

RESEARCH PAPER

Detecting moxifloxacin-induced QTc prolongation in thorough QT and early clinical phase studies using a highly automated ECG analysis approach

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BACKGROUND AND PURPOSE

Exposure–response (ER) modelling (concentration–QTc analysis) is gaining as much acceptance as the traditional by-time analysis of the placebo-adjusted change from baseline in the QTc interval ($\Delta\Delta\text{QTcF}$). It has been postulated that intensive ECG analysis and ER modelling during early-phase drug development could be a cost-effective approach of estimating QT liability of a new drug, in a small number of subjects.

EXPERIMENTAL APPROACH

We used a highly automated analysis of ECGs from 46 subjects from a crossover thorough QT/QTc study to detect $\Delta\Delta\text{QTcF}$ with moxifloxacin. Using these data, we also simulated (bootstrapped) 1000 datasets of a parallel study with eight subjects receiving moxifloxacin and eight others receiving placebo.

KEY RESULTS

The slope from the concentration–QTc analysis for moxifloxacin in 46 subjects was 4.12 ms of $\Delta\Delta\text{QTcF}$ per $\mu\text{g}^{-1}\text{ mL}^{-1}$; at mean C_{max} of $2.95\ \mu\text{g}\cdot\text{mL}^{-1}$, estimated $\Delta\Delta\text{QTcF}$ was 13.4 ms (90% confidence interval 11.3, 15.4 ms). In the 1000 simulated datasets, in 996 datasets, ER modelling showed that the upper bound of the 90% confidence interval for $\Delta\Delta\text{QTcF}$ at geometric mean C_{max} exceeded 10 ms. In 895 of these 996 datasets, the slope of the ER relationship was statistically significantly positive. Thus, with a small sample size (eight subjects on active drug and eight on placebo), moxifloxacin-induced QTc prolongation was demonstrated using ER analysis with statistical power of >80%.

CONCLUSIONS AND IMPLICATIONS

Our study adds to the growing body of data supporting intensive ECG collection and analysis in early-phase studies to estimate QT liability.

Abbreviations

ICH, International Conference on Harmonization; LOQ, limit of quantitation; SAD, single ascending dose; TQT, thorough QT/QTc

Tables of Links

TARGETS

Voltage-gated ion channels

HERG, K_v11.1 channels

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

Introduction

The International Conference on Harmonization (ICH) E14 guidance requires that all new drugs with systemic bioavailability be studied for their off-target effect on cardiac repolarization by a 'thorough QT/QTc' (TQT) study (ICH Harmonized Tripartite Guideline E14, 2005). Over 400 drugs have undergone TQT evaluation since 2005 (Stockbridge *et al.*, 2013). With experience on exposure–response modelling on data from many of these TQT studies, results obtained from concentration–QTc analysis are gaining as much acceptance as findings from by-time analysis of the placebo-adjusted change from baseline in the QTc interval after administration of a study drug (Stockbridge *et al.*, 2013). Given the high cost of performing a TQT study, which is usually done late in phase II of clinical drug development, there has been a move towards performing intensive ECG analysis in single ascending dose (SAD) studies (Valentin, 2010; Stockbridge *et al.*, 2013; Kothari *et al.*, 2015). While the sample size in these early-phase studies is statistically underpowered to demonstrate a placebo-adjusted change in mean QTc as described in the ICH E14 guidance, one could arguably obtain the same information by exposure–response modelling on early-phase data (Salvi *et al.*, 2010). A recent study, the International Consortium for Innovation and Quality in Pharmaceutical Development and the Cardiac Safety Research Consortium (IQ-CSRC) study, has shown that it is possible to detect drug-induced QTc prolongation with a limited number of healthy subjects using exposure–response modelling (Darpo *et al.*, 2015a).

A TQT study typically involves measurement of the QT interval in as many as 30 000 ECGs performed manually by trained readers or by a semi-automated process where annotations placed by a computer algorithm in each ECG are overread and corrected where necessary by an experienced cardiologist (Green *et al.*, 2012; ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2014). The ICH E14 Implementation Working Group has clarified in 2008 that fully automated analysis is not encouraged in TQT studies as they can yield misleading results in the presence of noisy ECG signals or when dealing with abnormal ECG rhythms, low amplitude P or T waves, or overlapping U waves (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2012). With improvement in performance of computer algorithms over the years, some recent studies have used a 'highly automated' process, where ECGs exceeding pre-specified cut-offs for confidence scores based on the quality of the ECG waveform that would limit the performance of the algorithm or those with outlier interval measurements are reviewed by a cardiologist, and ECG annotations are either retained if

acceptable or rejected if unacceptable; this hybrid process offers the advantage of higher throughput and less variability in QT measurements of the automated analysis with manual confirmation of automated annotations in those ECGs where they could have been inappropriately positioned (Kligfield *et al.*, 2010; Green *et al.*, 2012; ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2014).

