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CASE STUDY

GLOBAL GERMAN PHARMACEUTICAL COMPANY EMPLOYS
ELEM VIRTUAL HUMAN TRIALS AS
PRE-CLINICAL CARDIAC SAFETY ASSESSMENT

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GLOBAL GERMAN PHARMACEUTICAL COMPANY EMPLOYS ELEM VIRTUAL HUMAN TRIALS AS A PRE-CLINICAL CARDIAC SAFETY ASSESSMENT

A leading global pharmaceutical laboratory is committed to driving value through innovative solutions. To this end, the enterprise partnered with ELEM Biotech, the company at the forefront of computational modeling for **drug-induced cardiac risk prediction**. The objective of this partnership was to **explore potential predictive tools for improving early identification of cardiac risks and address ethical and cost-related concerns** associated with traditional testing methods.

Traditional methods of assessing drug-induced QT prolongation, involving extensive clinical trials and animal testing, are costly, time-consuming, and ethically require ethical approval. As **cardiac proarrhythmic risk has become a significant global concern, the need for more efficient, predictive methods in drug development has increased**. To address these issues, the International Council on Harmonization (ICH) established guidelines focused on evaluating a drug's potential to cause hERG channel block and QT prolongation, crucial for preventing Torsades des Pointes and sudden cardiac death.

While preclinical assays are sensitive, they are not sufficient due to complex translation to human cardiac physiology.

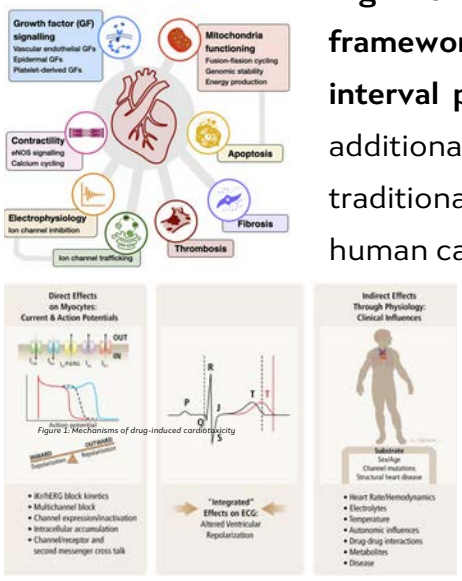

Figure 1: Mechanisms of drug-induced cardiotoxicity

Cardiac side effects remain a leading cause for terminating drug discovery programs, with up to 60% of candidates showing a potential proarrhythmic risk. The complexity of preclinical-clinical translation highlights **the need for human-based studies to assess risk accurately**. The Comprehensive In vitro Proarrhythmia Assay (CiPA) initiative aims to develop a more comprehensive assessment of proarrhythmic potential, not solely relying on hERG block and QT prolongation through in vitro and in vivo animal assays.

The new ICH E14/S7B Q&A guidance sets the stage for a paradigm shift in risk assessment, integrating mechanistic in vitro assays and silico simulations. This shift welcomes the potential for non-clinical studies to influence clinical risk assessment practices, aiming for greater specificity and reducing false positives in preclinical evaluations. Elem's work contributes to this evolution by **employing a High-Performance Computing (HPC) framework to assess drug concentration-QT interval prolongation** relationships, providing additional preclinical safety evidence beyond traditional QT studies, all within a virtual human cardiac population framework.

Furthermore, ELEM's V.HEART cloud-based platform **allows clients to easily set up virtual populations for personalized computational trials.**

PREDICTING QT INTERVAL CHANGES IN DRUG CANDIDATES WITH ELEM V.HEART- SAFETY

The recent paradigm shift in proarrhythmic risk assessment advocates integrating clinical, non-clinical, and computational evidence to comprehensively understand drug candidates' proarrhythmic potential. This study aims to predict concentration-response relationships specifically for QT as a clinical endpoint. **The objective of the study is to:**

Assess the use of full heart computational models replicating human cardiac populations to predict concentration-response relationships for QT interval changes, aligning with clinical trial recommendations.

Compare computationally derived concentration-response relationships of QT interval changes against clinical trial data for well-characterized compounds, including moxifloxacin, dofetilide, verapamil, and ondansetron.

Assess the accuracy and reliability of computational models in predicting critical concentrations and QT interval prolongation, aiming to bridge the gap between preclinical and clinical safety assessments.

Explore the potential of computational models to provide complementary information to preclinical and clinical safety packages, enhance trial design, and improve the understanding of preclinical-to-clinical translation in proarrhythmic risk assessment.

PREDICTING QT INTERVAL CHANGES IN DRUG CANDIDATES WITH ELEM V.HEART- SAFETY

RESULTS

Using clinical and computational data, the **study evaluated four compounds' concentration-QT (C-QT) relationships.** Linear regressions were performed, and confidence intervals were calculated to assess the accuracy of computational predictions compared to clinical trial data.

Dofetilide: The computational model aligned well with clinical trial data regarding slope and intercept, with critical concentration ratios close to clinical values.

Moxifloxacin: While the slope matched clinical data, the intercept exceeded the confidence interval, resulting in lower critical concentration ratios in computational predictions.

Ondansetron: Compared to clinical data, both slope and intercept were outside the confidence interval, leading to lower critical concentration ratios in computational predictions.

Verapamil: The computational model aligned well with clinical data for slope but varied slightly in intercept, resulting in conservative critical concentration ratios.

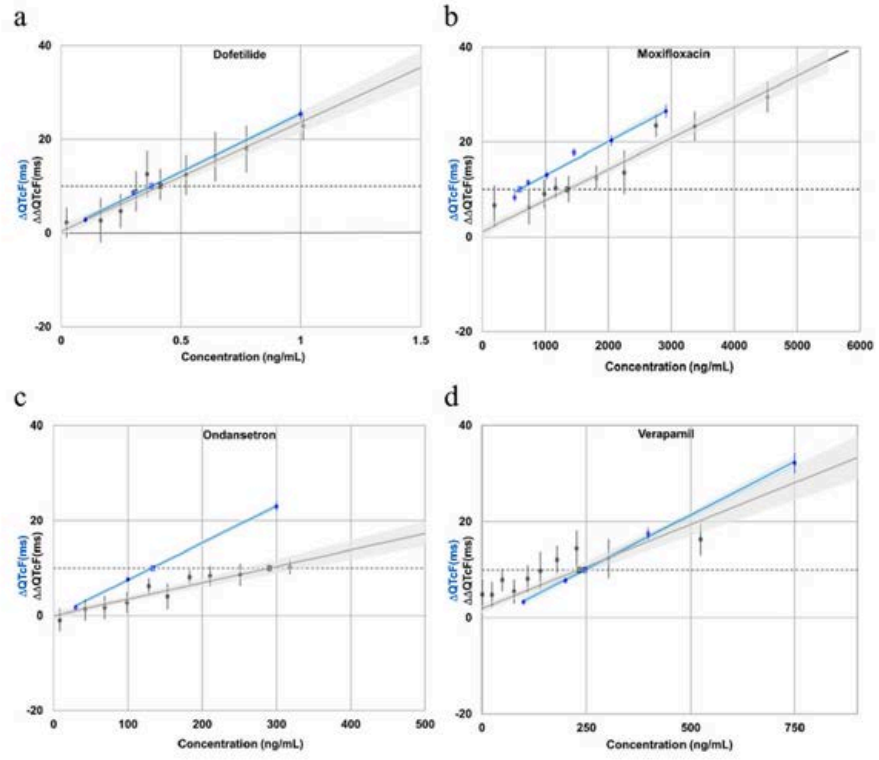

Figure 2: ΔΔQT clinical (black) and ΔQT computational (blue) for each drug assessed: a. dofetilide; b. moxifloxacin; c. ondansetron; d. verapamil. Each regression includes markers to indicate their predicted critical concentration (at the 10 ms threshold).

Computational predictions were conservative, with critical concentrations generally lower than clinical values. **Sex-related differences in QT interval prolongations were observed, albeit small, emphasizing the need for inclusion in cardiac safety trials** to enhance evidence on this aspect.

CONCLUSIONS

This study demonstrated the **viability of in silico cardiac safety trials in predicting QT-interval prolongation, with computational models showing consistent results with clinically observed values.** The effectiveness of ELEM's V.HEART-SAFETY platform was validated, highlighting its potential to revolutionize cardiac safety assessment in drug development.

Using V.HEART-SAFETY, the leading German pharmaceutical company can **efficiently and safely test new compounds in virtual exposure-QTc studies with diverse virtual human populations, including female and male hearts**, as soon as in vitro data is obtained. This approach facilitates a straightforward translation to clinical outcomes with human-based computational biomarkers, unlike animal experiments that require complex translational analysis.

The conservative nature of critical concentration predictions, with a maximum deviation of two-fold from clinical values and no instances of overestimation, highlights the precision of the methodology. The study suggests using virtual clinical studies during the preclinical phase and monitoring their predictive value retrospectively with clinical data, adding value to translational efforts.

