their TdP effect or physiological conditions and diseases such as hypokalemia, tachycardia, renal failure (main route of elimination for MOXI), or congenital long QT syndrome. Thus, MOXI prolongs the QTc interval at therapeutic dose levels but actual TdP events are contingent upon the presence of other clinical risk factors that may not be present in the healthy volunteer phase 1 trial setting as simulated.

Hence, to simulate a higher-risk clinical situation, the following scenarios were compared with placebo administration in a virtual 20-year-old female subject:

- Scenario 1: MOXI concentrations equivalent to the mean unbound heart tissue MOXI C_{max} with 400-mg single-dose administration in a healthy individual;
– Heart rate 60 beats per min and plasma potassium concentration of 4.2 mM
- Scenario 2: Supra-therapeutic MOXI concentrations 10-fold higher than the mean unbound heart tissue MOXI C_{max} with 400-mg single-dose administration in a healthy individual;
- Scenario 3: Therapeutic dosing as in scenario 1, but simulated in a virtual patient exhibiting pro-arrhythmic physiological factors;
– Tachycardia (heart rate 120 beats per min) and hypokalemia (plasma K^+ concentration of 1.5 mM)
- Scenario 4: Supra-therapeutic dosing as in scenario 2 in a virtual patient exhibiting the same pro-arrhythmic physiological factors as in scenario 3.

The results of the simulated pseudoECG from the CSS platform are shown in Fig. 5 for all scenarios.

Figure 5a shows that under normal physiological conditions (i.e., scenarios 1 and 2), MOXI prolongs QTc interval without causing any abnormal rhythms even at supra-
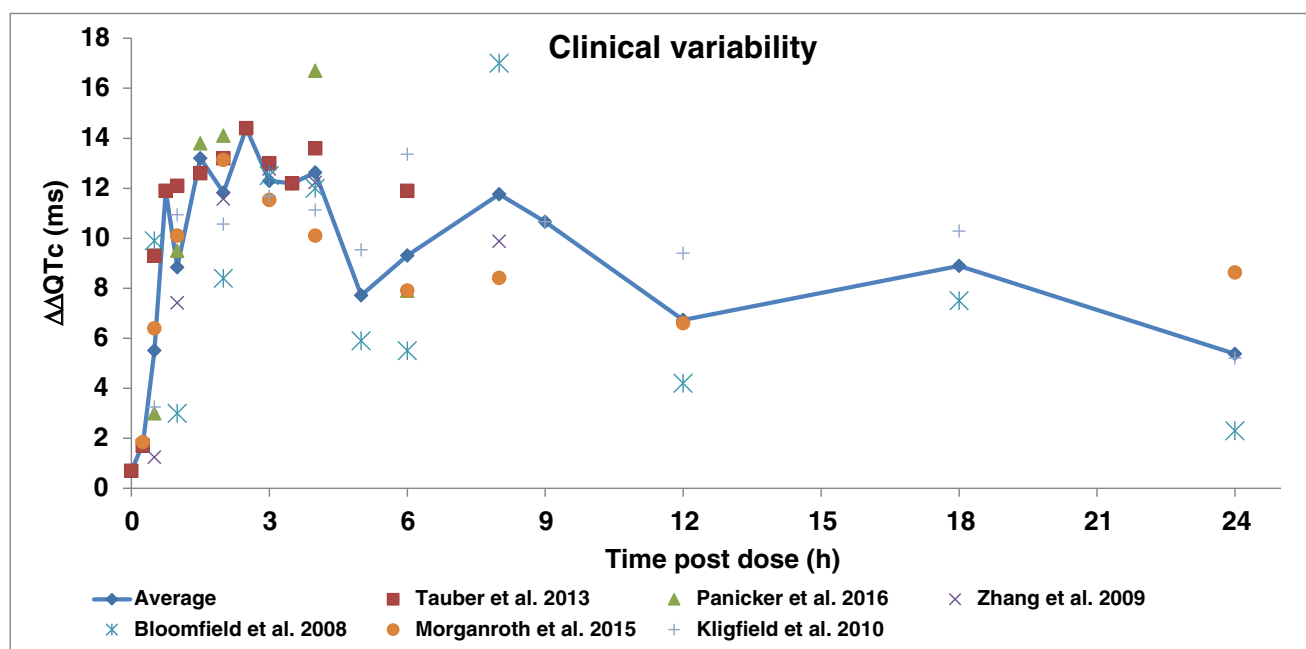
Fig. 3. Variability in clinical $\Delta\Delta$QTc after 400-mg MOXI oral dose