Here we have used the highly automated ECG analysis approach using the FAT-QT system (version 1.3.0, AMPS LLC, New York, NY, USA), a relatively new computerized ECG analysis system, to detect moxifloxacin-induced QTc prolongation using ECGs from a TQT study. Using the highly automated ECG measurement values from the TQT study, we also performed a simulation study to assess the statistical power of a single-dose study with eight subjects each in the moxifloxacin and placebo arms, to further investigate whether intensive ECG recording and analysis in SAD studies in early-phase drug development can provide an estimate of the QT liability of a drug that is robust enough to match the results obtained from a TQT study.

Methods

Group sizes

This evaluation was performed using ECGs from the moxifloxacin and placebo treatment arms of a previously published TQT study (Natekar *et al.*, 2011). The study protocol was approved by the Institutional Review Board of the sponsor as well as the study site. All subjects signed an informed consent document prior to enrollment in the study.

Part 1 – the TQT study. In the original study, 124 subjects were randomized to receive a single oral dose of moxifloxacin (400 mg) or placebo ($n = 62$ in each group) in a crossover design. The number of subjects in the original study was relatively large, because the TQT study design requires these subjects to be subsequently randomized to three parallel arms to receive either multiple doses of placebo or two doses of the investigational drug, as the investigational drug had a relatively long half-life. Most crossover thorough QT studies now include only 35–50 subjects, due to improvement in variability in QT interval measurements. Assuming a within-subject variability of 8 ms for the placebo-adjusted change from baseline in QTcF with moxifloxacin, 46 subjects were required to demonstrate a significant QTcF prolongation with an upper bound of 10 ms with a one-sided α of 95% and power of

80% in a crossover study. We therefore randomly selected 46 subjects from the original 124 subjects such that there were an equal number (23) of males and females.

Part 2 – single-dose phase I simulation study. In part 2 of the study, we assessed the ability to detect moxifloxacin-induced QT prolongation in an early-phase study-like scenario using the highly automated ECG analysis approach. Early-phase studies typically have a smaller sample size with 6–9 subjects in each SAD arm and 6–10 subjects in the placebo arm. We, therefore, performed a simulation study using the ECG and pharmacokinetic data from the 46 subjects described in part 1. Each simulated dataset had eight subjects in the placebo group and eight different subjects in the moxifloxacin treatment group to mimic a phase I single-dose study with a parallel design. One thousand such datasets were simulated. Although we could have used ECG data from all 124 subjects for the simulation study, we performed the simulation study using data from only the 46 subjects from part 1 so as to enable comparison of results from part 2 (phase I simulation) with those from part 1 (a formal TQT study).

Randomization

Part 1 – the TQT study. In the original TQT study, 124 subjects were randomized such that 62 received moxifloxacin on day 1 and placebo on day 3 and the other 62 received placebo first followed by moxifloxacin. For part 1 of our study, we randomly selected 46 subjects from the original 124 such that 23 were men and 23 were women. Of the 46 subjects, 21 had been randomized to receive moxifloxacin on day 1 and placebo on day 3, and 25 received placebo first followed by moxifloxacin.

Part 2 – single-dose phase I simulation study. In part 2, from the complete dataset of 46 subjects, a subset of eight subjects in the moxifloxacin treatment group were selected by random sampling with replacement using SAS (SAS Institute, Cary, NC, USA). After excluding subjects who were selected in the moxifloxacin group, another eight subjects were selected by random sampling with replacement. Thus, from the original dataset of 46 subjects who received moxifloxacin and placebo in a crossover design, a parallel design dataset was simulated such that no subject appeared in the moxifloxacin as well as the placebo groups. One thousand such data subsets with eight subjects in the moxifloxacin arm and eight subjects in the placebo arm were created.

Blinding

In the original study, the treatments were open labelled, but the ECG analysis was by readers in a central ECG laboratory who were blinded to the identity of the subjects, treatment allocation and the sequence of ECG recordings.

In the highly automated ECG analysis process employed in the present study, the cardiologist who performed the manual overread of the ECGs was blinded to the identity of the subjects, treatment allocation and the sequence of ECG recordings.

Statistical methods

Analyses were performed using SAS version 9.2.