Computational trials offer opportunities to enhance safety assessments, trial design, animal-human translation, and understanding of variability in drug response. They may also address gaps in clinical data, including the effects of sex-specific and pathological conditions, aiding in patient stratification early in drug development.

While not intended to replace existing methodologies, **the proposed framework is a complementary method for predicting drug-induced QT-interval prolongation, emphasizing clinical translational purposes.** Further research is warranted to explore the scope and limitations of more specific computational models, ultimately contributing to more informative clinical study designs and regulatory acceptance of in silico methodologies.

FUTURE WORK

Validating the model rigorously against large clinical datasets and benchmarking it against established methodologies is crucial to ensure its reliability and acceptance in drug safety assessment practices. This work paves the way towards providing credibility on computational approaches.

In addition, we are actively working on addressing the clinical bias paradigm by systematically analyzing and mitigating biases inherent in clinical trials. **By incorporating demographic variations, anatomical differences, age groups, comorbidities and medication histories, we aim to minimize bias and ensure the model's generalizability across different patient populations**. This approach not only enhances the model's accuracy but also fosters inclusivity and equity in drug development.

RECOMMENDATIONS

Implementation Steps:

- Integrate the computational model **into the early phases of drug development.**
- **Train research and development teams** on utilizing in silico methodologies.
- Establish a **feedback loop with clinical trials** to refine and validate the computational models continuously.
- **Engage with regulatory bodies** to promote the acceptance of in silico methods in drug safety assessments.

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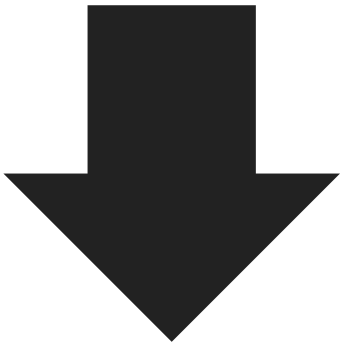

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Virtual clinical QT exposure-response studies – A translational computational approach

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A B S T R A C T

Background and purpose: A recent paradigm shift in proarrhythmic risk assessment suggests that the integration of clinical, non-clinical, and computational evidence can be used to reach a comprehensive understanding of the proarrhythmic potential of drug candidates. While current computational methodologies focus on predicting the incidence of proarrhythmic events after drug administration, the objective of this study is to predict concentration-response relationships of QTc as a clinical endpoint. *Experimental approach:* Full heart computational models reproducing human cardiac populations were created to predict the concentration-response relationship of changes in the QT interval as recommended for clinical trials. The concentration-response relationship of the QT-interval prolongation obtained from the computational car- diac population was compared against the relationship from clinical trial data for a set of well-characterized compounds: moxifloxacin, dofetilide, verapamil, and ondansetron. *Key results:* Computationally derived concentration–response relationships of QT interval changes for three of the four drugs had slopes within the confidence interval of clinical trials (dofetilide, moxifloxacin and verapamil) when compared to placebo-corrected concentration-ΔQT and concentration-ΔQT regressions. Moxifloxacin showed a higher intercept, outside the confidence interval of the clinical data, demonstrating that in this example, the standard linear regression does not appropriately capture the concentration-response results at very low concentrations. The concentrations corresponding to a mean QTc prolongation of 10 ms were consistently lower in the computational model than in clinical data. The critical concentration varied within an approximate ratio of 0.5 (moxifloxacin and ondansetron) and 1 times (dofetilide, verapamil) the critical concentration observed in human clinical trials. Notably, no other in silico methodology can approximate the human critical concentration values for a QT interval prolongation of 10 ms. *Conclusion and implications:* Computational concentration-response modelling of a virtual population of high- resolution, 3-dimensional cardiac models can provide comparable information to clinical data and could be used to complement pre-clinical and clinical safety packages. It provides access to an unlimited exposure range to support trial design and can improve the understanding of pre-clinical-clinical translation.

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1. Introduction

Managing proarrhythmic risk has been a challenge to clinicians and drug developers for decades. After the discovery of the hERG potassium channel as a main driver of proarrhythmic risk (Curran et al., 1995; Sanguinetti & Tristani-Firouzi, 2006), a wave of marketed drugs was withdrawn, and rigorous screening and selection processes were employed in discovery and development of new drugs. However, for late-stage decision-making, the prolongation of the QT interval as a biomarker for proarrhythmic risk has always been used as the relevant parameter in dedicated clinical studies. Since then, research on the ion channels involved in cardiac electrophysiology led to the insight that (1) proarrhythmic risk may also be brought about by unwanted interactions with ion channels other than hERG (Bril et al., 1996; Wu et al., 2008), and (2) in case of ion channel poly-pharmacology, the prediction of the potential collective effect on proarrhythmic risk becomes challenging (Sager, Gintant, Turner, Pettit, & Stockbridge, 2014). Moreover, the high investment for dedicated clinical QT (tQT) trials and the increased experience in concentration-QT (C-QT) analysis gained in past tQT studies paved the way for a modified de-risking strategy: the inclusion of a QT assessment in SRD/MRD (Single Rising Dose/Multiple Rising Dose) clinical studies (Darpo & Garnett, 2013). More recently, an emerging understanding of the translation of pre-clinical assays supported the integration of non-clinical data into proarrhythmic risk assessment in specific cases, as outlined in the recent ICH S7B/E14 Q&A document (*ICH guideline E14/S7B on clinical and nonclinical evaluation of QT/QTc interval prolongation and proarrhythmic potential - questions & answers*, 2024). This non-clinical data package includes a hERG assay and a non-rodent in vivo study, performed in compliance with 'best practice' as detailed in the guidance, and may be complemented by cellular or ex vivo studies and computational analyses of the cardiac action potential based on in vitro ion channel inhibition data.

When assessing proarrhythmic risk at a pre-clinical stage, verification of the translation of animal models or the prediction of clinical outcomes based on ion channel inhibition data is hampered by only anecdotal evidence of the incidence of pro-arrhythmias in man. Therefore, the validation of pro-arrhythmia models usually resorts to non-translatable biomarkers and rather coarse risk categories (Dutta et al., 2017). It is, however, important to note that to-date, the QT interval remains the key biomarker in early clinical studies, and potential prolongation of this interval is be part of the risk/benefit assessment for a drug candidate. We therefore decided to follow an approach that can be directly translated into a potential clinical effect, i.e., to predict the clinical concentration-QTc relationship. The pre-clinical use of computational modelling is aligned with the 3Rs principles (replace, reduce, and refine) for the ethical use of animals in medicine and has been recommended recently by both the European Medicines Agency (EMA) and the US Food and Drug Administration (Nuwer, 2022; *Recommendation to marketing authorisation holders, highlighting the need to ensure compliance with 3Rs methods described in the European Pharmacopoeia*, 2024). Recently, a white paper on the validation of proarrhythmia risk prediction models (Li et al., 2019), for regulatory use was published by a CIPA think tank. The document focuses on validation as an evaluation of "how good the model is for a given prediction task rather than how good is it as a representation of the real physiological system" (Li et al., 2019). In contrast to proarrhythmia risk prediction, the work described in this paper has a different aim: to represent the real physiological system response which is directly compared to the physiological human response during clinical trials for cardiac safety assessment using publicly available clinical datasets. This means that different training and validating datasets are not required. The approach described in this study provides a fully mechanistic response to ion channel block, which is based in the solution of the ion channel dynamics that quantifies the cardiomyocyte function embedded in a normal human anatomy to describe the transmembrane potential propagation. Any ion channel block can therefore be tested to observe the predicted mechanistic response of the entire system. The predictive capabilities of the population rely on the accurate description of the cardiomyocyte behavior (cell model), the cardiac tissue properties and the ion channel block produced by any given drug.