therapeutic MOXI concentrations. On the other hand, Fig. 5b indicates that in the presence of relevant physiological changes (i.e., hypokalemia and tachycardia), the same supra-therapeutic exposure could cause polymorphic arrhythmia. To put in clinical context, the supra-therapeutic MOXI concentration of 120 μM used in scenarios 2 and 4 is 10-fold higher than the mean unbound heart tissue MOXI C_{max} after 400-mg single oral dose administration. These exposures could be seen in a portion of the patient population under various clinical scenarios, such as drug–drug interactions (*viz.* inhibition of MOXI clearance), overdose or with intravenous bolus administration of high dose of MOXI (> 800 mg). Moreover, patients with renal impairment could show higher exposure at therapeutic doses as MOXI is predominantly cleared by renal filtration, which may underlie the reported TdP cases of MOXI in renally impaired patients (53). The results show that MOXI, an otherwise low TdP-risk drug at therapeutic dose in a healthy subject with normal physiology, could cause TdP when combined with elevated patient physiological risk factors.

DISCUSSION

Predictive Performance of PBPK-QST Model at Therapeutic and Supra-Therapeutic Exposure

PBPK modeling is widely utilized to estimate drug concentrations in various bio-phases for linking with PD models to establish exposure–effect relationships, without the requirement for a direct measurement of drug concentrations at site of action in clinic (54,55). These models can be semi- or fully mechanistic and can include multiple physiologically relevant pathways. For example, the contributions of transporters in lung uptake of MOXI are incorporated based on *in vitro* data, which is important in reproducing the observed clinical lung