Part 1 – the TQT study. The mean and 90% confidence intervals (CIs) of placebo-adjusted change from baseline for QTcF ($\Delta\Delta\text{QTcF}$) at each of the post-dose timepoints were estimated using repeated measures analysis of covariance with baseline QTcF as a covariate, gender and timepoint as factors and subject as random effect (Florian *et al.*, 2011). Concentration–QTc modelling was performed using a linear mixed-effects model with $\Delta\Delta\text{QTcF}$ as the dependent variable and drug plasma concentration as a continuous covariate (Garnett *et al.*, 2008).

Part 2 – single-dose phase I simulation study. Geometric mean C_{max} values were obtained, and concentration–response modelling was performed on each of the 1000 datasets using a linear mixed-effects model with ΔQTcF as the dependent variable and plasma concentration of moxifloxacin and timepoint as a covariate, treatment (moxifloxacin or placebo) as a factor and a random intercept per subject. Model-based estimates for the mean value of $\Delta\Delta\text{QTcF}$ and its 90% confidence bounds at the corresponding geometric mean of the maximum concentration (C_{max}) of moxifloxacin for each of the 1000 datasets were also calculated.

Further methods

Part 1 – the TQT study. We randomly selected 46 subjects from the original 124 subjects such that there were an equal number of males and females (23 of each).

Each subject had ECG recording at seven timepoints (one pre-dose and 0.5, 1, 1.5, 2, 4 and 6 h post-dose timepoints) during each treatment period. Six replicate ECGs were recorded at each timepoint in the original study using a digital electrocardiograph (Eli 250, Mortara Instrument Inc., Milwaukee, WI, USA) with a sampling rate of 1000 Hz. The replicates were recorded 1 min apart starting approximately 3 min before the specified timepoint and ending approximately 3 min after the timepoint. These ECGs had been electronically transmitted to the central laboratory of Quintiles Cardiac Safety Services, in Mumbai, India, and converted into the FDA compliant HL7 XML files.

Moxifloxacin plasma concentration was determined at each of the six post-dose timepoints at which ECGs were recorded and blood samples were drawn after the ECG recording. Blood samples of 10 mL were collected at each timepoint by separate venepuncture in Vacutainer® (Becton Dickinson, Franklin Lakes, NJ, USA) tubes containing heparin for the determination of plasma concentrations of moxifloxacin. Pharmacokinetic parameters were calculated using non-compartmental analysis to obtain C_{max} and T_{max} of moxifloxacin after single p.o. dose of 400 mg moxifloxacin. Concentrations below the limit of quantitation (LOQ) were calculated as zero.

ECG analysis. Of the six replicate ECGs recorded at each timepoint, only the first three ECGs were included in this analysis because most TQT studies use triplicate ECGs. The ECGs were analysed by a highly automated approach using the FAT-QT software (version 1.3.0, 2015; AMPS LLC), which embeds the Bravo algorithm: for each of the 12 ECG leads, a

representative 'median' beat is mathematically computed, and then automated annotations are computed on the global median beat, that is, on the Euclidean vector magnitude of the 12 median beats. On the global median beats, fiducial markers are placed with criteria based on adaptive thresholds techniques combining first and second derivatives of the ECG signal (Badilini and Sarapa, 2006).

Subsequently, quality scores were generated by the FAT-QT system based on 10 quality parameters for the ECG waveforms that included high-frequency noise and low-frequency noise of waveforms, QRS regularity, T wave characteristics and outlier values for ECG parameters. All ECGs where the quality score exceeded a pre-specified cut-off, customized for the central ECG laboratory, were then reviewed by a single trained cardiologist who was allowed to accept or reject the automated annotations; no manual change in placement of the annotations was permitted.

Statistical analysis

For this part of the evaluation, the ECG data of all 46 subjects recorded on days 1 and 3 were used. The first three replicate ECGs recorded at the pre-dose timepoint on days 1 and 3 were used as the baseline for the post-dose ECGs on the corresponding days. QTcF was calculated using RR, QT intervals from each ECG, and the average QTcF value from the triplicate ECGs was considered as the QTc interval at the particular timepoint.

The mean and 90% confidence intervals of placebo-adjusted change from baseline for QTcF ($\Delta\Delta\text{QTcF}$) at each of the post-dose timepoints were estimated using repeated measures analysis of covariance (Florian *et al.*, 2011). Concentration–QTc modelling was performed using a linear mixed-effects model with $\Delta\Delta\text{QTcF}$ as the dependent variable and drug plasma concentration as a continuous covariate (Garnett *et al.*, 2008).

