

The Effect of Moxifloxacin on QTc and Implications for the Design of Thorough QT Studies

DM Bloomfield¹, JT Kost², K Ghosh³, D Hreniuk¹, LA Hickey³, MJ Guitierrez⁴, K Gottesdiener¹ and JA Wagner¹

A number of issues have remained unanswered in the design of “thorough QT” (TQT) studies. In this randomized, placebo-controlled, two-period crossover study in 20 healthy subjects, replicate electrocardiograms (ECGs) were recorded on a digital 12-lead Holter recorder, extracted in a core ECG laboratory, and interpreted manually by a cardiologist. The observed within-subject variability was slightly greater when time-matched baselines were employed than when predose baselines were employed, whereas the magnitude of the increase in QTc was similar for both. Moxifloxacin 400 mg was associated with an observed 7.5–12.5 ms increase in the mean placebo- and baseline-corrected QTc interval. A PK-QTc model estimated a 3.9 ms increase in the QTc interval for every 1,000 ng/ml increase in moxifloxacin concentration. The QTc increases associated with moxifloxacin support the appropriateness of its use as a positive control in TQT studies. This crossover study failed to justify the use of time-matched baselines rather than the less resource-intensive predose definition of baseline.

The potential for some drugs to prolong the QT interval has generated intense interest and concern.^{1,2} Prolongation of the QT interval associated with torsade de pointes has been an important reason for the withdrawal or restriction of the use of several drugs.^{3–9}

A number of characteristics of cardiac repolarization contribute to the considerable difficulty of quantifying the effect of a drug on the QT/QTc interval. Intrinsic variability caused by the dependence of repolarization on heart rate and the status of the autonomic nervous system and gender have been documented as important sources of variability.^{10–20} Also, the end of the T wave is difficult to detect with precision. Although a number of different computerized automated methods for measuring the end of the T wave have been described, each has its own biases and limitations, with no single method having received general acceptance.²¹ In addition, there is no consensus about which lead should be used nor on how the measurement should be handled in the presence of a U wave.

The International Conference on Harmonisation E14 clinical guidance document attempted to achieve consensus in generating guidelines for evaluating the effect of a drug on the QTc interval.²²

This pilot study provides an assessment of the design and implementation of thorough QT (TQT) studies and focuses on two issues in depth: (i) selecting an optimal baseline (i.e., either predose baseline or time-matched baseline) and (ii) the effect of varying the number of replicates on within-subject variability.

RESULTS

Twenty healthy volunteers (10 men and 10 women) were enrolled in this study, and their mean age (SD) was 33.6 (ref. 8) years. The geometric mean for moxifloxacin peak plasma concentration (C_{\max}) was 2,236.8 ng/ml, and the largest geometric mean plasma concentration occurred at 4 h after the dose, while the median T_{\max} was at 2.0 h (Table 1).

Effect of replicates on variance

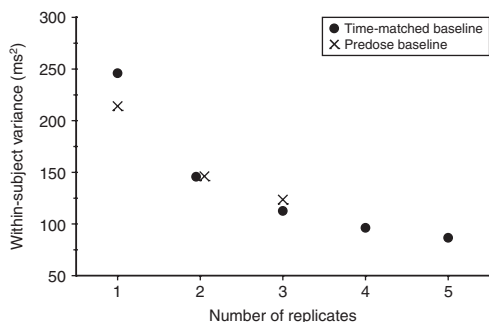
Because the comparison with respect to treatment outcomes in a crossover design is within-subject, the assessment of the effect of varying the number of electrocardiogram (ECG) replicates was carried out by focusing attention on the within-subject variance component. Figure 1 displays the observed mean value of the within-subject variance estimates relative to the number of ECG

¹Department of Clinical Pharmacology, Merck Research Laboratories, Merck and Company, Rahway, New Jersey, USA; ²Department of Biostatistics, Merck Research Laboratories, Merck and Company, Upper Gwynedd, Pennsylvania, USA; ³Visitation, Wayne, Pennsylvania, USA; ⁴Comprehensive Phase One, Fort Lauderdale, Florida, USA. Correspondence: DM Bloomfield (daniel_bloomfield@merck.com)

Table 1 Summary statistics for moxifloxacin pharmacokinetics

Pharmacokinetic end point	Time (h)	Geometric mean	95% CI	
C_{max} (ng/ml)	—	2,236.8	(2001.4, 2499.9)	
Plasma concentration (ng/ml)	1	1,784.2	(1527.5, 2084.0)	
	2	1,998.9	(1711.3, 2334.8)	
	4	2,012.4	(1722.9, 2350.6)	
	6	1,619.8	(1386.3, 1891.5)	
		Mean	Median	Minimum, maximum
T_{max} (h)	—	2.3	2.0	1.0, 6.0

CI, confidence interval.