disposition in tuberculosis patients (18). In the cardiac QST framework, heart tissue concentrations have been established as the relevant bio-phase for governing the QT effect rather than concentrations in plasma per *in vivo* animal work by Minematsu *et al.* (56). However, given that measurement of heart tissue drug concentrations is not feasible in the clinic due to ethical and practical considerations, suitable concentrations to estimate potential cardiac effects in patients are generally not well-informed. However, a sufficiently detailed PBPK model that exhibits good predictive performance can potentially help bridge the gap to estimate the concentrations in heart tissue. The robustness of this approach was not fully assessed in the present work, as no clinical or animal data on heart tissue MOXI concentrations was available to verify the PBPK-predicted distribution (i.e., fourfold higher exposure in heart as compared to plasma). Hence, the model predicted heart tissue MOXI concentration and unbound fraction were used "as is" without further refinement as input to the CSS model and drove the pseudoECG output. This approach demonstrated good predictive performance as shown in Fig. 4, where predictions based on the unbound heart tissue MOXI concentration (the most theoretically relevant bio-phase) closely matched the QTc prolongation profile observed in the clinic. Moreover, the CSS model was also able to recover the gender effect in QT prolongation effect, with predicted mean peak $\Delta\Delta$QTc value ~ 4.5 ms higher in females than in male subjects and similar to the ~ 4 ms difference reported by Taubel *et al.* (50). The ability to recapitulate this sex-related difference illustrates an advantage of the model-based QST framework for cardiac risk assessment, as the potential effects of covariates (e.g., food effects, concomitant medications, comorbidities) that affect drug PK and/or PD may be investigated beginning at early development stages.
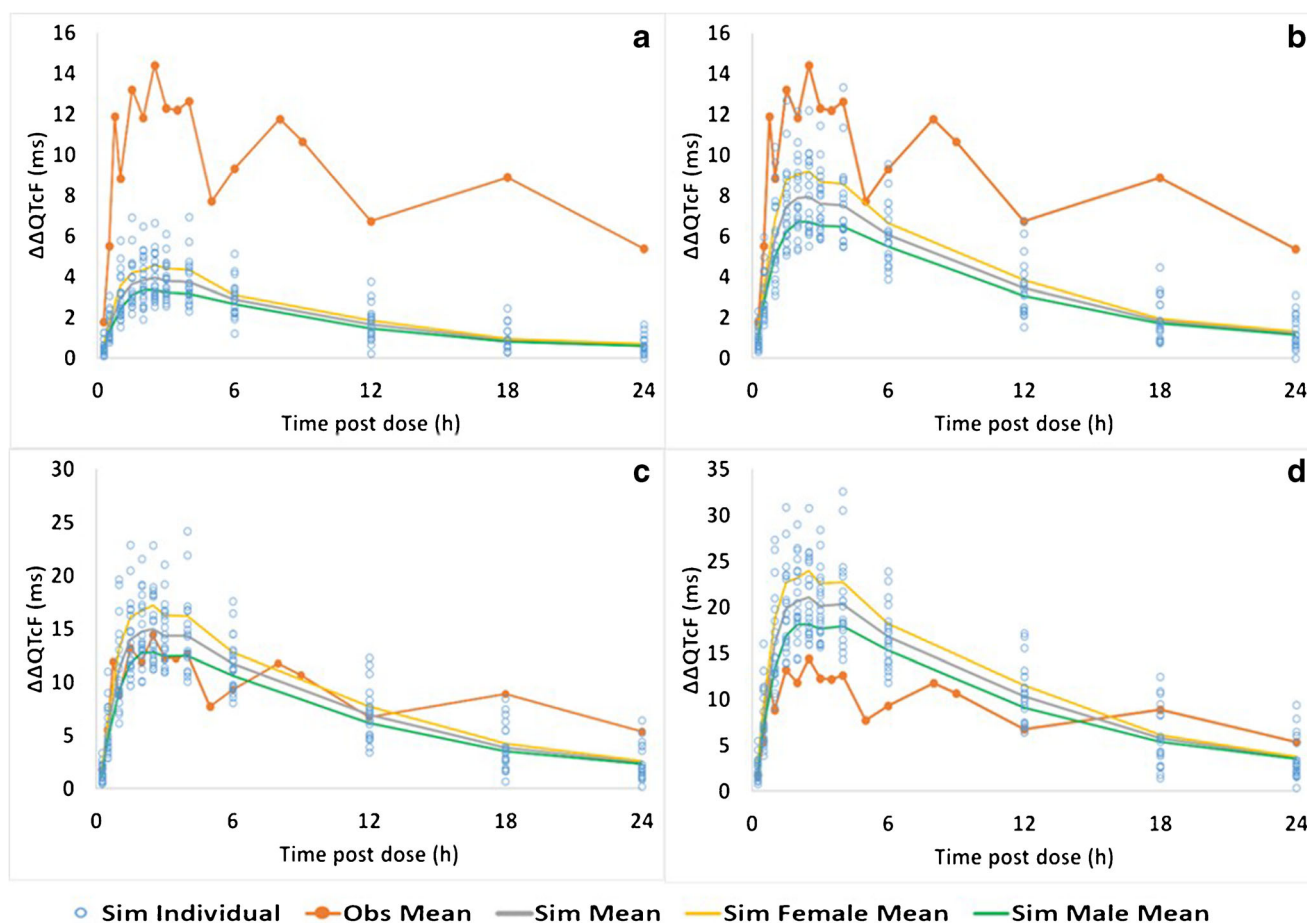
Fig. 4. TT 2006 model simulated ΔΔQTc-time (post-dose) profile using (**a**) unbound plasma, (**b**) total plasma, (**c**) unbound heart tissue, and (**d**) total heart tissue concentrations of MOXI as operational concentration to drive the QT response. The population variability of simulated 20 subjects (open circles), average of the population (thick gray line), average of 10 female population group (yellow line), and average of 10 male population group (green line) are also shown for each of simulation set