Part 2 – single-dose phase I simulation study. In part 2 of the study, we assessed the ability to detect moxifloxacin-induced QT prolongation in an early-phase study-like scenario by performing a simulation study using the original dataset of 46 subjects from the TQT study described in part 1.

From the original dataset of 46 subjects who received moxifloxacin and placebo in a crossover design, a parallel design dataset was simulated. A total of 1000 such data subsets with eight subjects in the moxifloxacin arm and eight subjects in the placebo arm were created.

In this part of the study, the ECG data from all six replicate ECGs recorded at each timepoint were included in order to reduce the within-subject and between-subject variability in ΔQTcF and the confidence limits of its estimate (Natekar *et al.*, 2011; Darpo *et al.*, 2015b). Thus, each data subset consisted of ECG data from 672 ECGs, with 336 ECGs from eight subjects in the moxifloxacin arm and 336 ECGs from eight subjects in the placebo arm.

Statistical analysis

For each of the 1000 datasets, concentration–response modelling was performed using a linear mixed-effects model with ΔQTcF as the dependent variable and plasma

concentration of moxifloxacin as a covariate. Model-based estimates for the mean value of $\Delta\Delta\text{QTcF}$ and its 90% confidence bounds at the geometric mean of the maximum concentration (C_{max}) of moxifloxacin for each dataset were also calculated.

The study was considered as positive (i.e. it could detect the moxifloxacin-induced QT prolongation) for a dataset if both of the following criteria were satisfied:

- the plasma concentration– $\Delta\Delta\text{QTcF}$ relationship showed a statistically significant ($P < 0.05$) positive slope, and
- the upper bound of the 90% two-sided confidence limit of the $\Delta\Delta\text{QTcF}$ at geometric mean C_{max} for the dataset exceeded 10 ms.

Datasets where the slope of the plasma concentration– $\Delta\Delta\text{QTcF}$ relationship was not statistically significant or the upper 90% confidence limit for mean $\Delta\Delta\text{QTcF}$ at moxifloxacin concentration corresponding to the mean C_{max} was < 10 ms were considered as 'false negative' studies. The overall statistical power of the entire study methodology was given by the percentage of datasets that showed a positive moxifloxacin effect using concentration– $\Delta\Delta\text{QTc}$ modelling.

Results

A total of 3857 ECGs recorded from 46 healthy adult subjects (23 women and 23 men) that included six replicates at each of seven timepoints (one pre-dose and six post-dose) were analysed. The annotations were initially placed by the automated ECG analysis algorithm, which also provided a quality score of each ECG. Based on the quality score, 491 (12.73%) ECGs were selected for cardiologist's review; 194 of these ECGs (5.02% of 3857 ECGs and 39.1% of ECGs with a poor quality score) were rejected after review by the cardiologist for incorrect placement of annotations.

Part 1 – the TQT study

Although six replicate ECGs were recorded at each timepoint, in part 1 of the study, only the first three replicates were considered at each timepoint as typical TQT studies are now performed with three or four replicate ECGs. A total of 1932 ECGs were included, of which 231 (12%) ECGs required cardiologist's review and 98 (5.1%) ECGs were rejected after review.

Assay sensitivity. The demonstration of moxifloxacin-induced QT prolongation by the highly automated approach for QTcF is shown in Figure 1. There was a statistically significant prolongation of placebo-adjusted change from baseline in the QTcF interval ($\Delta\Delta\text{QTcF}$) with moxifloxacin at all six post-dose timepoints (Figure 1). Maximum $\Delta\Delta\text{QTcF}$ was seen at 4 h post-dose (16.7 ms). The lower bound of the 90% two-sided confidence limits exceeded 5 ms, and the upper bound exceeded 10 ms at the 1, 1.5, 2, 4 and 6 h post-dose timepoints. Treatment \times period interaction was not statistically significant ($P = 0.23$).