**Figure 1** Within-subject variance according to the number of replicate electrocardiograms used at each time-point.

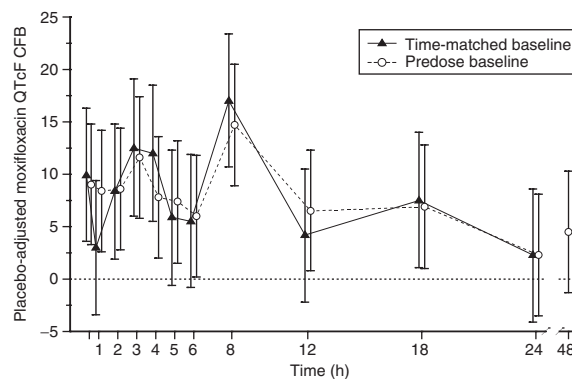
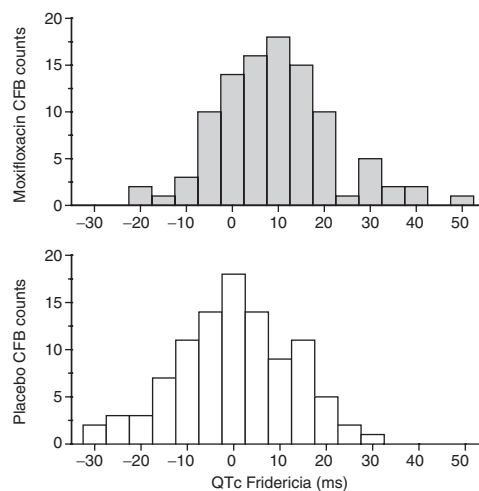
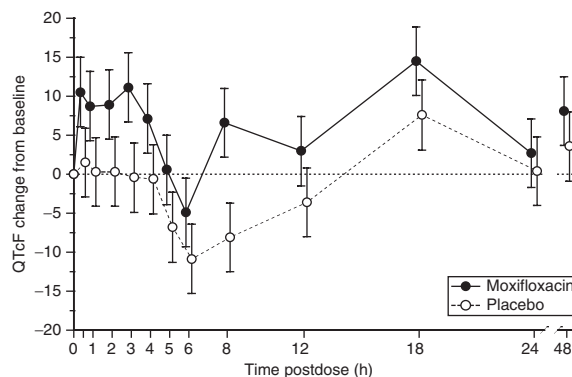
replicates and definition of baseline. As expected, the added benefit in going from one replicate to two replicates is much greater than the benefit realized in going from four replicates to five replicates. Using the predose definition of baseline (PDB), the within-subject variances of QTcF change from baseline (CFB) dropped from 214.1 (ms^2) when only one ECG was used, to 146.2 (ms^2) when two replicates were used, and to 123.6 (ms^2) when three replicates were used.

Definition of baseline

In order to assess the optimum method for determining “the change in QTc interval from baseline,” two definitions of baseline measurements were compared: PDB values and time-matched baseline (TMB) values. As shown in **Figure 1**, the observed mean (averaging over all possible subsets within a number of replicates) within-subject variance was slightly higher for the TMB definition when just one replicate was considered, and was quite comparable when two or three replicates were considered. Examination of the within-subject variances for QTc arising from the formal comparison of moxifloxacin vs. placebo (three replicates used) resulted in an observed within-subject variance for time-matched baseline values (148.5 ms^2) that was slightly greater than when predose baseline values were used (123.3 ms^2).

Effect of moxifloxacin on the change in QTc interval from baseline

The effect of moxifloxacin on the QTc CFB (using the PDB) is presented in **Figures 2–4** and in **Table 2**. **Figure 2** presents the

**Figure 2** Placebo-adjusted moxifloxacin QTcF CFB comparing the use of time-matched vs. predose baseline values. CFB, change from baseline.**Figure 3** QTc CFB (predose baseline) histograms: moxifloxacin vs. placebo (0.5, 1, 2, 3, and 4 h after the dose). CFB, change from baseline.**Figure 4** Change in QTc from baseline on account of treatment (moxifloxacin vs. placebo using predose baseline values).

placebo-adjusted moxifloxacin QTcF CFB (comparing the use of time-matched vs. predose baseline values). There was a rapid increase in QTc corresponding to peak moxifloxacin concentrations in the first 4 h after the dose, followed by a second increase in QTc at 8 h. The magnitudes of the increase in QTc were similar when TMBs were used as when the usual PDBs were used.