While the above results indicate that the estimated unbound heart tissue concentration predicted the QT prolongation reasonably well, it is noted that the PBPK model for MOXI is enriched based on non-clinical and clinical PK data to account for discrepancies between "bottom-up" mechanistic predictions of tissue uptake and clinically observed volume of distribution (*viz.* application of a Kp scalar). However, simulations such as those presented herein are often conducted during early clinical development, where data is generally more limited, and thus, there is greater uncertainty in the degree of tissue uptake. This uncertainty was reproduced herein as multiple potentially relevant bio-phase concentrations were considered to generate plausible CSS outputs reflecting an approximately fivefold range of mean peak ΔΔQTc values (i.e., ranging from approximately + 4 to + 20 ms for unbound plasma and total heart MOXI concentrations, respectively). Such an approach is useful in early development, where a set of predictions reflecting a range of potential clinical scenarios may be highly informative in cardiac risk assessment, clinical trial design, and overall clinical development strategy. Even though this work attempted to develop the most mechanistically plausible model based on currently available knowledge in the area,

it is worth noting certain uncertainties and assumptions associated with the model and input data. For example, the model did not explicitly account for formulation effects such as over encapsulation, because such information is rarely made publicly available and not well-defined in the clinical studies used for model verification. Secondly, we did not have fully detailed heart tissue model to mimic the exposure inside the cardiomyocyte cell, which has been suggested to be more suitable of exposure for some compounds (57–59). Such detailed heart tissue models exist (60,61), but they could be even more challenging to verify with limited measurements in heart tissue or cardiomyocytes. Moreover, the *in vitro* IC$_{50}$ data also reflects the concentration in the media not inside the cell; thus, combining such IC$_{50}$ without suitable correction with intra-cellular exposure model could produce misleading results. The translatability of *in vitro* experiments, typically conducted in HEK cells with heterologously expressed cardiac ion channels, to the *in vivo* situations or actual cardiomyocytes in terms of physiological functions is not fully established. Nonetheless, these are the challenges facing the general cardiotoxicity assessment area, rather than this work specifically, and more experimental data is required to complement such modeling
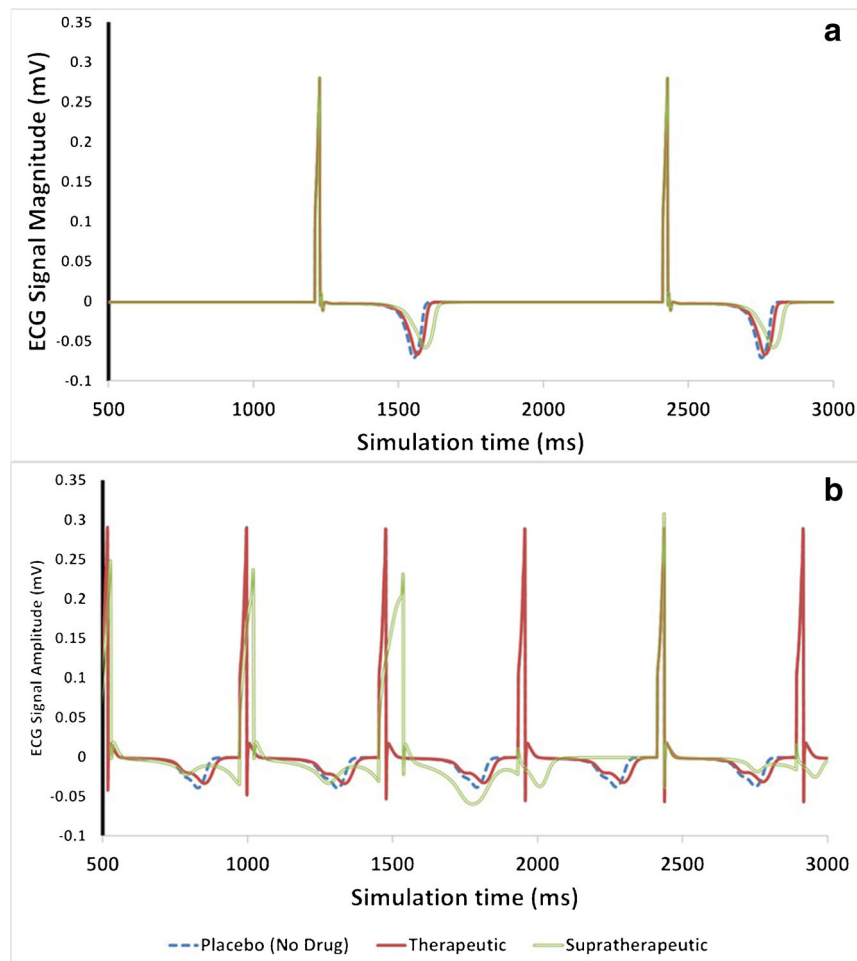
Fig. 5. Simulated pseudoECG traces at therapeutic (red thick line), supra-therapeutic (double green continuous line) exposure of MOXI and placebo (no drug) (dotted blue line) under normal physiological conditions (**a**), and tachycardia with hypokalaemia (**b**)

