ARTICLE

Evaluation of QT Liability for PF-05251749 in the Presence of Potential Circadian Rhythm Modification

Yeamin Huh[1],*, Danny Chen[2], Steve Riley[1] (iD) , Cheng Chang[1] and Timothy Nicholas[1]

PF-05251749 is a dual inhibitor of casein kinase 1 δ/ε, key regulators of circadian rhythm. As a result of its mechanism of action, PF-05251749 may also change the heart rate corrected QT (QTc) circadian rhythm, which may confound detection of drug-induced QTc prolongation. In this analysis, a nonlinear mixed effect model including a multioscillator function was developed in addition to fitting the prespecified linear mixed effect concentration-QTc model, to identify QTc liability of PF-05251749 in the presence of potential circadian rhythm change. The modeling results suggested lack of clinically meaningful QTc prolongation (upper bound of 90% confidence interval for ΔΔQTc < 10 milliseconds) and that the drug-induced QTc circadian rhythm change was not present. However, simulation results indicated that inference of drug-induced QTc prolongation could be misleading if the drug effect on QTc circadian rhythm is not properly addressed. The modeling and simulation results suggest that pre specification of the concentration-QTc model should be reconsidered for drugs with circadian rhythm modulation potential.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ As a result of the ability for prespecification, the linear mixed effect (LME) model has been widely used for concentration-heart rate corrected QT (CQTc) analysis. A recently published white paper also supported using a prespecified LME model for CQTc analysis. However, LME model does not have a flexibility to incorporate circadian rhythm variability for drugs with circadian rhythm modulation potential.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ The objective of this analysis was to evaluate QTc liability of PF-05251749 in the presence of potential QTc circadian rhythm change. Model-derived simulation followed to investigate if significant drug-induced QTc circadian rhythm change would affect the inference of the CQTc slope estimate.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Drug-induced QTc circadian rhythm change and drug-induced clinically meaningful QTc prolongation for PF-05251749 was not detected. The modeling and simulation results demonstrated that if the drug effect on QTc circadian rhythm was not properly addressed in CQTc model, inference of drug-induced QTc prolongation could be misleading.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Prespecification of the CQTc model should be reconsidered if circadian rhythm change is expected based on the pharmacology of the drug. Developing a more biologically based nonlinear mixed effect model in addition to fitting the prespecified LME model enables simultaneous evaluation of drug effect on QTc prolongation as well as physiological rhythm change for drugs with circadian rhythm modulation potential.

Circadian rhythm represents a biological process exhibiting 24-hour cycles, which plays a critical role for the optimal functioning of organisms. Disruption of the circadian rhythm is observed in patients with Alzheimer's disease (AD) and various types of mood disorders as evidenced by sleep-cycle alterations.[1–4] Therefore, entrainment of misaligned circadian behavior is a promising target for the treatment of AD or major depressive disorder.[5,6]

The circadian system of the body is regulated by an endogenous biological clock located in the suprachiasmatic nucleus of the anterior hypothalamus. The suprachiasmatic nucleus

ensures rhythmic expression of the clock genes period (*PER1*, *PER2*, and *PER3*) and cryptochrome (*CRY1* and *CRY2*), which are ultimately translated into physiological circadian rhythm.[7] Casein kinases 1 (*CK1*) δ/ε were shown to phosphorylate *CRY* and *PER* proteins, leading to proteasome-mediated degradation and inhibition of circadian locomotor output cycles kaput (*CLOCK*)/brain, muscle Arnt-like 1(*BMAL1*) transcriptional activity, acting as a negative feedback for clock genes.[8] A mutation in the human *CK1* δ gene has been linked to familial advanced sleep phase syndrome characterized by early sleep and early morning awakening.[9] Also, *CK1*-inhibiting

---

[1]Global Product Development, Pfizer Inc, Groton, Connecticut, USA; [2]Early Clinical Development, Pfizer Inc, Cambridge, Massachusetts, USA. *Correspondence: Yeamin Huh (yeamin.huh@pfizer.com)

compounds showed a remarkable period-lengthening effect in clock cell lines.[10] These findings suggest that pharmacologic manipulation of *CK1* can significantly alter the circadian rhythm.