The distributions of the responses to moxifloxacin and placebo over the first 4 h are illustrated in **Figure 3**, with the distribution

Table 2 Placebo-adjusted moxifloxacin QTc FCF means and 90% CIs by time-point

Baseline adjustment method	Time	Mean difference ^a	90% CI for mean difference ^a	Nominal P value ^b
Predose	0.5	9.0	(3.3, 14.8)	0.0103
	1.0	8.4	(2.6, 14.2)	0.0169
	2.0	8.6	(2.8, 14.4)	0.0145
	3.0	11.6	(5.8, 17.4)	0.0011
	4.0	7.8	(2.0, 13.6)	0.0274
	5.0	7.4	(1.5, 13.2)	0.0387
	6.0	6.0	(0.2, 11.8)	0.0900
	8.0	14.7	(8.9, 20.5)	<0.0001
	12.0	6.5	(0.8, 12.3)	0.0631
	18.0	6.9	(1.0, 12.8)	0.0529
Time-matched	24.0	2.3	(-3.5, 8.1)	0.5143
	48.0	4.5	(-1.3, 10.3)	0.1975
	0.5	9.9	(3.6, 16.3)	0.0105
	1.0	3.0	(-3.4, 9.4)	0.4372
	2.0	8.4	(1.9, 14.8)	0.0324
	3.0	12.5	(6.0, 19.1)	0.0017
	4.0	12.0	(5.5, 18.5)	0.0026
	5.0	5.9	(-0.6, 12.3)	0.1347
	6.0	5.5	(-0.8, 11.9)	0.1518
	8.0	17.0	(10.7, 23.4)	<0.0001
12.0	4.2	(-2.2, 10.5)	0.2785	
18.0	7.5	(1.1, 14.0)	0.0550	
24.0	2.3	(-4.1, 8.6)	0.5524	

CFB, change from baseline; CI, confidence interval.

^aBased on least-squares means arising from a mixed effects linear model with treatment, time, period, treatment sequence, and treatment-by-time interaction as fixed effects. ^bNot adjusted for multiplicity.

of the moxifloxacin responses shifted to the right (i.e., higher QTc CFB) as compared to placebo. The overall spread of the QTc CFB responses appears similar between moxifloxacin and placebo, as assessed through the categorical representation in the histograms in **Figure 3**. **Table 3** contains counts of the maximum QTc CFB response over the 48-h period after the dose, and provides further evidence that moxifloxacin prolongs the QTc interval. None of the subjects had absolute QTc intervals >450 ms.

Figure 4 illustrates the change in QTc from baseline (PDB) brought about by treatment. Moxifloxacin appears to be associated with a ~10 ms increase in QTc CFB, which becomes evident from the first measurement at 30 min and persists over the first 4 h after the dose. In contrast, the mean change in QTc interval associated with placebo over this same 4-h period remained close to zero. Interestingly, there is a transient decrease in the change in QTc from baseline at 5 and 6 h after the dose, similar in both the moxifloxacin and placebo treatment groups. This drop in QTc was associated with small but consistent increases in heart rate that occurred following the meal (which was given to all subjects 4 h after the dose). A secondary increase in QTc appears after 6 h, persisting through 24 h.

Table 3 Categorical analysis of maximum CFB QTc FCF (ms) values by treatment

Treatment	Count (%)		
	QTc CFB ≤ 30	30 < QTc CFB ≤ 60	60 < QTc CFB
Placebo (n = 20)	19 (95.0)	1 (5.0)	0 (0.0)
Moxifloxacin (n = 20)	12 (60.0)	8 (40.0)	0 (0.0)

Largest QTc CFB over 48 h using predose baseline and average of three replicate measurements.

CFB, change from baseline.

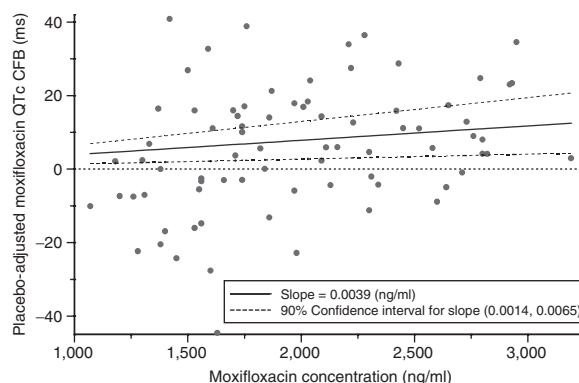


Figure 5 Predicted placebo-corrected QTc CFB mean values and upper 95% pointwise confidence limits by plasma concentration. Two observations with moxifloxacin concentrations <1,000 were excluded. The confidence interval is not adjusted for multiplicity. CFB, change from baseline.