results to test or establish hypotheses and move the field of cardiac risk assessment forward.

Although the estimated unbound heart tissue MOXI concentrations resulted in the best match to the observed clinical data when used as the input for the CSS model, it is noted that PK-PD relationships for cardiac parameters (e.g., QT prolongation) are predominantly reported as correlations with plasma drug concentrations. This correlation is shown in Fig. 6, which depicts the $\Delta\Delta$QTc values predicted from the CSS using unbound heart tissue MOXI concentration as input versus concentrations at the corresponding time points (unbound heart tissue and total plasma MOXI concentrations in open and closed circles, respectively).

Figure 6 shows robust correlations with the predicted $\Delta\Delta$QTcF, with slopes of 6.86 and 3.51 ms/μg $\times$ mL^{-1} of MOXI in total plasma and unbound heart tissue bio-phases, respectively, with the steeper slope for the plasma correlation reflecting the lower MOXI concentrations in the total plasma *vs.* the unbound heart tissue bio-phases. Notably, when compared with published slopes from clinical studies, the "bottom-up" predicted slope of 6.86 ms/μg $\times$ mL^{-1} for the plasma correlation was similar to slightly higher in magnitude. For example, the value reported by Panicker *et al.* (49) from a

bootstrapping analysis was 5.9 ms/μg $\times$ mL^{-1}, whereas an earlier population PK-PD study by Florian *et al.* reported
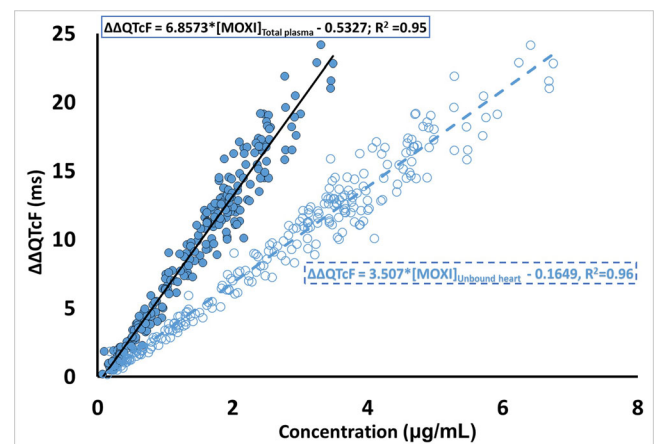

Fig. 6. Relationship between simulated MOXI concentrations and $\Delta\Delta$QTcF (filled blue circles represent the total plasma concentration *vs.* $\Delta\Delta$QTcF obtained with unbound heart tissue concentration as operating concentration and open blue circles represent the unbound heart tissue concentration *vs.* $\Delta\Delta$QTcF obtained with unbound heart tissue concentration as operating concentration)

slopes from individual studies ranging from 1.6 to 4.8 ms/μg × mL^{-1} and a mean estimate of 3.1 ms/μg × mL^{-1} (45). In the latter case, it is noted that this may be influenced by clinical trial size, as clinical studies with larger populations are known to have slopes higher than 3.1 ms/μg × mL^{-1} (62). Regardless, this suggests that the bottom-up approach outlined in the present work provides reasonable, if possibly somewhat conservative, predictions of QTc prolongation for MOXI.