Most computational models for proarrhythmic risk assessment focus on single cell simulations or populations of single cells (Dutta et al., 2017; Fogli Iseppe et al., 2021; Mirams et al., 2011; Passini, Margara, & Rodriguez, 2021; Trovato, Mohr, Schmidt, Passini, & Rodriguez, 2020; Yang & Clancy, 2012) or cell strands (Patel, Wisniowska, & Polak, 2018). Others employ multi-scale models from atoms to tissue segments (Patel et al., 2018; Yang et al., 2020). Few solve biventricular anatomies to assess proarrhythmic risk (Hwang et al., 2019; Margara et al., 2021; Matsuno, Yao, Perdikaris, & Kuhl, 2019; Yoshinaga et al., 2018), and their value has already been identified. This work goes beyond the strand or single cell population paradigm to create a full heart population reflecting inter-subject variability of ion channel density that reproduces a healthy human study population to quantify biomarkers that can be directly compared to clinical trial data in drug concentration-response analyses. Going beyond single cells and cell strands, the generation of a full-heart 3D virtual human populations allows us to compute in a direct way a virtual electrocardiogram for each subject to quantify QT-prolongation after the administration of a drug as compared to baseline. On these grounds, it is remarkable that recent studies (Gonzalez-Martin et al., 2022) have identified that *there is not a strict direct proportionality between the action potential duration and the QT-interval duration in normal human hearts*. The use of a framework like the one proposed in this work may provide translatable information on the potential risk of subjects to develop drug induced QTc prolongation. The goal of the present study is to provide a proof of concept as a step towards the validation of the computational method and the virtual population against small-scale clinical trials with reference compounds (moxifloxacin, dofetilide, ondansetron, and verapamil) having different profiles of ion channel inhibition (see Table 4) and diverging impact on the QT interval. Within the large number of compounds and pharmacological profiles of interest we selected compounds for which high-quality clinical data from small-scale trials were available as the most appropriate comparator to our approach. Using sets of virtual ECGs, concentration-response analyses for the virtual population were performed to achieve results comparable to SRD/MRD concentration-response analyses (Darpo et al., 2015; Vicente et al., 2019).

2. Methods

This in silico study was performed using Alya (Santiago et al., 2018; Vazquez et al., 2016), a multi-physics, multi-scale, finite element-based simulation tool developed at the Barcelona Supercomputing Center, commercialized by ELEM Biotech, and capable of running efficiently on supercomputers, with tested scalability up to 100.000 cores (Casoni et al., 2014; Houzeaux, Vazquez, Aubry, & Cela, 2009). Alya was used to reproduce the electrophysiologic behavior of the human heart employing the monodomain approximation to the anisotropic electrical cardiac propagation (Margara et al., 2021; Santiago et al., 2018). The human heart anatomy was obtained from a young deceased donor without cardiac history, and with anatomically normal ventricles (20yo, BMI 19.1). Normality was assessed by the donor's clinical history, the size of the heart, its volume mass, the ventricular wall thickness and its structure. The measured myocardial volume of the heart is 170.6 cm3, and its trabecular volume is 8.7%. The anatomy is shown in Fig. 1.

The data was collected from the University of Minnesota's Visible Heart® Lab (VHL) library. It was reconstructed from high-resolution magnetic resonance images (MRI) of ex-vivo sequences, including detailed representation of ionic currents, electrical activation, heterogeneity of tissue and with a rule-based description of the cardiac fiber orientation. A detailed description of the mathematical model construction can be found in Gonzalez-Martin et al. (Gonzalez-Martin et al., 2022). The finite element simulations in this study employed the model

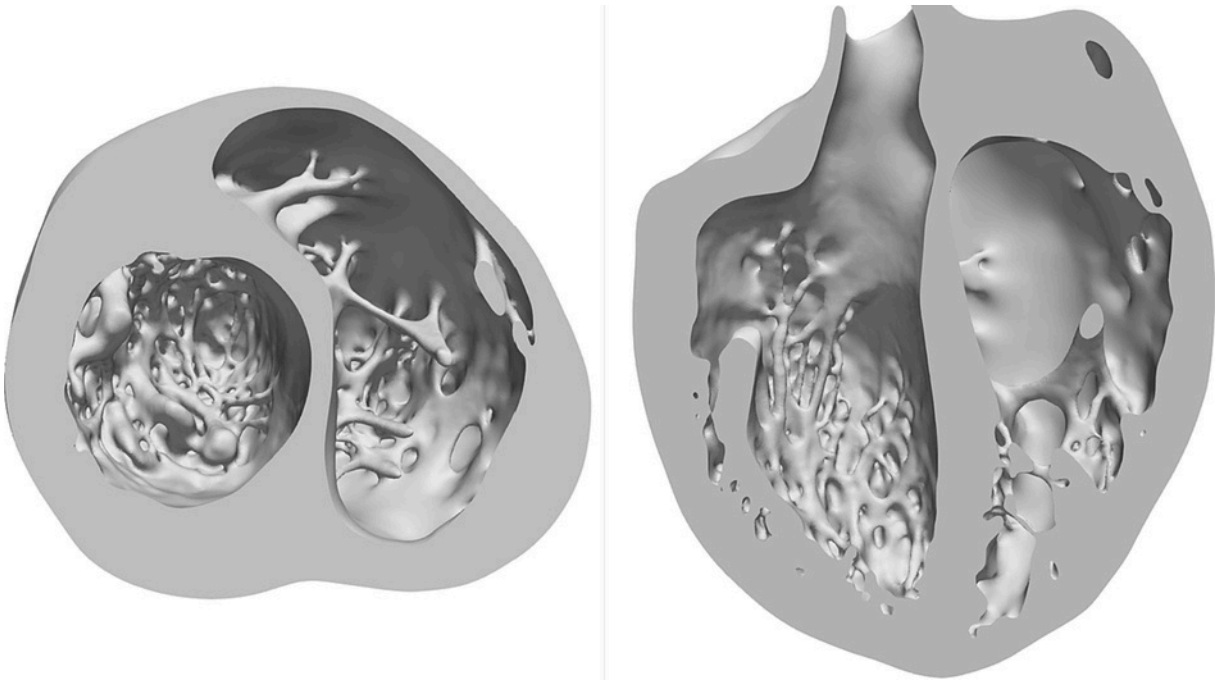


Fig. 1. High resolution, finite element model of the normal human heart anatomy used in this study.

"C" exactly as described in (Gonzalez-Martin et al., 2022) as baseline, from which the population was built.

Briefly, a volumetric finite element mesh (37.3 million tetrahedral elements) was created (Aguado-Sierra et al., 2022; Gonzalez-Martin et al., 2022), with a regular element side length of 328 μm (Santiago et al., 2018), which demonstrated mesh convergence (Gonzalez-Martin et al., 2022). Next, fiber orientation was incorporated using a rule-based approach (Doste et al., 2019). Subsequently, transmural cell heterogeneity (endocardial, M-cells, and epicardial) was assigned based on the O'Hara cell model (O'Hara, Virag, Varro, & Rudy, 2011), and the diffusion coefficients were employed to reproduce clinically observed total activation times and conduction velocities of the myocardium. Electrical activation was simulated following the normal activation sequences in humans (Durrer et al., 1970) at a basic cycle length of 1000 ms (60 bpm). The tissue was paced at each of the specified locations with a magnitude of 5 mA/cm3 applied for 5 ms at each of the specified locations assuming a cell membrane capacitance of 1 μF using a hemispherical stimulus of 0.2 cm radius.

2.1. Inter-subject variability and sex-specific phenotype definition

The O'Hara-Rudy model of a human ventricular cell (O'Hara et al., 2011) with modified conductances described by Dutta et al. (Dutta et al., 2017), (except for the Markov chain model of hERG), was used to reproduce action potentials. Sex-specific ion channel subunit expression (Fogli Iseppe et al., 2021; Yang & Clancy, 2012) was introduced to generate male and female phenotypic cohorts (see Table 1). The experimentally-calibrated population-of-models approach (Muszkiewicz et al., 2016) was used to produce the spectrum of phenotypes. The inter-subject phenotypic variability was created following the variability measured in normal human hearts (Walmsley et al., 2013). The conductance variations were reduced to an approximate ±20% (well within normal human variability) applied to five of the main ionic currents that determine action potential duration (IKr, IKs, INaL, ICaL, INa) on both male and female phentoypes. To create a population with the greatest variability, the 5 ion channels were varied in a combinatorial manner to produce 32 phenotypes for males and females. All selected conductance values reproduced normal QRS and QT-interval durations on the full heart anatomy.

Table 1
Gender-specific baseline conductance differences (Fogli Iseppe et al., 2021).

Ion Channel	Epi		Mid		Endo	
	Male	Female	Male	Female	Male	Female
IKs	1.04	0.87	1	1	1	0.83
IKr	1.09	0.875	1	1	1	0.79
IK1	0.98	0.74	1	1	1	0.86
Ito	0.6	0.26	1	1	1	0.64
INaK	1	1	1	1	1	1
IpCa	0.88	1.6	1	1	1	1.6
Iup	1	1	1	1	1	1
Calmodulin	1.07	1.41	1	1	1	1.21
INaCa	1	1.15	1	1	1	1.15

2.2. Characterization of QT interval and QRS duration in virtual population

Virtual electrocardiograms (or pseudo-ECGs) were calculated by positioning the biventricular model within a generic torso (Gonzalez-Martin et al., 2022). Importantly, the torso was employed to provide the coordinate references of each lead, but not to calculate the voltage gradient through its mass and tissues. Fig. 2 shows the derivation I of the pseudo-ECG produced by a female and male subjects at baseline and after the administration of 1 Cmax of moxifloxacin. The pseudo-ECGs were calculated to obtain biomarkers, such as QRS and QT, at baseline and after the administration of drugs to quantify drug effects. Data were analysed using R Project for Statistical Computing (v4.2.2) and Python (v3.9). An automatic algorithm was used to evaluate the QT-interval duration in all the populations. The algorithm quantified the QT-interval from the known activation time of the heart anatomy until the end of the T-wave, which was defined as the time when all three leads (Lead I, II and III) returned to baseline, i.e., the standard deviation between the leads was lower than 0.3 mV.