PF-05251749 is a novel, brain-penetrable, highly selective, dual inhibitor of *CK1* δ/ε developed to treat multiple diseases related to the disrupted circadian rhythm. The inhibition of *CK1* disrupts *CLOCK/BMAL1* transcriptional activity, and the disruption of the *BMAL1* gene was reported to induce a time-of-day prolongation in heart rate corrected QT (QTc) intervals during the light phase.[11] As a result of this mechanism of action, PF-05251749 may also change the QTc circadian rhythm, which may confound the detection of drug-induced QTc prolongation. Therefore, the primary objective of this modeling analysis was to evaluate the QT prolongation risk of PF-05251749 in the presence of potential circadian rhythm change based on recently conducted single-ascending dose and multiple-ascending dose phase I clinical studies of PF-05251749.

Because the purpose of a concentration-QTc (CQTc) analysis is to provide a valid inference of the QTc prolongation potential of a drug, prespecification of the model is preferred.[12] The ability to estimate biologically relevant parameters in a model with oscillatory time functions is likely to be dependent on the study design. Therefore, a more simple linear mixed effect (LME) model with nominal time as factor variables has been used as a prespecified CQTc model. However, because the diurnal variation is described as a fixed factor effect, the LME model may not be flexible enough to account for any changes in circadian rhythms. To address the potential QTc circadian rhythm change by PF-05251749, a nonlinear mixed effect (NLME) model with oscillatory time functions was considered in this analysis. Subsequently, the developed NLME model was used for model-based clinical trial simulations, and the performances of LME and NLME models were compared in evaluating QTc prolongation risk of a drug with QTc circadian rhythm modulation potential.

METHODS
Study design
The CQTc analysis was conducted using pooled data from studies B8001001 and B8001002. Briefly, study B8001001 was a phase I study with single escalating oral doses of PF-05251749 and study B8001002 was a phase I study with multiple escalating doses of PF-05251749. A summary of the study designs and pharmacokinetic-electrocardiogram (ECG) matched time points are provided in **Table S1**.

Both studies were placebo-controlled studies conducted under fasted conditions (except one fed group in B8001001), and concomitant medications were not allowed. Electrocardiogram measurement procedures were the same between those studies in that triplicate 12-lead ECGs were collected in a supine position both at baseline and during treatment (except a single ECG was measured in part B of study B8001001). The PF-05251749 concentrations of both study data were analyzed at the same lab, Pfizer Inc (Groton, CT). Therefore, there were no specific concerns on pooling data from studies B8001001 and B8001002 to be used in the concentration-QTc analysis.

The different study conditions such as fed and single ECG measurement were evaluated as potential covariates in the covariate analysis.

Both studies were conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The final protocols were approved by the institutional review boards.

LME model development
The following LME model was used to describe the concentration-QTc relationship:

$$QTcF_{ij} = QTcF_0 + \eta_i 1 + NMTM_m \cdot \left[time = m \right] + COV$$
$$+ \left(slope + \eta_i 2 \right) \cdot conc_{ij} + \varepsilon_{ij} \qquad (1)$$

where i indexes the ith individual, j indexes the jth measurement time, m indexes the nominal time since first dose, and $QTcF_{ij}$ is the arithmetic mean of Fridericia corrected QT (QTcF) triplicates (or single QTcF for part B of study B8001001). The intercept parameter $QTcF_0$ represents the mean QTcF response in the absence of drug ($conc_{ij} = 0$) at day 1 predose (baseline; time = 0); $NMTM_m$ represents the mean QTc difference for other nominal times relative to nominal time 0; COV is important covariates identified in the covariate analysis; slope represents the population mean slope; conc is the observed PF-05251749 plasma concentration around the time of the ECG collection; $\eta_i 1$ and $\eta_i 2$ (etas) represent subject-specific random effects, which are assumed to be normally distributed with mean 0 and variance-covariance matrix ω^2 (omega); and ε (epsilon) represents the residual random variable with mean 0 and variance σ^2 (sigma). An unstructured matrix was used for the random effects covariance matrix.