Although the ΔΔQTcF is a useful comparator for evaluating performance of the PBPK-based CSS simulations, it is important to note that the reliance on QTc as a metric of torsadogenic potential has significant drawbacks (13,63). This is exemplified by MOXI, as reports by Abbasi *et al.* (64), Okada *et al.* (39), and simulations done previously by our group (14) have shown with single cell level as well as three-dimensional heart wall simulations that MOXI does not cause early after depolarization (EAD) or arrhythmia at up to 100-fold supra-therapeutic concentration with healthy cardiac electrophysiology. While this is in contradiction to the reported TdP cases with MOXI in clinic at lower than 100-fold exposure levels (65,66), these cases, as well as 274 records[1] of MOXI-associated TdP (obtained by mining of the US FAERS database using the OpenVigil 2.1 portal (67) for TdP cases reported with MOXI) indicates that almost all cases were multifactorial. Specifically, the MOXI-associated cases involved other contributing factors, such as additional QT prolonging co-medications, disease conditions such as renal failure, or physiology modifications that could influence cardiac electrophysiology such as electrolyte imbalance or abnormal heart rate. It should be emphasized that these findings are highly consistent with pseudoECG simulations presented herein, which also show that MOXI itself is less likely to cause TdP even at 10-fold higher than therapeutic exposure in a healthy subject but could cause arrhythmia when administered to patients with other physiological risk factors or co-medications with torsadogenic potential (refer to Fig. 5). Hence, this demonstrates that the application of the cardiac QST modeling framework shown in this study could potentially help bridge the translational gap by starting with assumption-rich exploratory simulations in early clinical development with iterative refinement through learn-confirm-predict cycles through later stages of drug development. Such an approach is envisioned to guide both appropriate labeling and rational pharmacotherapy of new drugs to avoid or reduce post-marketing TdP risk.

Differences in Reported IK$_r$ IC$_{50}$ Values and Its Impact on QST Model Prediction

Lab to lab variability in cell-based *in vitro* assays, such as those used to provide input values for use in the present analysis, is well-documented (43,68). This can be seen in the reported IK$_r$ IC$_{50}$ values of MOXI from different laboratories and different experimental protocols provided in Table I as well as in the results of an extensive literature search carried out for IC$_{50}$ values of many drugs and drug-like compounds for various cardiac ion channels (available on tox-

database.net) (43). However, despite the known variability in lab-based parameters which can potentially strongly influence QST modeling outputs, the impact of this variability is not commonly assessed and reported. To minimize the effects of such variability within the current study, we applied a step-wise strategy to identify the most physiologically relevant experimental input value of IC$_{50}$ for QST modeling. Results were first triaged based on cell line, where HEK cells were preferred over CHO cell or other non-human cell lines due to their human origin. Of those based on HEK cells, Alexandrou *et al.* (26) and Chen *et al.* (30) used the same experimental protocols, mimicking human physiology with minor difference in operating temperatures. While Lacroix and colleagues (34) have also run their study in the physiological temperature, there were other settings that differed from human physiology (higher value of the holding potential and short pulses) which resulted in their removal from consideration.

Of the remaining two studies, as the experimental conditions used by Alexandrou *et al.* (26) were the most physiologically relevant, their reported IC$_{50}$ value of 29 μM was used for all the simulation results presented in this publication. However, it is noted that the experimental conditions were very similar differing only in experimental temperature (35–37 *vs.* 34–36°C; Alexandrou *et al.* (26) and Chen *et al.* (30), respectively). While these differences appear minor, Table I shows that even small temperature changes may have resulted in an approximately 20% difference in IC$_{50}$ values, albeit inter-lab, inter-operator, or even inter-run variability may also contribute to the differences. These sources of variability, which seem to be typical for the Patch Clamp technique, may influence decisions taken on the cardiac safety assessment (68). To evaluate the potential impact of the input IC$_{50}$ value on the model predictions, a sensitivity analysis conducted to study the impact of varying the IC$_{50}$ from 1 to 300 μM on ΔΔQTcF was conducted (Fig. 7).