The acceptance of the quantifications from the automatic algorithm was performed after inspection of the calculations by an expert and in case of failure of the automatic algorithm, the expert performed the

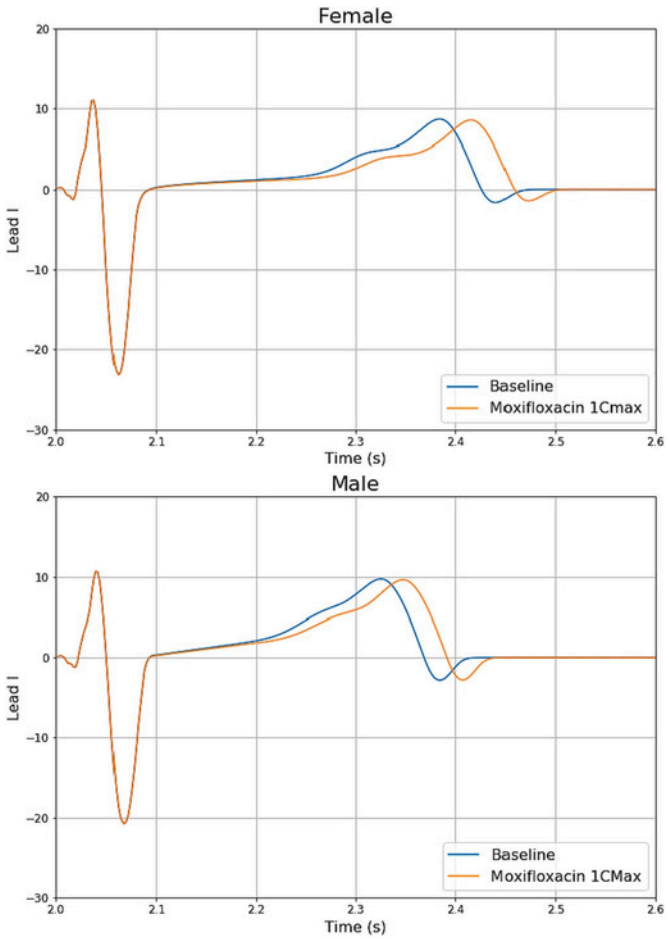


Fig. 2. Pseudo-ECG waveforms of two virtual subjects; a female and a male at baseline and after the administration of 1Cmax concentration of moxifloxacin.

evaluation manually, ensuring that the QT-interval selected follows the same criteria as the automatic algorithm.

The 64 baseline virtual subjects were characterized by their QT interval as shown in Fig. 3. The QT interval values are within the normal population ranges (Mason et al., 2007). These baseline QT-intervals were used to determine the QT-interval prolongation after the admin- istration of drugs. It is noticeable that female subjects have longer QT- interval durations in comparison to males.

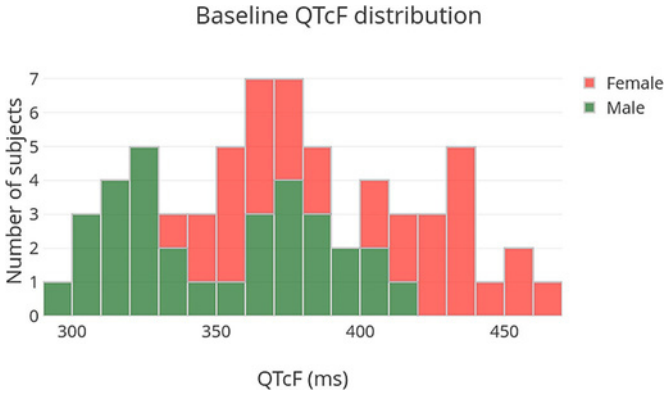


Fig. 3. Histogram showing the distribution of the QT interval duration in milliseconds of 64 virtual human subjects, at a basic cycle length of 1000 ms obtained within the full baseline population and coloured by sex phenotype (green: male, red: female). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The calculated effect on the QT interval (ΔQT) versus plasma concentration was compared with IQ-CSRC results (Darpo et al., 2015) in the case of moxifloxacin, ondansetron and dofetilide, and CiPA results (Vicente et al., 2019) in the case of verapamil. Importantly, the study by Florian et al. (Florian, Tornøe, Brundage, Parekh, & Garnett, 2019) was not used for comparison of moxifloxacin given that the currently accepted value of critical concentration published in the S7B/E14 Q&A training material (*ICH E14/S7B Q&As Training Material Examples Supplemental File (Table 1C)*, 2024) is determined to be 1866 ng/mL, which is closer to the IQ-CSRC study data (Darpo et al., 2015), than the one obtained by Florian et al. (Florian et al., 2019). Moxifloxacin was selected because it is commonly employed as a positive control for cardiac drug safety studies. Dofetilide was selected because it is a primarily hERG channel blocker, ondansetron was selected because it is also a QT-positive drug; and verapamil was selected because it is a balanced ion channel-blocking drug. QT values in clinical data are corrected for heart rate (QTcF), while computational data does not require correction due to the implementation of a standard 1000 ms basic cycle length in the entire population. For simplicity, the results will be described as QTcF for both clinical and computational data in the results.

2.3. Population subsampling

From the entire 64 subject population, a uniform distribution subsampling based on QT interval duration was applied to cover a full normal range of QT values with the least number of subjects, independently of the observed frequency of a QT value in a patient population in order to verify that the response is not biased by selecting only a short baseline QT interval range. This also reduces the computation time and reproduces more closely the sampling number of the *small scale human trials* used *as comparators*. Sixteen specific virtual subjects were selected to obtain an approximately uniform distribution of QT-interval values and an equal split between male and female sex. These 8 males and 8 female virtual subjects were administered moxifloxacin, dofetilide, ondansetron and verapamil.

2.4. Drug pore block model

The effects of the drug affecting the ion channel conductances were incorporated using a multi-channel conductance-block formulation (Mirams et al., 2011) using the following equation:

$$gnew = gbaseline \left[1 + \left(\frac{D}{IC50}\right)_h\right]^{-1} \quad (1)$$

where gnew is the ion channel conductance after the drug administration; gbaseline is the ion channel conductance in baseline conditions; *D* is the free plasma concentration, *IC50* corresponds to the drug concentration at which ion channel conductance is reduced by 50%, and *h* corresponds to the Hill coefficient.

2.5. Data for reference drugs

The simulations with dofetilide and ondansetron were performed at three different concentrations, while for verapamil four different concentrations were investigated (see Table 2). For moxifloxacin three concentrations were employed in a sex-specific manner (see Table 3), since it has been shown (Florian et al., 2019) that women had about 40% higher observed maximum moxifloxacin concentrations than men. A Cmax of 2918 ng/mL was observed in women as compared to 2054 ng/mL in men following equivalent moxifloxacin oral dosing. These percentage differences were applied in a sex-specific manner within our simulations. Specific pharmacokinetic properties can be accounted for using adjusted concentrations for specific sub-populations, as it was done for moxifloxacin. Generally, the model works with exposures

Table 2 Ondansetron, Dofetilide and Verapamil concentrations employed throughout the study. K-fold values were derived from clinical Cmax values reported in Crumb et al. (2016) (Crumb, Vicente, Johannesen, & Strauss, 2016).

Ondansetron		Dofetilide k-		Verapamil	
k-fold therapeutic Cmax	*Plasma Conc. (ng/mL)*	fold therapeutic Cmax 0.11	*Plasma Conc. (ng/mL)*	k-fold therapeutic Cmax 0.25	*Plasma Conc. (ng/mL)*
0.29	30	0.32	0.1	0.5	99.6
0.95	100	1.08	0.3	1	199.2
2.85	300		1	2	398.4
					750

Table 3 Moxifloxacin concentrations employed throughout the study. K-fold values were derived from clinical Cmax values reported in Crumb et al. (2016) (Crumb et al., 2016).

Moxifloxacin			
Female		Male	
k-fold therapeutic Cmax	*Plasma Conc. (μg/mL)*	k-fold therapeutic Cmax	*Plasma Conc. (μg/mL)*
0.33	0.7	0.2325	0.5
0.66	1.5	0.465	1
1.32	2.9	0.93	2.1

resulting from doses administered by any route. In our case the concentrations were chosen to cover the entire range of exposures reached in the clinical comparator studies.