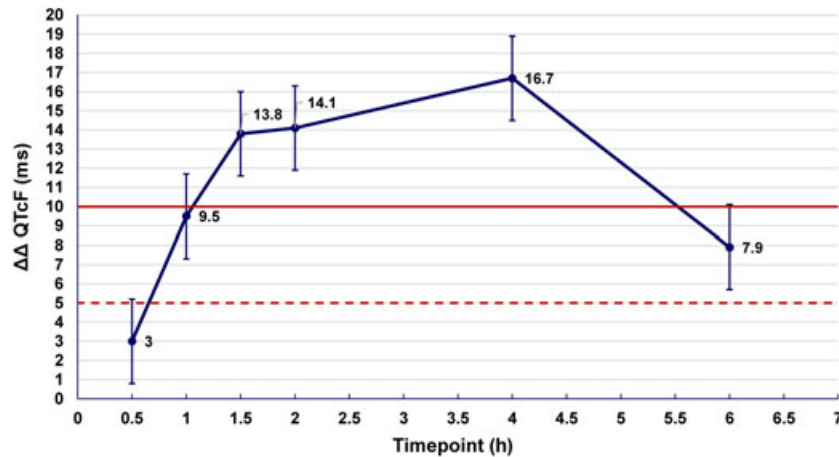


Figure 1

Mean placebo-adjusted change from baseline in QTcF ($\Delta\Delta\text{QTcF}$) and its 90% two-sided confidence limits by a highly automated analysis approach using the Bravo algorithm in 46 healthy subjects after a single oral dose of 400 mg moxifloxacin. The important thresholds for $\Delta\Delta\text{QTcF}$ and its 90% confidence limits are shown at 5 ms (dotted line) and 10 ms (bold line).

Concentration- $\Delta\Delta\text{QTcF}$ evaluation. PK-PD evaluation was performed using a linear mixed-effects model with $\Delta\Delta\text{QTcF}$ as the dependent variable, drug plasma concentration as a continuous covariate and intercept as a random effect using data from 46 healthy subjects after a single 400 mg oral dose of moxifloxacin. The slope of the concentration- $\Delta\Delta\text{QTcF}$ relationship was $4.12 \text{ ms}\cdot\mu\text{g}^{-1}\cdot\text{mL}^{-1}$ (SE = 0.61; $P < 0.001$) (Figure 2). Using this model, the estimated mean $\Delta\Delta\text{QTcF}$ at the geometric mean concentration of moxifloxacin ($2.9478 \mu\text{g}\cdot\text{mL}^{-1}$) was 13.4 ms (90% two-sided confidence limits 11.3, 15.4 ms).

Part 2 – single-dose phase I simulation study

In part 2 of the study, a total of 1000 datasets, each with eight subjects on moxifloxacin and eight on placebo, were created from the TQT ECG data by bootstrapping so as to simulate early-phase studies; all six replicate ECGs recorded at each timepoint were included in the simulation study. The mean of the observed peak plasma concentration of moxifloxacin (C_{max}) in each of the 1000 datasets ranged from 2.22 to $3.70 \mu\text{g}\cdot\text{mL}^{-1}$ (Figure 3) with the median value being $2.96 \mu\text{g}\cdot\text{mL}^{-1}$. A linear mixed-effects model similar to that used in part 1 of the study was used to determine the slope of the relationship between

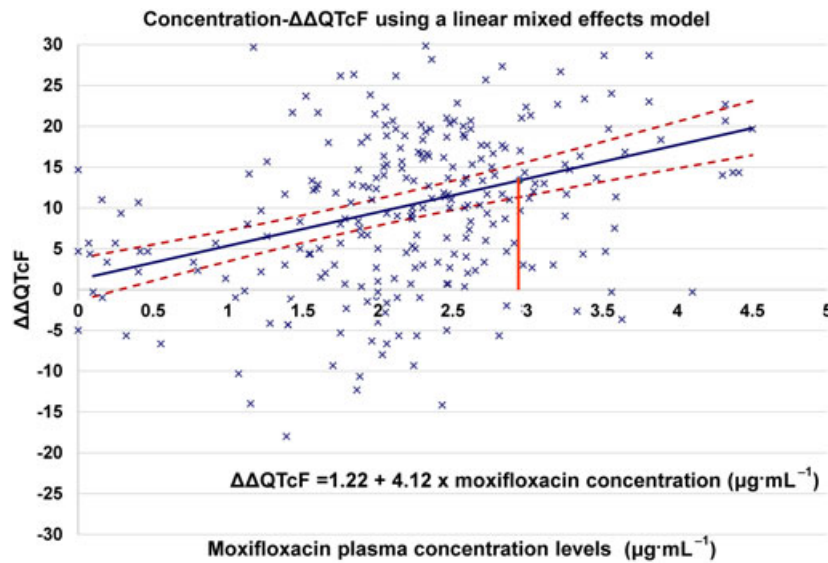


Figure 2

Moxifloxacin plasma concentration versus placebo-adjusted change from baseline in QTcF ($\Delta\Delta\text{QTcF}$) in 46 healthy subjects after administration of 400 mg moxifloxacin. The mean predicted $\Delta\Delta\text{QTcF}$ at various concentrations of moxifloxacin is shown as a solid line, and its two-sided 90% confidence limits are shown by the broken lines. At the mean geometric C_{max} value of $2.9478 \mu\text{g}\cdot\text{mL}^{-1}$ (indicated by vertical line), the model-based value of mean $\Delta\Delta\text{QTcF}$ was 13.4 ms (90% two-sided confidence limits 11.3, 15.4 ms).