A statistically significant period effect was observed in this study in the PDB analysis ($P < 0.0001$) but not in the TMB analysis ($P = 0.1066$); however, treatment effect was estimated after adjusting for period effects.

PK/QTc analysis

The concentration–response relationship was assessed by comparing the concentration of moxifloxacin (range: 1,000–3,200 ng/ml) with the placebo-adjusted QTc CFB (**Figure 5**) using a linear mixed-effects model. The estimated slope was 0.0039 ms/(ng/ml), thereby suggesting that for every 1,000 ng/ml increase in moxifloxacin concentration within this range, there is an increase of ~3.9 ms in the QTc CFB.

Alternative models (i.e., nonlinear) were also considered for characterizing the relationship between placebo-adjusted QTc and moxifloxacin concentration. However, in the absence of some obvious functional form in the data (**Figure 5**), a linear relationship was chosen as the most straightforward means of demonstrating the increase of placebo-adjusted QTc with rising moxifloxacin concentrations.

DISCUSSION

This pilot study was conducted to gain experience in the design and implementation of TQT studies for new drugs in development. Using a placebo-controlled crossover design with TMB on day –1 of each period, ECGs were collected on a 12-lead digital Holter and analyzed at a centralized ECG laboratory using state-of-the-art techniques for manually annotating the ECG and measuring the ECG intervals. This study used the design

and procedures of a typical TQT study and was sufficiently large to demonstrate a statistically significant increase in QTc interval associated with moxifloxacin treatment. The associated estimates of the within-subject variance for QTc CFB, using varying numbers of replicates, provide useful information for the design of future thorough QTc studies.

Methodology and analysis: The measurement of QTc interval is associated with a large amount of variability. This study utilized three approaches for reducing variability in the QTc interval. First, replicate ECGs were extracted from each time-point and then averaged, thus increasing the precision of the estimate of the QTc interval at that time-point. Second, an environment was created with the intention of minimizing intrinsic physiologic variability as well as external variability due to noise and motion artifact. In order to minimize the variability in autonomic tone within and between treatment periods, subjects were kept supine and resting quietly for 10 min prior to, and 5 min after, each time-point. Because all replicate ECGs were restricted to within 5 min of the nominal time-point, this 10-min rest period ensured that subjects were resting quietly for at least 5 min prior to the extraction of any ECG included in the analysis. Third, a core laboratory was used for measuring ECG intervals using a standardized and validated methodology. All ECGs from a given subject were analyzed by a single cardiologist so as to eliminate inter-reader variability within a subject.

Variance: The within-subject variance of 123.3 ms^2 for QTc CFB observed in this study (three replicate ECGs, PDB) appears approximately similar to what has been reported in other studies.^{23–25} However, it is important to emphasize that these estimates of variance are highly dependent on the method of analyzing the QTc interval at the central laboratory, as well as on the statistical model used for analyzing the data. In this study, a technician manually annotated the QTc intervals from all the ECGs and analyzed the ECG waveform without the help of any computer-generated annotations. The ECGs with their annotations were then reviewed by a cardiologist.

Definition of baseline: In the E14 guidance document, the primary end point for a TQT study is referred to as “the change in QTc interval from baseline,” although the document does not specify exactly which baseline measurements should be used. Mention is made of TMB measurements in order to reduce the variability caused by diurnal variation in QT/QTc intervals. In this study, placebo-controlled change in QTc from baseline was evaluated comparing two definitions of baseline: TMB (collected over a 24-h period on day –1 of each period) and PDB (collected 1 h before the dose was administered).

Data from this study did not support the use of TMB values for use in future TQT studies that employ a placebo-controlled crossover design. First, the estimated within-subject variability was slightly greater when TMB values were used in the model than when PDB values were used. From the viewpoint of improving the precision of the estimates of treatment difference, TMB did not seem to justify the added cost. Second, the magnitude of the effect of moxifloxacin on the change in QTc from baseline was similar whether TMB values were used

or PDB values were used. Third, any theoretical concern about diurnal variation in the QTc interval is accounted for in a placebo-controlled crossover study. This is because the change in QTc interval (from predose baseline) on placebo is calculated for each time-point and then subtracted from the change in QTc at the corresponding time-point on active treatment within each subject. Furthermore, the process of collecting TMB values requires additional cost, time, and overheads, when compared with collecting only PDB values. On the basis of these findings, the primary assessment of moxifloxacin for use as a positive control was carried out using the PDB values.