The IC50 and Hill coefficient values for the seven ion channels used for the analysis were taken from (Dutta et al., 2017) (dofetilide) and (Crumb et al., 2016) (rest of the compounds) and are listed in Table 4. The range of values reported for dofetilide is particularly large and 2 nM reported in (Crumb et al., 2016) is at the very low end. We therefore decided to use the value reported by Dutta et al. (Dutta et al., 2017) for their simulation study.The pore block was calculated for each ion channel for all components assessed and at all concentrations of interest.

The plasma protein binding (PPB) of moxifloxacin varies depending on the sources, from 30 to 50% (Moxifloxacin, n.d.). In our study, the PPB of moxifloxacin was assumed to be 35% (Bergogne-B´ér´ézin, 2002). The free fraction in plasma was assumed to be the mean of the extreme values reported for the remaining compounds: 27% for ondansetron (*Zofran FDA label*, n.d.), 35% for dofetilide (Mounsey & DiMarco, 2000) and 12% for verapamil (Verapamil, n.d.). A literature review on the variability of PPB values across the literature for each compound can be found in Table 5.

To initialize the finite element simulation, each 0-dimensional model of each cell type is solved until it has reached a steady state of the calcium transient (root mean square error, RMSE, between consecutive beats smaller than 1.e-7(μM) for 3 consecutive beats). The results of the single cell models provided initial conditions to the 3D finite element simulations. Simulations on the 3D mesh of at least 3 beats were solved to achieve steady state of the pseudo-ECG.

Table 5
Literature review on interstudy PPB variability. Yellow-shaded PPB values represent the values selected for this study.

Drug	PPB 60–	Study
Dofetilide	70%	(Mounsey & DiMarco, 2000)
	60–70%	(Dofetilide, n.d.)
Moxifloxacin	26–50%	(Bergogne-B´ér´ézin, 2002)
	30–50%	(Moxifloxacin, n.d.)
	45%	(Siefert et al., 1999)
	54%	(Østergaard, Klitmøller Sørensen, Dahl Knudsen, & Frimodt-Møller, 1998)
	52%	
	40%	(Müller et al., 1999)
	30–45%	(Stass & Kubitza, 1999)
	70–76%	(Turnidge, 1999)
Ondansetron	88%	(*Zofran FDA label*, n.d.)
	86%	(Verapamil, n.d.)
	90%	(Sube & Ertel, 2017)
		(Singh, Ellrodt, & Peter, 1978)
Verapamil	93.7%	(Echizen, Brecht, Niedergesä¨ss, Vogelgesang, & Eichelbaum, 1985)
	90%	(Keefe, Yee, & Kates, 1981)

Statistical Analysis. Linear mixed effect (LME) Concentration-QT (C-QT) models are used as the analysis recommended by the E14/S&B Q&A document for assessing the QT interval prolongation risk of new drugs in early phase clinical pharmacology and tQT studies. Linear mixed models are an extension of simple linear models to allow both fixed and random effects and are specifically used when there is non-independence in the data, as it arises from a hierarchical structure. Fixed effects are the coefficients (intercept, slope) and random effects are the variances of the intercepts or slopes across groups. These models, despite being over-parameterized, appropriately address the overall modelling objective in comparison to simple linear models. The prespecified LME C-QT model includes ΔQT (or ΔΔQT) as the dependent variable, for which the fixed effect parameters are intercept, slope, influence of baseline on intercept, treatment, and nominal time from first dose. Subject identity is present as a random effect parameter on both intercept and slope terms (Garnett et al., 2018). The lack of a control population (or placebo group) in our computational study prevents us from defining the treatment variable, and therefore we are unable to account for the variance introduced by the placebo effect. That is why we cannot use the recommended LME model for this application and why a simple linear model was selected as the best approach to fit the data.

The results of the IQ-CSRC study (Darpo et al., 2015) were used for comparison in case of moxifloxacin, dofetilide and ondansetron. The results of the CiPA study (Vicente et al., 2019) were used for comparison in case of verapamil. A linear regression was performed for all datasets. Importantly, a recommended mixed linear model could not be employed to compare all the datasets because of the lack of a placebo in this

Table 4
Ion channel inhibition data for reference compounds used in this study.

Drug		Cav1.2	hERG	Kir2.1	Kv4.3	KvLQT1 /minK	Nav1.5-late	Nav1.5-peak	Drug data
Moxifloxacin	*IC50 (nM)*	–	93,041	–	–	50,321	382,337	–	Crumb et al. (2016)
	h	–	0.6	–	–	1.0	1.1	–	
Ondansetron	*IC50 (nM)*	22,551	1492	–	–	– – –	19,181	–	Crumb et al. (2016)
	h	0.8	1.0	–	–		1.0	–	
Dofetilide	*IC50 (nM)*	– –	4.9	–	18.8	–		–	Dutta et al. (2017)
			0.9	–		–	– –	–	
	h		499	–	0.8	–	–	–	
Verapamil	*IC50 (nM)*	202	1.1	–			–	–	Crumb et al. (2016)
	h	1.1			– –				

computational trial. Furthermore, the number of concentration samples computed were small, therefore a mixed linear model as in the clinical trial was not appropriate. The placebo correction for the data published of the CiPA study (Vicente et al., 2019) was performed as the ΔQT minus the respective mean ΔQT per time point for all placebo subjects. In the IQ-CSRC study (Darpo et al., 2015), however, the authors applied placebo correction as the difference between the model-derived ΔQT at concentration of interest and model-derived ΔQT for placebo (concentration =0). In this study, the placebo correction for all the clinical data was performed as the ΔQT minus the respective mean ΔQT per time point for all placebo subjects. The statistical analysis was done using the function ggpredict, within the package ggeffects (v1.1.4) in R.

The term 'critical concentration' was introduced in the most recent S7B/E14 Q&A document (*ICH guideline E14/S7B on clinical and nonclinical evaluation of QT/QTc interval prolongation and proarrhythmic potential - questions & answers*, 2024) and corresponds to the concentration at which a drug causes a mean effect on the QTc-interval of 10 ms. Critical concentrations, corresponding to total concentration values, were computed from the clinical and computational datasets following the previously described definition. In other words, they represent the concentration at which the clinical and computational c-QT relationships intersect on 10 ms ΔQT. Both values were reported for the entire populations along with the values observed for each sex. The critical concentration ratio was calculated as the critical concentration value of the computational trial divided by the critical concentration value estimated from the human clinical trial data for both regressions, the non-corrected ΔQT data and the placebo-corrected ΔQT for each drug studied.

3.Results

The calculated concentration–QT (C-QT) relationship using a linear regression of both the clinical (non-adjusted ΔQT) and computational (ΔQT) data of the four compounds are shown in Fig. 4; shaded areas represent the 90% confidence interval (CI) of the predicted effect. Linear regressions obtained from the female and male virtual subpopulation are displayed in the right column in red and green, respectively. Similarly, the calculated concentration–QT values using a linear regression of both the clinical (placebo-adjusted ΔQT, ΔΔQT) and computational (ΔQT) data are shown in Fig. 5. The slope of the C-QT relationship and the intercept for the clinical and computational results with ΔQT and ΔΔQT can be found in Tables 6 and 7, respectively. The critical concentrations (from both ΔQT or ΔΔQT regressions) derived from the clinical data and the virtual population are also shown in Tables 6 and 7.

The regression for dofetilide demonstrates that the response of the simulated population falls within the confidence interval of the IQ-CSRC (Darpo et al., 2015) clinical trial data when compared to both the ΔQT (Fig. 4a, b) and the ΔΔQT (Fig. 5a) slopes and intercepts of the linear regressions. The ratio of critical concentrations from clinical data and computational predictions were 0.67 (ΔQT, Table 6) and 0.93 (ΔΔQT, Table 7).

For moxifloxacin, the regression shows the same slope both for ΔQT (Fig. 4b and c) and ΔΔQT (Fig. 5b). The estimated slope, therefore, falls within the confidence interval of the clinical trial data; however, the intercept for both comparisons is outside the 90% confidence interval, shifting the ratio of critical concentrations to 0.32 (ΔQT, Table 6) and 0.44 (ΔΔQT, Table 7). The high intercept for moxifloxacin could be due to the use of a low percentage of protein binding (35%) in comparison to some of the reported values between 30 and 50% concentration. This produces a higher free fraction of drug which was applied to the model (65% versus a mean of 60%). A full uncertainty quantification on the input parameters will provide a clearer understanding on their effect on the computational models.

For ondansetron (Figs. 4c and 5c), both slope and intercept are outside the 90% confidence interval in comparison to the IQ-CSRC clinical trial data (Darpo et al., 2015). Very similar to moxifloxacin, the critical concentration ratios are 0.34 (ΔQT, Table 6) and 0.46 (ΔΔQT, Table 7).

In the case of verapamil, the simulations result in slopes within the confidence interval for both ΔQT (Fig. 4d) and ΔΔQT (Fig. 5d), but only the intercept of the ΔΔQT comparison (Fig. 5d) falls inside the 90% confidence interval. The critical concentration ratios were calculated as 0.42 (ΔQT, Table 6) and 1.06 (ΔΔQT, Table 7).