Covariate analysis was performed for sex, fed condition, study, PM dosing, and cerebrospinal fluid sampling cohort (part B of study B8001001) effect on baseline. A stepwise covariate model-building procedure approach was employed involving both forward addition (based on a significance level of $P < 0.05$) and backward elimination (based on a significance level of $P < 0.001$).

The LME model recommended in the scientific white paper on concentration-QTc modeling (white paper model) was also tested for the placebo-corrected QTcF change from baseline (ΔΔQTcF) estimation.[12] The mathematical expression is as follows:

$$\Delta QTcF_{ij} = \Delta QTcF_0 + \eta_i 1 + NMTM_m \cdot \left[time = m \right]$$
$$+ TRT + BASE \cdot \left(QTc_{i,j=0} - \overline{QTc_0} \right)$$
$$+ \left(slope + \eta_i 2 \right) \cdot conc_{ij} + \varepsilon_{ij}$$

where $\Delta QTcF_{ij}$ is the change from baseline in $QTcF_{ij}$, TRT is a treatment specific intercept, BASE is a fixed effect of baseline $QTc_{i,j=0}$, and $\overline{QTc_0}$ is overall mean of $QTc_{i,j=0}$.

NLME model development
Because the LME model addresses time effect as a fixed factor, it will not be able to capture a potential drug effect on QTc circadian rhythm. Therefore, a NLME model with cosine functions was fitted to the data to describe a 24-hour

circadian variation. The following NLME model was used to describe the 24-hour circadian variation as well as concentration-QTc relationship:

$$\text{Model 1: } QTcF_{ij} = QTcF_0 \cdot e^{\eta_i 1} \cdot circ + COV$$
$$+ \left(slope + \eta_i 2 \right) \cdot conc_{ij} + \varepsilon_{ij}$$

$$circ = 1 + A_1 \cdot \cos\left(\frac{2\pi \cdot (t - \varphi_1)}{24} \right) + A_2 \cdot \cos\left(\frac{4\pi \cdot (t - \varphi_2)}{24} \right)$$

where circ is a circadian rhythm function consisting of two amplitudes (A_1 and A_2) and two phases (φ_1 and φ_2), t is a 24-hour clock time, and COV is the selected covariates from LME model development.

The drug effect on QTc circadian rhythm was evaluated in terms of either phase delay or period lengthening with additional parameters on cosine functions. The following equations were used to describe drug-induced QTc circadian rhythm change:

$$\text{Model 2: } circ = 1 + A_1 \cdot \cos\left(\frac{2\pi \cdot \left(t - \left(\varphi_1 + E_{D1} \cdot [trt = yes] \right) \right)}{24} \right)$$
$$+ A_2 \cdot \cos\left(\frac{4\pi \cdot \left(t - \left(\varphi_2 + E_{D1} \cdot [trt = yes] \right) \right)}{24} \right)$$

$$\text{Model 3: } circ = 1 + A_1 \cdot \cos\left(\frac{2\pi \cdot \left(t - \left(\varphi_1 + E_{D2} \cdot conc_{ij} \right) \right)}{24} \right)$$
$$+ A_2 \cdot \cos\left(\frac{4\pi \cdot \left(t - \left(\varphi_2 + E_{D2} \cdot conc_{ij} \right) \right)}{24} \right)$$