In a parallel group study design in which subjects receive either the active drug or the placebo, the use of TMB may be justified in order to improve precision in the estimates of treatment difference, in the presence of a true diurnal variation. For crossover designs (such as the one employed in this study) with complete data, the estimated treatment difference is unbiased even in the presence of diurnal variation because this estimate is derived through within-subject treatment differences.

Effect of moxifloxacin: This study clearly demonstrated that moxifloxacin is associated with an increase in QTc interval. Between 1 and 4 h after a 400 mg dose of moxifloxacin, the QTc interval increased ~10 ms above the PDB value. This effect of moxifloxacin was partially negated by a similar but smaller increase in QTc interval following treatment with placebo. For the first 4 h after the dose, the maximum mean of the placebo-corrected change in QTc from baseline was 12.1 ms and occurred at 3 h after the dose. Other studies have demonstrated similar effects of a single dose of moxifloxacin (6–12 ms), although each study utilized slightly different methodologies, thereby making it difficult to attempt a formal comparison of the results.^{23–25} The PK-QTc analysis pointed to a linear relationship between the plasma concentration of moxifloxacin and the increase in QTc interval from baseline, suggesting a 3.9 ms increase in QTc for every 1,000 ng/ml increase in plasma moxifloxacin concentration.²³

The early increase in QTc interval reversed almost back to baseline values 5–6 h after the dose, and then increased again and remained elevated above the predose baseline value for up to 48 h after the dose. The drop in QTc interval at 5–6 h after the dose relative to the QTc interval in the first 4 h was likely related to changes in the study environment. First, all the subjects were given a meal at 4 h after the dose. Second, because the number of procedures was greater during the first 4 h, the subjects were required to remain supine and resting. After the meal, the subjects were able to ambulate more because the procedures were farther apart. In the 5–6 h postdose time frame, there was an increase in heart rate in both the moxifloxacin- and placebo-treated groups, which was probably related to the meal and the increased activity. Conceivably, the drop in the QTc interval during this time frame may be an artifact of the heart rate correction formula for QT interval.

Implications for the design of a thorough QTc study: These data support the use of moxifloxacin as a positive control in thorough QTc studies. In formal terms, a conclusion that moxifloxacin increases QTc interval at a particular time-point was to be reached if the lower limit of the 90% confidence interval for

the mean CFB difference (moxifloxacin – placebo) in QTc was >0 at that time-point. This criterion was met over all nominal time-points examined through 18 h.

The observed magnitude of the maximum increase in QTc CFB after moxifloxacin was ~10 ms, and as high as ~15 ms when the placebo-corrected change was calculated. Although this increase in QTc CFB is greater than the guidance in the International Conference on Harmonisation E14 (which indicates that the positive control should demonstrate the ability to detect changes as small as ~5 ms), smaller increases in QTc CFB were identified at lower moxifloxacin concentrations. Quantitatively, the analysis of the PK-QTc relationship was consistent with an increasing monotonic relationship between moxifloxacin concentration and the QTc CFB. Given the fact that it was possible to identify smaller increases in QTc CFB at lower moxifloxacin concentrations, the methodology used in this study, if appropriately powered, would have been sufficient to identify changes in QTc CFB as low as 5 ms.

The sample size necessary for a TQT study depends on the assumed within-subject variance based on the number of replicate ECGs to be used and the maximum allowable placebo-adjusted true mean CFB increase in QTc (10 ms according to the E14 guidance). In addition, the sample size requirements for a TQT study depend on the assumed true effect of the study drug. On the basis of the QTc changes observed with placebo in this and other studies, we suggest that sample size calculations should be based on the assumption that a drug candidate causes a true increase in QTc of up to 2.5 ms, particularly if that drug has a preclinical signal suggestive of an effect on ventricular repolarization.

In conclusion, the data from this study show that, for the purpose of conducting a placebo-controlled crossover TQT study, the use of time-matched baselines, as compared to the use of the less resource-intensive standard PDB, would not be justified. Using the study design and procedures of a typical thorough QTc study, it was demonstrated that moxifloxacin is associated with a statistically significant increase in the placebo-corrected change in QTc from predose baseline and therefore could be used as positive control to establish assay sensitivity.