Overall, the critical concentrations were closer to clinical values when compared to placebo-corrected ΔQT clinical regressions. The computational predictions of the critical concentration were rather conservative: on average about a factor of two lower than clinical data for moxifloxacin but never significantly higher.

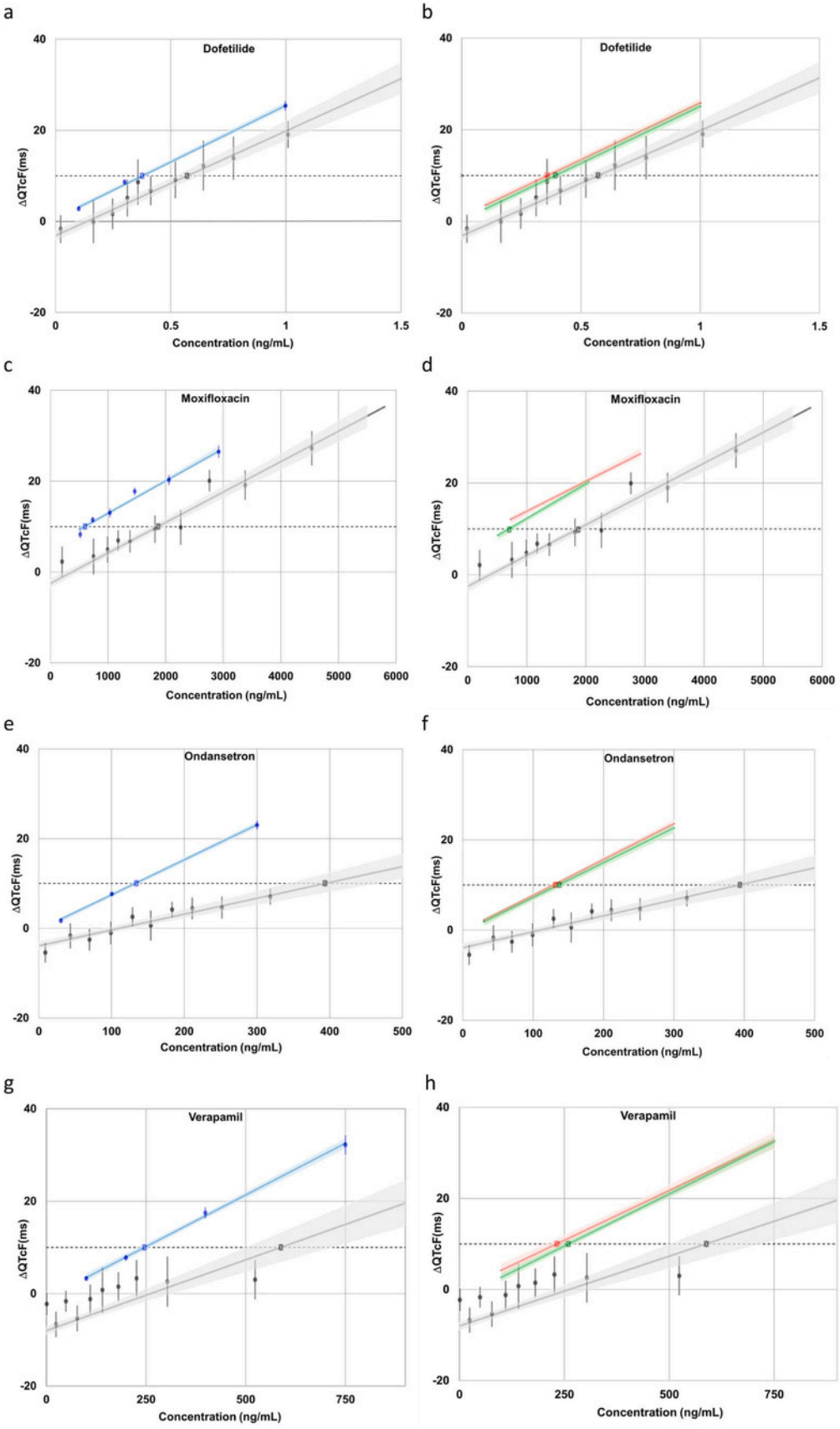
The existence of sex-related differences of drug-induced QT interval value prolongations is controversial. In this study, QT-interval values for all four compounds exhibited small sex-related differences, showing just slightly larger QT-prolongation values on female subjects as observed in Fig. 4b, d, f and h. Regressions for females showed a slight increase in intercept value and hence a minimal reduction in the critical concentration ratio for moxifloxacin, ondansetron and dofetilide, but not for verapamil. The slopes obtained from the linear regressions exist within the confidence interval of each other for ondansetron, dofetilide and verapamil. The largest difference was observed in moxifloxacin, where average plasma concentrations administered to each sex were different. In this case, a 0.2 ratio of computational versus clinical critical concentration difference was observed. Given the lack of data on sex-specific, drug-induced QT-interval prolongation information, any small differences are required to be included in any study to increase the evidence on sex-related differences in cardiac safety trials.

4.Discussion

This study proposes a novel computational approach with a virtual population of healthy subjects using 3D biventricular heart simulations to predict the clinical concentration-QT relationship of reference drugs. The methodology to reproduce a healthy population incorporated a realistic anatomical description, and a normal inter-subject electrophysiological phenotype variability including sex differences.

QT intervals (Fig. 3) and QT-interval prolongations (Figs. 4 and 5) obtained from the computational framework were within the ranges observed in clinical trial subjects (Darpo et al., 2015; Vicente et al., 2019). The clinical studies were all relatively small (e.g., 9 subjects on active and 6 on placebo in the IQ-CSRC study), and variability across clinical studies must also be borne in mind. The computational model was successful in identifying the QT effect of four (4) QT prolonging drugs; therefore, the presented methodology has the potential as a screening tool in early development for detection of drugs that may cause clinical QT prolongation. We anticipate the following benefits of virtual clinical trials: (1) A prediction of the expected clinical QT effect of a compound before being administered in a clinical trial, which is not possible with an hERG assay and standard non-clinical studies; (2) The testable exposure range may be expanded virtually to reach multiples of the clinical high exposure (*ICH guideline E14/S7B on clinical and nonclinical evaluation of QT/QTc interval prolongation and proarrhythmic potential - questions & answers*, 2024), as desired, without risks. (3) Compounds that are poorly tolerated at high concentrations in healthy volunteers or compounds that can only be tested in patients receiving co-medications can be studied in a virtual environment without confounding factors. (4) Virtual clinical studies may complement clinical datasets used to understand the translation of pre-clinical animal models traditionally used for drug candidate selection.

Observations from the computational population have highlighted a variety of aspects when compared to human clinical trials: (1) a reduced inter-subject variability in the virtual populations, (2) factors impacting the regression models and parameters (slope, intercept, and, consequently, the critical concentration for the threshold of 10 ms) and (3) existence and treatment of placebo controls.



(caption on next page)

Fig. 4. ΔQT clinical (black) and ΔQT computational (blue) using the entire computational population on the left, and with sex-specific regressions on the right (male in green and female in red). (a & b) dofetilide, (c & d) moxifloxacin, (e & f) ondansetron and (g & h) verapamil. The solid green line with green shaded area (90% confidence interval) determines the model-predicted ΔQT for the male population as a function of plasma concentration. The solid red line with red shaded area (90% confidence interval) determines the model-predicted ΔQT for the female population as a function of plasma concentration. The solid black line with grey shaded area and the black dots with vertical bars denote the clinically observed effect on ΔQT. The red markers on left panels indicate the critical concentration calculated from clinical and computational data. Similarly, critical concentrations were marked for the regressions of male and female subjects on the panels to the right. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