the QTc effect and drug plasma levels in each of the 1000 datasets; treatment was used as a fixed effect as has been carried out in several previous studies (Badilini and Sarapa, 2006; Florian *et al.*, 2011). The concentration– $\Delta\Delta\text{QTcF}$ relationship for each of the 1000 datasets, its *P*-value and the model-derived estimate of the $\Delta\Delta\text{QTcF}$ at mean C_{max} of moxifloxacin for each dataset were calculated. The mean slope of the concentration– $\Delta\Delta\text{QTcF}$ relationship for the 1000 datasets was $5.9 \text{ ms}\cdot\mu\text{g}^{-1}\cdot\text{mL}^{-1}$ (90% confidence interval 2.9, 9.3), while the mean estimated $\Delta\Delta\text{QTcF}$ at mean C_{max} was 16.5 ms (90% confidence interval 9.5, 24.1 ms); the upper bound of the 90% confidence interval for $\Delta\Delta\text{QTcF}$ at mean C_{max} was 23.9 ms (mean; 90% confidence interval 14.9, 34.1 ms).

In 895 of the 1000 datasets, both criteria for a positive study were met, and moxifloxacin-induced QT prolongation could be demonstrated. Of the remaining 105 datasets, in 101 datasets, the estimated 90% two-sided upper confidence bound for moxifloxacin was >10 ms but the *P*-value for the slope was ≥ 0.05 , and in four datasets, the 90% two-sided upper confidence bound for $\Delta\Delta\text{QTcF}$ was <10 ms and the *P*-value for the slope was ≥ 0.05 (Figures 4, 5).

Discussion

This study used a highly automated QT/QTc measurement approach using the Bravo algorithm in the FAT-QT system, which measures the QT interval on the global median beat derived from all 12 ECG leads. In part 1 of the analysis, the highly automated approach was able to demonstrate assay sensitivity with a statistically significant placebo-adjusted prolongation of QTcF with moxifloxacin. The ICH has stated that assay sensitivity with moxifloxacin is demonstrated if the 90% two-sided lower confidence bound of mean $\Delta\Delta\text{QTcF}$ exceeds >5 ms (ICH Harmonized Tripartite Guideline E14 Questions and Answers, 2014). This criterion was satisfied at five post-dose timepoints in our study with 46 subjects and triplicate ECGs at each timepoint, with maximum mean $\Delta\Delta\text{QTcF}$ of 16.7 ms at 4 h post-dose timepoint; by concentration–effect modelling, the mean $\Delta\Delta\text{QTcF}$ at mean C_{max} was 13.4 ms (90% two-sided confidence limits 11.3, 15.4 ms). These observations are consistent with those reported by Florian *et al.*

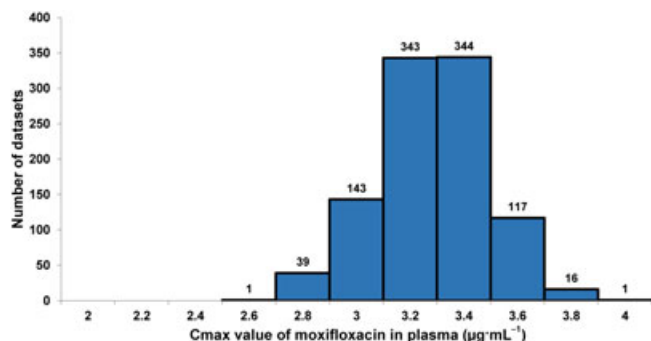


Figure 3

Distribution of peak plasma concentration (geometric mean C_{max}) values for moxifloxacin in the 1000 datasets.