METHODS

Study design. This single-blind, randomized, placebo-controlled, two-period crossover study in 20 healthy subjects was conducted for the purpose of evaluating the effect of moxifloxacin on the QTc interval, and also to estimate the variability of the CFB QTc interval. Each period consisted of a day of TMB measurements (day –1) and a treatment day (day 1). No treatment was administered on day –1, and subjects were randomly assigned to receive either moxifloxacin 400 mg (Avelox; Bayer Pharmaceuticals, West Haven, CT) or grossly matching placebo on day 1. There was a 10-day washout interval, after which the subjects crossed over to the alternate treatment. During both treatment periods, moxifloxacin/placebo was administered to subjects by a clinical research unit staff member who was unblinded to the specifics of the treatment but who was otherwise not involved in the conduct of the study. Subjects were dosed at the same time of day during each period. Standardized meals were provided 4 and 8 h after the dose and a snack was provided 12 h after the dose. For each of the treatment periods, the subjects were admitted to the clinical research center the evening before day –1 and were discharged from the clinical research unit after

completing the 24-h postdose procedures on day 1. For subjects who received moxifloxacin, blood samples were taken before the dose and at 1, 2, 4, and 6 h after the dose on day 1 of each period.

ECG recording. The ECGs were recorded using Mortara H12+ Digital Holter Recorders (Mortara Instrument, Milwaukee, WI). ECG collection using this method has been validated against the use of standard 12-lead ECG machines.²⁶ ECGs were simultaneously recorded (using dual snap ECG electrodes on the six standard precordial positions) with a Holter recorder and a bedside 12-lead ECG machine. Limb leads were placed in the modified foreshortened position. The Holter recorder was activated 10 min before the planned dose time on day 1. The subjects were asked to rest quietly in a supine position for 10 min before and 5 min after each prescribed ECG time-point. Measurements were taken at the following time-points 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 18, and 24 h on day –1 and day 1, with additional ECG measurements also taken at 48 h after the dose on day 1. Predose baseline ECGs were collected for 1 h before the dose on day 1. On day 1 of each period, a single 12-lead ECG was printed and reviewed by the investigator at 2, 4, 6, and 24 h after the dose for safety evaluation.

ECG analysis and interpretation. The flash cards containing the ECG data from each Holter ECG session were analyzed in an ECG core laboratory (Covance Central Diagnostics, Reno, UT). ECG data from the Holter were initially reviewed by a Holter technician and a fully annotated record, indicating any 10-s periods with significant artifact, technical failure, or any nonsinus beats, was compiled. Once annotated, 10-s periods of the Holter recording were extracted at the pre-specified time-points. In order to accurately extract replicate measurements at each nominal time-point, a 10-min window centered around each nominal time-point was defined. One ECG was extracted as close to the center of the window as possible, with subsequent replicates obtained from the next available 10-s periods on both sides of the nominal time-point. Any 10-s periods with artifact, technical failure or nonsinus beats, as determined above, were rejected and the 10-s period next closest in time was substituted. At least three replicate ECGs were extracted at all time-points; five replicate ECGs were extracted from the 2, 4, and 6 h time-points.

ECG digital waveforms were transferred from Holter recorder to the Digitography digital on-screen system. All 12 leads of each ECG were displayed simultaneously and measurements were made in three consecutive complexes in the lead with the longest QT. All ECGs were annotated and analyzed manually, without the aid of automation. Duration values were electronically determined from the annotations. Fridericia's correction ($QTc = QT/RR^{1/3}$) was used for correcting HR. The final value for each QTc in each ECG was determined by the average of three consecutive complexes. The initial manual annotation of the ECG was performed by a medical technician prior to review by a board-certified cardiologist. Both the medical technician and the cardiologist were blinded as to time-point, treatment, period, and replicate number.

Statistical analysis. The statistical analyses used in this study focused primarily on two issues: (i) the determination of the optimal baseline (i.e., either predose baseline or time-matched baseline) and (ii) the influence of varying numbers of replicates on the precision of the results. Although the primary comparison of interest in a QTc study is the comparison between the treatments (i.e., a comparison of postdose QTc values after placebo vs. those obtained after active treatment), the baseline-adjusted treatment difference is often the end point analyzed (as described in the E14 guidance). This adjustment is performed to correct for potential observed baseline imbalances between the treatment periods. This study evaluated the relative merits of using TMB (matched to the clock time of the previous day) vs. the standard PDB (before the dose on day 1). Period-specific baselines were used. It is noted that it may be appropriate to analyze data arising from a crossover design without incorporation of baseline measurements; however this consideration was outside the scope of the present investigation.