$$\text{Model 4: } circ = 1 + A_1 \cdot \cos\left(\frac{2\pi \cdot \left(t - \varphi_1 \cdot \left(1 + E_{L1} \cdot [trt = yes] \right) \right)}{24 \cdot \left(1 + E_{L1} \cdot [trt = yes] \right)} \right)$$
$$+ A_2 \cdot \cos\left(\frac{4\pi \cdot \left(t - \varphi_1 \cdot \left(1 + E_{L1} \cdot [trt = yes] \right) \right)}{24 \cdot \left(1 + E_{L1} \cdot [trt = yes] \right)} \right)$$

$$\text{Model 5: } circ = 1 + A_1 \cdot \cos\left(\frac{2\pi \cdot \left(t - \varphi_1 \cdot \left(1 + E_{L2} \cdot conc_{ij} \right) \right)}{24 \cdot \left(1 + E_{L2} \cdot conc_{ij} \right)} \right)$$
$$+ A_2 \cdot \cos\left(\frac{4\pi \cdot \left(t - \varphi_2 \cdot \left(1 + E_{L2} \cdot conc_{ij} \right) \right)}{24 \cdot \left(1 + E_{L2} \cdot conc_{ij} \right)} \right)$$

where E_D is a phase delay parameter and E_L is a period-lengthening parameter. Bracketed expressions are indicator functions that = 1 if the condition in bracket is true and = 0 otherwise.

Simulation

To investigate the effect of drug-induced QT circadian rhythm change on the inference of concentration-QT slope estimate as well as QT liability, a simulation study was performed based on the selected final NLME model (model 1 in **Table 2**). The design of the simulated trials was identical to the CQTc analysis data set for PF-05251749, including pharmacokinetic concentrations. QTcF data were simulated under

the following four different scenarios: (i) clinically meaningful QT prolongation (slope = 0.00242 milliseconds/ng/mL, such that the predicted mean $\Delta\Delta QTc$ at steady-state maximum concentration (C_{max}) of supratherapeutic dose $\geq$ 10 milliseconds) in the presence of concentration-dependent phase delay (no delay and maximum 1, 2, 3, 4, and 5 hours of delay) in a QT circadian rhythm, (ii) clinically meaningful QT prolongation in the presence of concentration-dependent period lengthening (no lengthening and maximum 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, and 1.5-fold lengthening) in a QT circadian rhythm, (iii) no QT prolongation (slope = 0) in the presence of a phase delay in a QT circadian rhythm, and (iv) no QT prolongation in the presence of period lengthening in a QT circadian rhythm. For concentration-dependent circadian rhythm change, the maximum effect (E_{max})-half maximal effective concentration (EC50) function was used with EC50 of 50 ng/mL such that the maximum QTc rhythm change is achieved at a very low concentration within the observed range. A total of 1,000 data sets were simulated for each scenario, and then both the LME (Eq. 1) and NLME models (true model) were fitted to investigate the performance of each model for detecting QTc prolongation in the presence of QTc circadian rhythm change.

For the drug-induced QT prolongation case, bias in slope estimate and the false-negative rate in detecting QTc liability were used to evaluate model performance. The bias in slope estimate was calculated with an estimation error as follows:

$$\text{Bias in slope estimates} = \frac{1}{N} \sum_{i=1}^{N} \left(\text{slope estimate}_i - \text{true slope} \right)$$

The false-negative rate in detecting QT liability was calculated based on the upper bound of the 90% confidence interval (CI) of the $\Delta\Delta QTcF$ estimate:

$$\text{False} - \text{negative rate} = \frac{1}{N} \sum_{i=1}^{N} I[\text{Upper bound of 90\%}$$
$$\text{CI of } \Delta\Delta QTcF_i < 10\,ms] \times 100$$

$$\Delta\Delta QTcF_i = \text{slope estimate}_i$$
$$\times SS\ C_{max}\ \text{of supratherapeutic dose}$$

where I is an indicator function that = 1 if the condition in bracket is true and = 0 otherwise.