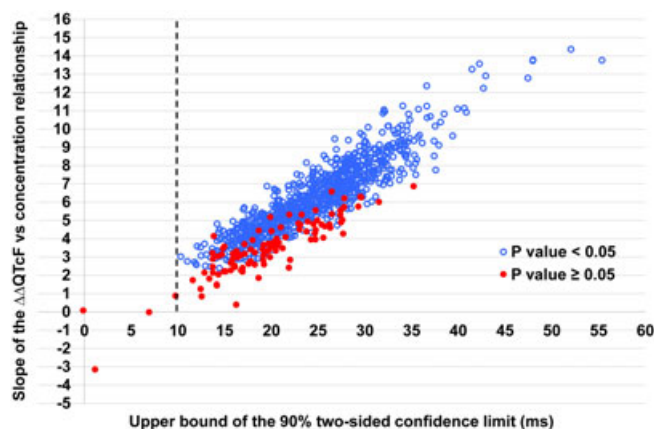


Figure 4

Distribution of the slope of the placebo-adjusted change from baseline for QTcF ($\Delta\Delta\text{QTcF}$) versus concentration relationship for moxifloxacin and the upper confidence limit for $\Delta\Delta\text{QTcF}$ at mean C_{max} for the 1000 simulated datasets. Only four datasets had an upper confidence limit for $\Delta\Delta\text{QTcF}$ that was less than 10 ms (seen as falling to the left of the vertical line). For 105 datasets, the slope of the $\Delta\Delta\text{QTcF}$ versus concentration relationship for moxifloxacin did not reach statistical significance ($P \geq 0.05$).

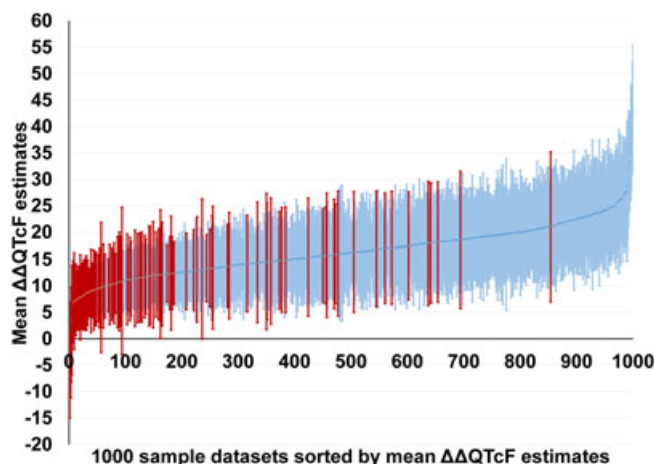


Figure 5

Estimated mean $\Delta\Delta\text{QTcF}$ and its two-sided 90% confidence limits at mean C_{max} of moxifloxacin for each of the 1000 datasets from the phase I simulation study. Data are sorted in ascending order of the mean $\Delta\Delta\text{QTcF}$. Results from 895 datasets with a positive result are shown in blue (lighter bars), while results from the remaining 105 datasets that could not demonstrate a drug-induced $\Delta\Delta\text{QTcF}$ prolongation are shown in red (darker bars).

from an analysis of 20 TQT studies. They found that the mean $\Delta\Delta\text{QTcF}$ by by-time analysis ranged from 7.7 to 16.7 ms and by concentration–effect modelling ranged from 6.4 to 12.9 ms. For reasons not clear, they too observed lower estimate of mean $\Delta\Delta\text{QTcF}$ by the concentration–effect model than with the by-time analysis (Florian *et al.*, 2011). The slope of the concentration response in our study ($4.12 \text{ ms}\cdot\mu\text{g}^{-1}\cdot\text{mL}^{-1}$ of moxifloxacin) was also within the range of $1.6\text{--}4.8 \text{ ms}\cdot\mu\text{g}^{-1}\cdot\text{mL}^{-1}$ reported by Florian *et al.* (2011).

In the last few years, there has been a move to investigate if drug-induced QT prolongation of the extent defined in the ICH E14 guidance can also be detected by intensive ECG recordings in SAD studies during early-phase development (Salvi *et al.*, 2010). However, evidence to support this concept is still emerging. Using a highly automated approach performed with the COMPAS system (iCardiac Technologies Inc., Rochester, NY, USA), Darpo *et al.* (the IQ-CSRC study) could demonstrate moxifloxacin-induced QTcF prolongation using 10 replicate ECGs recorded in nine subjects receiving moxifloxacin and six receiving placebo in a parallel study (Darpo *et al.*, 2015b). In another simulation study, Ferber *et al.* used subsampling from three conventional TQT studies to simulate a single-dose early-phase study with small sample sizes (Ferber *et al.*, 2015). Using a concentration–effect modelling similar to that in the IQ-CSRC study, they found that to detect a drug-induced QTc effect of 12–14 ms with good consistency required a sample size of nine or more subjects on drug and six on placebo.