For the primary evaluation of moxifloxacin, the average (arithmetic mean) of the first three replicate measurements was taken before analysis, for both the baseline definitions. The QTc CFB values were analyzed in a

mixed-effects linear model appropriate for a two-period crossover design, with treatment, hour, sequence, period, and treatment-by-hour interaction as fixed effects, and with subject-within-sequence as a random effect (resulting in a compound symmetric covariance structure over all repeated observations within a subject). (Other models with a two-part covariance structure, one component accounting for the within-period correlation and the other accounting for the across-period correlation, were also investigated. However, because the results and conclusions were similar between the two models, the simpler covariance structure was chosen for this article.)

In order to assess the potential use of moxifloxacin as a positive control, two-sided 90% confidence intervals (equivalent to one-sided 95% confidence intervals) were constructed for the true mean CFB differences (moxifloxacin – placebo) at each hour, using each of the above baseline definitions. If the lower limit of the confidence interval was >0 at a particular time-point, it was concluded that moxifloxacin increases QTc CFB, as compared to placebo, at that time-point. Although no multiplicity adjustment was employed for this exploratory pilot study, it is recognized that, in order to assess a formal hypothesis comparing moxifloxacin to placebo, it may be preferable to specify a primary time-point of interest, or to adjust for multiplicity if multiple time-points are used in the assessment.

In order to quantify the benefit associated with using replicates, estimates of the within-subject variances using varying numbers of replicates were computed. As per the study design, five replicates were collected at 2, 4, and 6 h, and three replicates were collected at all other time-points. In the assessment of the effect of replicates, the estimated variance components were computed using the CFB data from the 2-, 4-, and 6-h time-points only. Note that five replicates were available for use with the TMB, whereas only three replicates were available for use with the PDB (because only three replicates were recorded at baseline). Within both of the baseline definitions and within each of the numbers of replicates, separate estimates for the within-subject variance were computed for all possible sets of replicates. For example, using two replicates for TMB, there are 10 ways to select two replicates from the group of five replicates available. The average of the within-subject variances arising from these 10 replicate sets was then used as the final within-subject variance component estimate for the TMB definition of baseline using two replicates.

In order to examine the relationship between drug concentration and QTc prolongation, PK/QTc analyses were performed on the placebo-adjusted moxifloxacin CFB QTc values. A linear mixed-effects model was used, with moxifloxacin plasma concentration as a continuous variable and with subject as a random effect (compound symmetry assumed). The model was fitted without an intercept. The estimated slope and corresponding two-sided 90% confidence interval were reported, and the predicted mean CFB QTc difference (moxifloxacin – placebo) and 90% pointwise confidence interval bounds were plotted.

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CONFLICT OF INTEREST

Drs Bloomfield, Kost, Hreniuk, Gottesdiener, and Wagner are employees of Merck and may hold Merck stock. Dr Ghosh and Ms Hickey are employees of Visitation. Dr Guitierrez is an investigator supported by clinical research grants from Merck.

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- Roden, D.M. Drug-induced prolongation of the QT interval. *N. Engl. J. Med.* **350**, 1013–1022 (2004).
- Fenichel, R.R. *et al.* Drug-induced torsades de pointes and implications for drug development. *J. Cardiovasc. Electrophysiol.* **15**, 475–495 (2004).