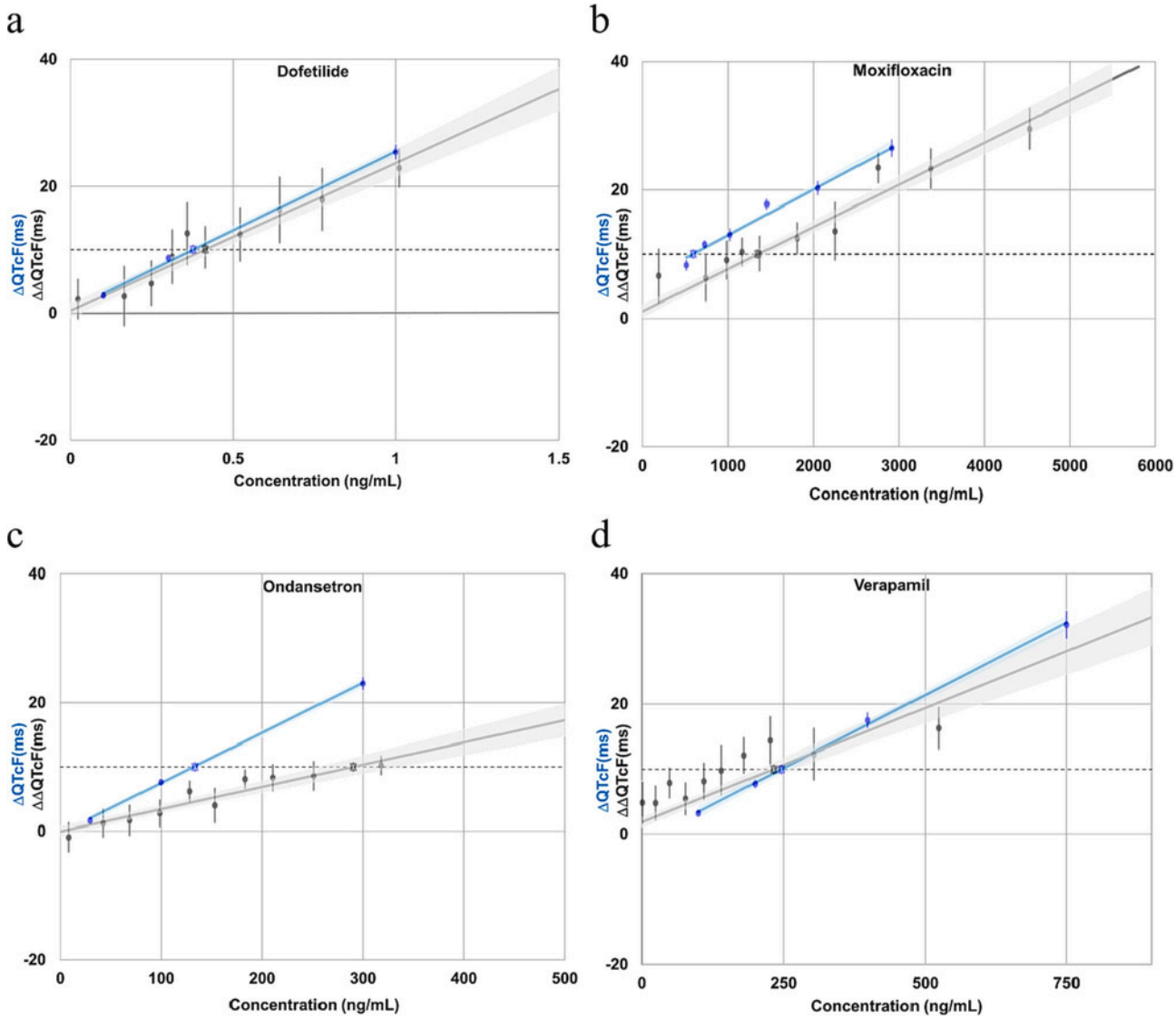


Fig. 5. ΔΔQT clinical (black) and ΔQT computational (blue) for each drug assessed; a. dofetilide; b. moxifloxacin; c. ondansetron; d. verapamil. Each regression includes markers to indicate their predicted critical concentration (at the 10 ms threshold). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4.1. Inter-subject variability

In this study, only inter-subject variability of ion channel expression is included in the computational model as the source of variation, which mostly corresponds to the electrophysiological properties of the cardiac tissue. Participants in clinical trials are heterogeneous regarding age, sex, ethnicity, individual physiological, hormonal, and anatomical characteristics, to mention just a few. To keep variability low, in small-scale clinical trials healthy subjects are deliberately selected to restrict heterogeneities. However, some level of variability will remain. Yet, this computational population provides the opportunity to study sex differences for QT prolonging drugs (Benton, Sale, Flockhart, & Woosley, 2000; Darpo et al., 2014).

Another source of variation in the clinical data which is missing in the computational approach is the accuracy of exposure determination. Depending on the analyte, a deviation of the measured exposure of ±15% may be expected (*ICH Guideline M10 on Bioanalytical Method Validation*, 2024). Given the comparatively large range of exposures tested both clinically and in the simulations, exposure determination is not expected to contribute significantly to the larger variability in the clinical data.

Table 6
Slope, intercept of concentration-QTc relationship and critical concentrations estimated from computational and clinical (non-adjusted ΔQT) data of Fig. 4.

Drug Trial			Coef	Std err	t	P < |t|	[95.0% Conf. Interval]	Critical Conc. (ng/mL) (F, M)	Critical Conc. ratio
Moxifloxacin	*Computational*	*Intercept*	5.67	0.4846	11.70	<0.001	[4.69, 6.64]	596 (427,699)	0.32
		Slope	0.01	0.00029	25.04	<0.001	[0.01, 0.01]		
	Clinical	*Intercept*	−2.51	0.6070	−4.136	<0.001	[−3.71, −1.31]	1866	
		Slope	0.01	0.0003	19.891	<0.001	[0.01, 0.01]		
Ondansetron	*Computational*	*Intercept*	−0.52	0.3823	−1.373	0.177	[−1.29, 0.24]	134 (131,137)	0.34
		Slope	0.08	0.0021	37.735	<0.001	[0.07, 0.08]		
	Clinical	*Intercept*	−3.89	0.5379	−7.229	<0.001	[−4.95, −2.83]	393	
		Slope	0.04	0.0040	8.898	<0.001	[0.03, 0.04]		
Dofetilide	*Computational*	*Intercept*	0.67	0.4028	1.654	0.105	[−0.14, 1.48]	0.38 (0.36, 0.39)	0.67
		Slope	24.82	0.6651	37.319	<0.001	[23.48, 26.16]		
	Clinical	*Intercept*	−3.13	0.6786	−4.616	<0.001	[−4.47, −1.80]	0.57	
		Slope	22.98	1.6321	14.077	<0.001	[19.76, 26.19]		
Verapamil	*Computational*	*Intercept*	−0.98	0.6459	−1.517	0.134	[−2.27, 0.31]	246 (232, 259)	0.42
		Slope	0.04	0.0015	30.372	<0.001	[0.04, 0.05]		
	Clinical	*Intercept*	−8.03	0.5705	−14.081	<0.001	[−9.15, −6.91]	588	
		Slope	0.03	0.0036	8.401	<0.001	[0.02, 0.04]		

Table 7
Slope, intercept of concentration-QTc relationship and critical concentrations estimated from computational and clinical (placebo-adjusted ΔQT) data of Fig. 5.

Drug Trial			Coef	Std err	t	P < |t|	[95.0% Conf. Interval]	Critical Conc. (ng/mL) (F, M)	Critical Conc. ratio
Moxifloxacin	*Computational*	*Intercept*	5.67	0.4846	11.70	<0.001	[4.69, 6.64]	596 (427,699)	0.44
		Slope	0.01	0.0003	25.04	<0.001	[0.01, 0.01]		
Moxifloxacin clinical	*Clinical*	*Intercept*	1.09	0.6077	1.80	0.073	[−0.10, 2.29]	1356	
		Slope	0.01	0.0003	19.46	<0.001	[0.01, 0.01]		
Ondansetron	*Computational*	*Intercept*	−0.52	0.3823	−1.373	0.177	[−1.29, 0.24]	134 (131,137)	0.46
		Slope	0.08	0.0021	37.735	<0.001	[0.07, 0.08]		
Ondansetron clinical	*Clinical*	*Intercept*	−0.03	0.4997	−0.066	0.947	[−1.02, 0.95]	290	
		Slope	0.03	0.0037	9.378	<0.001	[0.03, 0.04]		
Dofetilide	*Computational*	*Intercept*	0.67	0.4028	1.654	0.105	[−0.14, 1.48]	0.38 (0.36, 0.39)	0.93
		Slope	24.82	0.6651	37.319	<0.001	[23.48, 26.16]		
Dofetilide clinical	*Clinical*	*Intercept*	0.39	0.6871	0.563	0.574	[−0.97, 1.74]	0.41	
		Slope	23.25	1.6527	14.069	<0.001	[20.00, 26.51]		
Verapamil	*Computational*	*Intercept*	−0.98	0.6459	−1.517	0.134	[−2.27, 0.31]	246 (232, 259)	1.06
		Slope	0.04	0.0015	30.372	<0.001	[0.04, 0.05]		
Verapamil clinical	*Clinical*	*Intercept*	1.86	0.4969	3.749	<0.001	[0.89, 2.84]	232	
		Slope	0.04	0.0032	11.021	<0.001	[0.03, 0.04]		

4.2.Impact of input parameters

There is relevant variability related to experimental ion channel electrophysiology data. Uncertainty in the Hill coefficient (h) and the half-maximal inhibitory concentration (IC50) of reported concentration-effect curves can be particularly high. For example, dofetilide displays a large inter-laboratory and cross-platform variability (Kramer et al., 2020). However, the range of variation of the IC50 values under the same experimental protocol is approximately 3-fold (*ICH E14/S7B Q&As Training Material Examples Supplemental File (Table 1C)*, 2024).
The use of the IKr dyn model requires hERG data generated with the Milnes protocol which are not available for all compounds. Moreover, estimating the IKr dyn parameters requires some assumptions to be made and increases the number of parameter uncertainties in the input data. The use of the original IKr model from the Ohara-Rudy model allows for usage of compound data that is more easily available experimentally. It is therefore remarkable that the full heart simulation could reproduce the exposure response data so closely to clinical trial data.