In part 2 of the present study, we used the bootstrapping technique to create 1000 data subsets by repeated subsampling of data from the TQT study to simulate a smaller early-phase clinical trial with intensive ECG assessment. Concentration–effect modelling was used to estimate moxifloxacin-induced QT prolongation in 1000 datasets created from the TQT study data. With eight subjects in the placebo group and eight subjects in the moxifloxacin group, we could detect a significant drug-induced QTc prolongation in 895 of the 1000 datasets, indicating a statistical power of around 89%. If we consider studies where the upper bound of the 90% CI of the estimated $\Delta\Delta\text{QTcF}$ at mean C_{max} exceeded 10 ms but the P -value of the slope of the concentration–effect model was ≥ 0.05 as having an indeterminate outcome, a further 101 studies fell in this category.

In a simulation study using data from three TQT studies, Ferber *et al.* found that if six subjects were included in the active treatment group and six in the placebo group, only 68% of samples had a statistically significant positive slope of the plasma concentration/ $\Delta\Delta$ QTcF relationship (Darpo *et al.*, 2015b; Ferber *et al.*, 2015). This increased to 76% with 9 subjects and 82% with 12 subjects. Our results are similar and show that with eight subjects on active treatment and eight subjects on placebo, a significant QTcF prolongation can be detected with $>80\%$ power.

Before extrapolating our results to a SAD study, one should note that while only a single therapeutic dose of moxifloxacin was used in our study, in a typical SAD study, there would be several dose groups for the active compound using ascending doses. It is therefore likely that there may be one or more dose groups receiving smaller doses and some receiving larger (supra-therapeutic) doses. This would considerably enhance the power of the study to detect the QT effect using concentration–effect modelling by providing more data points at higher concentrations, thereby providing better estimates of the slope and mean $\Delta\Delta\text{QTc}$ and thereby lowering the false negative rate (Kothari *et al.*, 2015). Including subjects from different dose groups would also increase the overall sample size and thus reduce the confidence limits for the estimated $\Delta\Delta\text{QTc}$, thus reducing the false positive rate (Kothari *et al.*, 2015).

Most previous studies with an early-phase design have had 10 replicate ECGs recorded at each timepoint in each subject in an attempt to reduce within-subject and between-subject variability (Sparve *et al.*, 2014; Darpo *et al.*, 2015a).

This is because the between-subject and within-subject variability decreases with increasing replicates (Beasley *et al.*, 2005; Natekar *et al.*, 2011), but the optimum number of ECGs needed in early-phase studies needs to be further investigated. The present study suggests that recording six replicate ECGs may be adequate if consistency in QT interval measurement can be ensured.

Our study has some limitations. Firstly, we only studied the ability of a single-dose phase I-like study design to detect a significant QTc prolongation with moxifloxacin, a drug with a known QT liability. It would also be important to ensure that this study design also had the ability to exclude the absence of a QT liability with a drug proven to be negative in a TQT study (i.e. to exclude false positive results). Secondly, the half-life of moxifloxacin is 12 h, and therefore, the wash-out period between the two treatments in the crossover study should have been a minimum of 60 h (five half-lives) while it was 48 h in the present study. However, when treatment \times period interaction was studied, this was not statistically significant, suggesting the absence of a carry-over effect.

In conclusion, the present study shows that moxifloxacin-induced QTc prolongation could be demonstrated in a thorough QT study, using a highly automated ECG analysis method and the Bravo algorithm. More importantly, we also studied the performance of the highly automated approach in detecting QTc changes in simulated early-phase clinical trials, by subsampling subjects to create 1000 datasets from the TQT study data. Using concentration–effect analysis, we found that QTcF prolongation could be reliably detected with a small sample size (eight subjects on active drug and eight on placebo) with a power of more than 80%. Our study therefore adds to the growing body of data supporting intensive ECG collection and analysis in early-phase studies that, when interpreted along with preclinical data from the proposed Comprehensive *in vitro* Pro-arrhythmia Assessment, may provide a good estimate of the QT liability of a new drug (Cavero and Holzgrefe, 2014).

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Author contributions

G.K.P., D.K., and A.D. performed the research. G.K.P., D.K., and S.K. designed the research study. F.B. contributed essential tools. G.K.P., D.K., and P.K. analysed the data. G.K.P., D.K., and P.K. wrote the paper. G.K.P., D.K., F.B., P.K., S.K. drafted the manuscript.

Conflict of interest

G.K.P., P.K. and S.K. are employees of Quintiles Cardiac Safety Services. F.B. is a member of AMPS, a limited liability

company from New York that owns the software used for the ECG analysis of the study. D.K. and A.D. are consultants to Quintiles Cardiac Safety Services.

Declaration of transparency and scientific rigour

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research recommended by funding agencies, publishers and other organisations engaged with supporting research.

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