- Lasser, K.E., Allen, P.D., Woolhandler, S.J., Himmelstein, D.U., Wolfe, S.M. & Bor, D.H. Timing of new black box warnings and withdrawals for prescription medications. *JAMA* **287**, 2215–2220 (2002).
- Pratt, C.M. *et al.* Dose-response relation between terfenadine (Seldane) and the QTc interval on the scalar electrocardiogram: distinguishing a drug effect from spontaneous variability. *Am. Heart J.* **131**, 472–480 (1996).
- Pratt, C.M., Hertz, R.P., Ellis, B.E., Crowell, S.P., Louv, W. & Moyé, L. Risk of developing life-threatening ventricular arrhythmia associated with terfenadine in comparison with over-the-counter antihistamines, ibuprofen and clemastine. *Am. J. Cardiol.* **73**, 346–352 (1994).
- Honig, P.K., Woosley, R.L., Zamani, K., Conner, D.P. & Cantilena, L.R. Jr. Changes in the pharmacokinetics and electrocardiographic pharmacodynamics of terfenadine with concomitant administration of erythromycin. *Clin. Pharmacol. Ther.* **52**, 231–238 (1992).
- Davies, A.J., Harindra, V., McEwan, A. & Ghose, R.R. Cardiotoxic effect with convulsions in terfenadine overdose. *BMJ* **298**, 325 (1989).
- Wysowski, D.K., Corken, A., Gallo-Torres, H., Talarico, L. & Rodriguez, E.M. Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and Food and Drug Administration regulatory actions. *Am. J. Gastroenterol.* **96**, 1698–1703 (2001).
- Khongphatthanayothin, A., Lane, J., Thomas, D., Yen, L., Chang, D. & Bubolz, B. Effects of cisapride on QT interval in children. *J. Pediatr.* **133**, 51–56 (1998).
- Ahnve, S. & Vallin, H. Influence of heart rate and inhibition of autonomic tone on the QT interval. *Circulation* **65**, 435–439 (1982).
- Bazett, H.C. An analysis of time relations of electrocardiograms. *Heart* **7**, 353–367 (1920).
- Browne, K.F., Zipes, D.P., Heger, J.J. & Prystowsky, E.N. Influence of the autonomic nervous system on the Q-T interval in man. *Am. J. Cardiol.* **50**, 1099–1103 (1982).
- Cappato, R., Alboni, P., Pedroni, P., Gilli, G. & Antonioli, G.E. Sympathetic and vagal influences on rate-dependent changes of QT interval in healthy subjects. *Am. J. Cardiol.* **68**, 1188–1193 (1991).
- Fridericia, L.S. Die Systolendauer im Elektrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Med. Scand.* **53**, 469–486 (1920).
- Hnatkova, K. & Malik, M. "Optimum" formulae for heart rate correction of the QT interval. *Pacing Clin. Electrophysiol.* **22**, 1683–1687 (1999).
- Magnano, A.R., Holleran, S., Ramakrishnan, R., Reiffel, J.A. & Bloomfield, D.M. Autonomic nervous system influences on QT interval in normal subjects. *J. Am. Coll. Cardiol.* **39**, 1820–1826 (2002).
- Magnano, A.R., Holleran, S., Ramakrishnan, R., Reiffel, J.A. & Bloomfield, D.M. Autonomic modulation of the u wave during sympathomimetic stimulation and vagal inhibition in normal individuals. *Pacing Clin. Electrophysiol.* **27**, 1484–1492 (2004).
- Magnano, A.R., Talathoti, N., Hallur, R., Bloomfield, D.M. & Garan, H. Sympathomimetic infusion and cardiac repolarization: the normative effects of epinephrine and isoproterenol in healthy subjects. *J. Cardiovasc. Electrophysiol.* **17**, 983–989 (2006).
- Malik, M. Is there a physiologic QT/RR relationship? *J. Cardiovasc. Electrophysiol.* **13**, 1219–1221 (2002).
- Malik, M., Färbon, P., Batchvarov, V., Hnatkova, K. & Camm, A.J. Relation between QT and RR intervals is highly individual among healthy subjects: implications for heart rate correction of the QT interval. *Heart* **87**, 220–228 (2002).
- McLaughlin, N.B., Campbell, R.W. & Murray, A. Comparison of automatic QT measurement techniques in the normal 12 lead electrocardiogram. *Br. Heart J.* **74**, 84–89 (1995).
- Food and Drug Administration, HHS. International Conference on Harmonisation; guidance on E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs; availability. Notice. *Fed. Regist.* **70**, 61134–61135 (2005).
- Démolis, J.L., Kubitzka, D., Tennezé, L. & Funck-Brentano, C. Effect of a single oral dose of moxifloxacin (400 mg and 800 mg) on ventricular repolarization in healthy subjects. *Clin. Pharmacol. Ther.* **68**, 658–666 (2000).
- Morganroth, J. *et al.* Evaluation of vardenafil and sildenafil on cardiac repolarization. *Am. J. Cardiol.* **93**, 1378–1383, A6 (2004).
- Extramiana, F., Maison-Blanche, P., Cabanis, M.J., Ortemann-Renon, C., Beauvais, P. & Leenhardt, A. Clinical assessment of drug-induced QT prolongation in association with heart rate changes. *Clin. Pharmacol. Ther.* **77**, 247–258 (2005).
- Sarapa, N. *et al.* Electrocardiographic identification of drug-induced QT prolongation: assessment by different recording and measurement methods. *Ann. Noninvasive Electrocardiol.* **9**, 48–57 (2004).