For no QT prolongation case, model performance was evaluated with a false-positive slope rate (lower bound of 95% CI for slope > 0) and a false-positive study rate for QTc liability (upper bound of 90% CI for $\Delta\Delta QTcF$ at supratherapeutic dose $\geq$ 10 milliseconds).

RESULTS

LME model results

Key assumptions about the relationship between concentration and QTc interval were graphically evaluated first as recommended in the scientific white paper on concentration-QTc modeling.[12] The exploratory plots are provided in **Figure S1**. The exploratory plots suggested

that (i) drug effect on heart rate is not significant, (ii) QTcF is independent of heart rate, (iii) lack of hysteresis between drug concentrations and ΔQTcF, and (iv) a linear concentration-QTcF relationship is a reasonable assumption. Therefore, QTcF was selected as a sufficient correction method, and the LME model was used for CQTc model development.

Parameter estimates and visual predictive check of the final LME model are summarized in **Table 1** and **Figure S2**, respectively. Covariate analysis identified gender and study as important covariates on intercept, so those effects were included in the final model. Females tend to have 16.5 milliseconds higher baseline than males as expected,[13] and study B8001002 subjects tended to have 19.9 milliseconds lower baseline than study B8001001 subjects. The slope estimate

Table 1 Parameter estimate of the final linear mixed effect model

Parameter	Estimate	RSE, %[a]
$QTcF_0$ (millisecond)	410	0.671
Slope (millisecond/ng/mL)	0.00136	26.0
$NMTM_{0.5}$ (millisecond)	−2.60	29.7
$NMTM_{1.5}$ (millisecond)	−0.601	129
$NMTM_{2.5}$ (millisecond)	−1.73	56.4
$NMTM_3$ (millisecond)	2.91	32.3
$NMTM_4$ (millisecond)	1.05	91.6
$NMTM_5$ (millisecond)	7.16	13.0
$NMTM_8$ (millisecond)	−3.53	26.8
$NMTM_{12}$ (millisecond)	−1.68	57.7
$NMTM_{24}$ (millisecond)	−0.643	115
$NMTM_{48}$ (millisecond)	−6.28	17.7
$NMTM_{73.5}$ (millisecond)	0.263	379
$NMTM_{144}$ (millisecond)	−0.497	192
$NMTM_{144.5}$ (millisecond)	−2.24	44.0
$NMTM_{145.5}$ (millisecond)	1.41	70.6
$NMTM_{147}$ (millisecond)	4.32	22.6
$NMTM_{149}$ (millisecond)	5.30	18.2
$NMTM_{168}$ (millisecond)	−0.581	165
$NMTM_{217.5}$ (millisecond)	3.14	31.8
$NMTM_{312}$ (millisecond)	−1.37	70.5
$NMTM_{312.5}$ (millisecond)	−1.44	69.4
$NMTM_{313.5}$ (millisecond)	1.39	72.7
$NMTM_{315}$ (millisecond)	4.10	24.2
$NMTM_{317}$ (millisecond)	5.44	17.9
$NMTM_{336}$ (millisecond)	−0.877	110
$NMTM_{369}$ (millisecond)	−0.149	648
P1002 (millisecond)	−19.9	16.2
SEXF (millisecond)	16.5	26.1
σ (millisecond)	6.61	1.53
ω_{int} (millisecond)	15.0	13.1
ω_{slope} (millisecond)	0.00169	35.5

$NMTM_m$, mean QTcF difference for other nominal times relative to nominal time 0; P1002, mean baseline QTcF difference for Study B8001002 relative to Study B8001001; $QTcF_0$, mean QTcF response in the absence of drug and covariate effects at Day 1 predose (baseline; time=0); RSE, relative standard error; SEXF, mean baseline QTcF difference for females relative to males; σ, residual random variability; ω_{int}, intersubject variability for baseline QTcF; ω_{slope}, intersubject variability for slope.
[a]RSE values for intersubject variability terms (ω_{int} and ω_{slope}) were calculated based on the variance.

was positive and statistically significant, but the magnitude was small, indicating a 1.36-millisecond increase in QTcF per 1,000 ng/mL increase in PF-05251749 concentration.