An IC$_{50}$ of around 30 μM, similar to the 29- and 35-μM values from Alexandrou *et al.* (26) and Chen *et al.* (30), gave the predicted ΔΔQTcF closest to clinically observed. In contrast, higher values as estimated under less physiologically-relevant conditions would have underpredicted the magnitude of QTc
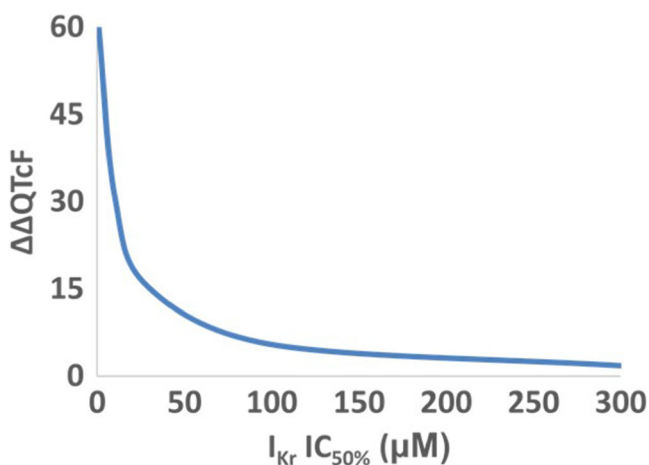
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[1]   Many records are apparent duplicates attributed to reporting by different organizations/individuals; refer to csv file available as the Supplementary Material 2

Fig. 7. Sensitivity analysis of predicted ΔΔQTcF with respect to input I$_{Kr}$ IC$_{50}$ value for MOXI

prolongation. Thus, it is very important to supply the *in vitro* input parameters obtained from the experiments conducted at operating conditions mimicking *in vivo* situations as quality of QST prediction depends heavily on the quality of input information supplied to mechanistic model. It is noted that to aid in translation of IC_{50} values obtained from different cell lines and different temperatures, the CSS contains validated mathematical inter-system scaling factors (ISSF) (69). However, these factors are only intended to help correct values to better match human physiology, and as such, the ISSF should be used cautiously as they are not intended to replace physiologically relevant *in vitro* experimentation.

Impact of QST Model Selection on the Results

The TT2006 model was primarily used to run the pseudoECG simulations in the current work, given the existence of numerous peer-reviewed publications demonstrating their utility in adequately predicting the clinically observed QT prolongation. This model is also relatively fast to run, especially at the pseudoECG level. However, the ORd model and its variants (70,71) have recently been proposed as a method to run single cell simulations to propagate the impact of *in vitro* IC_{50} value of ion current inhibition to action potential duration (APD). Although the ORd model to run the pseudoECG simulations offers some additional insights, its overall impact to this work remains limited or negligible, when compared to the use of the TT2006 model. Moreover, the simulations with the ORd model take significantly longer time and show some numerical instability at pseudoECG level in some individuals, when running simulations of unbound plasma and unbound heart tissue concentration exposure. Thorough evaluations of available cardiomyocyte models in terms of the numerical accuracy and their ability to recover observed APD and/or ECG signals are needed to establish the utility of the models to simulate clinical endpoints and/or regulatory use such as CiPA initiative. The impact of model selection on our results is shown in Fig. 8.

The ORd model resulted in larger QT prolongation estimates as compared to the TT2006 model, at the same exposure levels (Figs. 4 and 8). The ORd model showed over- and under-estimation of clinically observed QT prolongation profile of MOXI with unbound heart and unbound plasma concentrations, respectively. As such, it is likely that the observed QT prolongation profile is more adequately matched to the total plasma exposure with the ORd model, while the TT2006 model showed best predictions with unbound heart tissue concentrations. As stated above, the aim of the current study was to test physiologically plausible scenarios; therefore, this work does not focus on total plasma concentrations, which would deviate from the hypothesis that only the free drug can be pharmacologically active. Further experimental and *in silico* work is needed to determine and establish the utility and/or accuracy of various QST models and input data choices to continuously grow the current knowledge base.