Uncertainties of model input parameters through experimental variability and their impact should be quantified using appropriate statistical methods and then translated into probabilities of the predicted risk (Pathmanathan, Cordeiro, & Gray, 2019). Recent uncertainty quantification (UQ) studies of computational electrophysiology models have paved the way for their application in the models employed in this work (Pathmanathan et al., 2019; Pathmanathan et al., 2020). The translation of the experimental variability in ion channel inhibition into uncertainty of the concentration-response regression that comes on top of the variability caused by the virtual study population is technically feasible. Current UQ work is focused on the single cell electrophysiology model at the conductance variability level (Pathmanathan et al., 2019) with subsequent analysis of the uncertainty of the ion channel IC50 experimental variability (Matsuno et al., 2019) a combined simplified strand model and full heart anatomy. For the reference compounds studied here the variability of ion channel IC50 data found in the literature may arise mainly from diverging experimental protocols applied in the different reports and therefore does not allow the calculation of true confidence intervals for repeated measurements under the recommended conditions (*ICH guideline E14/S7B on clinical and nonclinical evaluation of QT/QTc interval prolongation and proarrhythmic potential - questions & answers*, 2024). Our group is currently conducting the appropriate sensitivity analyses and UQ quantifications from the full computational framework as performed previously (Pathmanathan et al., 2020).

The uncertainty of experimental determination of plasma protein binding (Wang et al., 2014) presents further challenges for the prediction of clinical data. Generally, as a rule of thumb, a factor of two is considered an acceptable range, although repeated measurements usually vary less than two-fold (Wang et al., 2014) under carefully set conditions. A two-fold shift of free drug concentration would inevitably result in a two-fold shift in the slope of the regression when total plasma level is plotted on the x-axis. The published plasma protein binding for the compounds of the current study is quite consistent (shown in Table 5); however, the number of reports is limited, and conditions of determination are not always known.

When performing simulations of pre-clinical compounds, the choice of the exposures to be used will present further challenges. Usually, human PK is predicted based on pre-clinical data with some uncertainty, however, our approach is designed to produce a C-QT relationship which covers a range of a factor of 10 (Tables 2, 3). This range should suffice to cover uncertainties in human dose and PK prediction.

4.3.Treatment of placebo controls

A third source for discrepancies between simulations and clinical data may arise from the treatment of placebo controls: The placebo effect observed in clinical studies is comprised of many unrelated effects such as circadian rhythm, post-prandial effects, emotional status, and many more. In case all study participants follow the same daily routine, as it is the case in most small-scale clinical trials, the placebo adjustment (ΔΔQT) will reduce background effects as outlined above in contrast to an analysis with pretreatment as reference (ΔQT). Since placebo treatment so far is not implemented in our computational model, simulated ΔQT already represents values free of any placebo-type background factors. Therefore, it is not surprising that discrepancies between simulations and clinical data are smaller when simulated ΔQT is compared with clinical ΔΔQT. It is desirable to increase the confidence on the methodology to develop computational models that reflect the time course after application of placebo to a healthy population to enable virtual ΔΔQT analyses. The potential need to create a control population with a placebo effect would be to provide a statistically relevant analysis to reproduce the same type of data as in clinical trials, as the inability of reproducing a computational ΔΔQT represents a hurdle for the validation of these models. A control population could be approximated by reproducing the daily variability that is portrayed when placebo effect is measured throughout the main biological mechanisms underlying circadian rhythm, such as sex hormones.

4.4.Limitations

As previously stated, the computational framework was created from the current state of the art mechanistic modelling of the behavior of the human cardiomyocyte. It relies on the mathematical model that describes the ion channel dynamics of the cardiomyocyte. Therefore, any dynamics not explicitly integrated or incorrectly considered in the model may affect the overall behavior of the population. The use of a variety of normal human anatomies and variability in tissue properties could further introduce some variability in the response to drug administration. Any of the parameters involved in describing the ion channel block on human cardiomyocytes would have a potential effect on the results of the in silico trial. The model will predict the modified action potential dynamics provided conductance variations produced by any drug, however it is subject to errors given an inaccurate description of the percentage of protein binding of the drug, it's IC50 and hill coefficient or uncertainties regarding the clinical exposure to the drug. These variables determine the amount by which an ion channel is blocked, and this is the most important input to the model to reproduce the expected clinical behavior. The uncertainty to IC50, hill coefficient, PPB%, and clinical concentrations will reflect on the effect of a drug at the population level.

In this manuscript, the linear model was the only statistical tool that could be employed to quantify the slope of the computational data, given the lack of a control population. This model is unable to capture any non-linearities within the clinical or computational data. Non-linearities may arise from measurement errors and can impact the comparison to clinical data (Bonate, 2013). The computational data may also produce a non-linear concentration-response behavior, and this is entirely due to the inherent non-linear cardiomyocyte ion channel dyanamics. Furthermore, it needs to be acknowledged that a concentration response relation is expected to be non-linear due to the very nature of pharmacology, however, exposure QT modelling in clinical studies is performed using linear regression in the vast majority of cases since a linear model is a good approximation for the narrow range of exposures covered in such studies. When performing linear regression care must be taken that conclusions are only drawn in a concentration range where linearity is approximately given. This is the case for the "critical concentration" we are considering in our study.

Only four compounds were tested in this study. The extension to a larger number of compounds with diverse ion channel profiles, within the limits of available clinical data, is currently in progress to fully assess the predictive capabilities of the computational model.

Other quantifiable intervals within the ECG were not assessed in this work. Future work will involve the evaluation of QRS, J-T peak, Tpeak-Tend, but comparison to clinical data will be dependent on their availability in the literature for the compounds assessed.

4.5.Conclusion and Outlook

This study was able to approximate the complexity of anatomically normal human heart electrophysiology to predict clinically observed QT-interval prolongation. The computational model prediction provided conservative values of critical concentration up to a two-fold difference to clinically observed values. It was also capable of providing the slope within the confidence interval of the concentration-QT prolongation for at least three of the four drugs assessed. Within the limitations of only four drugs evaluated and considering factors like for example uncertainty in ion channel inhibition data or plasma protein binding for which their impact on the comparison of calculated and clinical data has been discussed, we conclude the presented methodology could be used to predict the C-QT relationship of drug candidates with the above-mentioned limitations. A conservative prediction of the critical concentration with a maximum deviation by a factor of two and no case of over-estimation is remarkably precise given that the critical concentration determined in small-scale clinical trials may vary as well. An indication on observed ranges of slopes in C-QT relationships for moxifloxacin in through QT studies is given in Florian et al. (Florian et al., 2019). We, therefore, use virtual clinical studies for decision making in the preclinical phase and monitor their predictive value once clinical data become available in a retrospective fashion. If a computational approach can predict the critical concentration for these 4 mildly QT prolonging drugs, in a reasonable way, this is of clear value from a translational point of view.

Computational trials offer multiple opportunities to augment the safety assessment and related decision making along the drug development process. They enable access to an unlimited dosage or exposure range to support specific trial design. They may also support an improved animal-human translation, filling gaps in clinical data, and help to better understand sources of variability and error propagation that may lead to unexpected clinical results. Animal-human translation, however, will be limited in cases of species-specific metabolites contributing to the ion channel inhibition profile. Sex-specific computational models may help to compensate for the underrepresentation of females in early clinical trials. Similarly, models that reflect certain pathological conditions of human hearts that would prevent inclusion of patients with comorbidities in clinical trials may contribute to an early understanding of the patient stratification for the use of a given drug. Further research is required to assess scope and limitations of such more specific computational models. The proposed framework was not developed to substitute single cell populations simulations or the CIPA approach. The proposed framework has been developed as a complementary method that aims to predict clinical endpoints of drug-induced QT-interval prolongation to incorporate the non-linear relationships between anatomy and cardiomyocyte electrophysiology function (Gonzalez-Martin et al., 2022) for clinical translational purposes.

Finally, employing virtual clinical trials will enable more informative clinical study designs giving a stronger basis for dose selection strategies and decisions towards the regulatory acceptance of in silico

methodologies.

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CRediT authorship contribution statement

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Elem Biotech owns the commercial rights to Alya, the computational finite element solver employed in this study. However, any other commercial or academic finite element solver could be employed to reproduce this work.

Elem Biotech owns the commercial rights to Alya, the solver employed in this study.

Data availability

The methodology can be replicated using any finite element solver given all the parameterisation information provided in this paper. Quantified markers can be made available upon request.

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