CONCLUSIONS

The assessment of pro-arrhythmic potential and risk of TdP of novel molecules is often challenging given the complex interplay of multiple factors involved requiring a comprehensive understanding of not only the drug-dependent parameters but also the systems characteristics such as human anatomy, physiology, and pathophysiology, as well as external factors, e.g., dosing scheme, concomitantly taken drugs, and co-morbidities. While the method demonstrated herein appears to predict the cardiotoxicity for a well-characterized drug such as MOXI reasonably well, it is envisioned that PBPK-QST modeling be applied throughout development where the component models are refined iteratively through further study per the "learn-confirm-predict-apply" paradigm with enrichment of clinical knowledge of the compound in order to guide decision-making during drug development. Application of a progressive PBPK-QST cardiac risk assessment paradigm starting in early development could guide drug development decisions and later define a clinical "safe
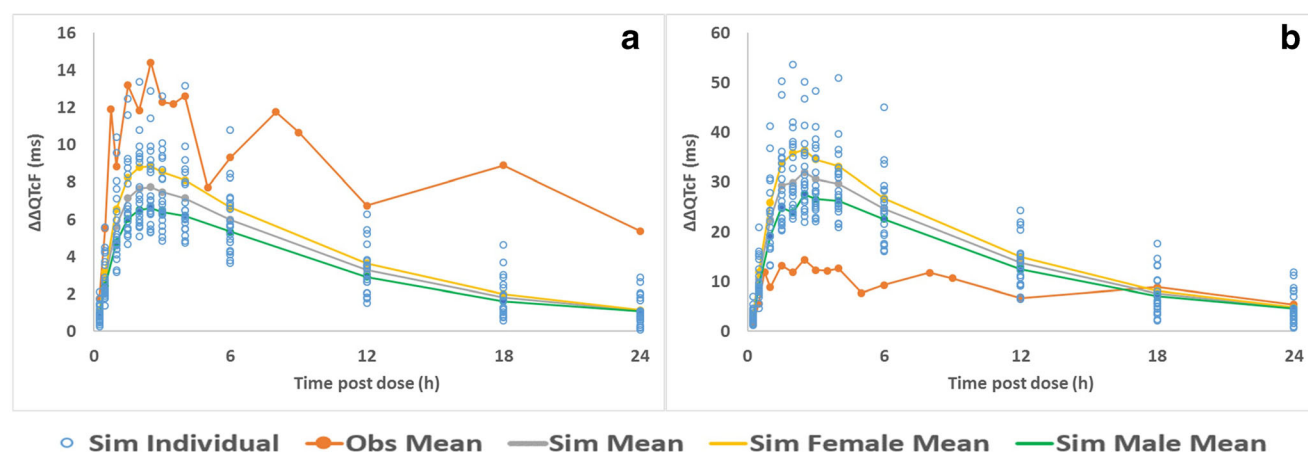

Fig. 8. ORd model simulated $\Delta\Delta$QTc-time (post-dose) profile using (**a**) unbound plasma and (**b**) unbound heart tissue concentrations of MOXI as operational concentration to drive the QT response. The population variability of simulated 20 subjects (open circles), average of the population (thick gray line), average of 10 female population group (yellow line), and average of 10 male population group (green line) are also shown for each of the simulation set

space" for post-approval risk management to identify high-risk clinical scenarios. Additional collaborative initiatives are needed to further understand the impact of input data sources and model selections toward establishing good practices to progress the field forward. Further work demonstrating the performance of the QST framework for other drugs and different formulations, routes of administration, co-medications, and diets as well as in the recapitulation of real TdP cases would help to validate this approach and build confidence in proposed progressive risk assessment strategy.

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AUTHOR CONTRIBUTION

Participated in research design: NP and SP
Conducted Experiments: NP and SP
Performed data analysis: NP
Contributed to the writing of the manuscript: NP, SP, AB, KR, BW, OH, DeH, andDaH.

COMPLIANCE WITH ETHICAL STANDARDS

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