NLME model results
Parameter estimates of the NLME models and the final model visual predictive check are summarized in **Table 2** and **Figure S3**, respectively. One and two cosine functions were tried for placebo data, and two cosine functions described the data better, with an objective function value (OFV) drop of 28.77 points (degrees of freedom = 2). Therefore, two cosine functions were used to describe the circadian rhythm for THE NLME models. All parameters were precisely estimated with small relative standard error, except the circadian rhythm change parameters (E_D or E_L). Circadian rhythm change parameters from models 2–5 were not statistically significant, nor did they induce a significant OFV reduction (at a significance level of $P < 0.001$) when compared with model 1, indicating that the presence of drug-induced QT circadian rhythm change was not supported in the current study data. Therefore, the selected final NLME model was model 1. Estimates of the baseline-related parameters ($QTcF_0$, mean baseline QTcF difference for females relative to males (SEXF), and mean baseline QTcF difference for Study B8001002 relative to Study B8001001 (P1002) in **Table 2**) were similar to the estimates from the LME model (**Table 1**). The statistically significant positive slope from the NLME model was also similar to the LME model slope estimate. The parameter estimate indicated an 1.21-millisecond increase in QTcF per 1,000 ng/mL increase in PF-05251749 concentration.

ΔΔQTcF prediction
The developed LME and NLME models as well as the prespecified white paper model were used to produce predictions of ΔΔQTcF. As summarized in **Figure 1**, the ΔΔQTcF prediction results were similar across the three different models. All of the models indicated that the upper bound of 90% CI for ΔΔQTcF was less than 10 milliseconds at steady-state C_{max} of both the therapeutic and supratherapeutic doses. Therefore, the QT prolongation of a regulatory threshold is unlikely at the expected PF-05251749 therapeutic exposure.

Model-derived clinical trial simulation
Detectable drug-induced QTc circadian rhythm change was not observed in the analysis data set. However, the following key question remains: If there were detectable drug-induced QTc circadian rhythm changes, would they affect the inference of CQTc slope estimate? To investigate this question, a simulation study was performed using the final NLME model. **Figure 2** shows the fitted QTc circadian rhythm (red line) as well as the QTc rhythm change scenarios for phase delay and period lengthening.

QTc prolongation case in the presence of QTc circadian rhythm change
Estimation errors of the CQTc slope for phase delay scenarios are summarized in **Figure 3**. For the LME model that incorporates the nominal time effect as a fixed factor, bias in the slope estimate was dependent on the differing degrees of phase delay. The slope estimates were negatively biased in

Table 2 Parameter estimate of the developed nonlinear mixed effect models

Parameter	Model 1 (RSE[a], %)	Model 2 (RSE[a], %)	Model 3 (RSE[a], %)	Model 4 (RSE[a], %)	Model 5 (RSE[a], %)
$QTcF_0$, millisecond	410 (0.686)	410 (0.688)	410 (0.689)	410 (0.687)	411 (0.687)
SEXF, millisecond	16.5 (26.8)	16.6 (26.6)	16.5 (26.7)	16.6 (26.7)	16.5 (26.8)
A_1	0.00732 (17.5)	0.00573 (23.8)	0.00625 (27.5)	0.00706 (16.1)	0.00749 (18.3)
ϕ_1, hour	25.1 (1.96)	23.9 (4.21)	24.9 (2.46)	26.1 (2.07)	25.1 (1.93)
A_2	0.00899 (7.48)	0.00874 (7.10)	0.00885 (7.40)	0.00906 (7.07)	0.00901 (7.55)
ϕ_2, hour	12.9 (1.66)	11.7 (6.31)	12.6 (3.08)	13.7 (2.34)	12.9 (2.15)
σ, millisecond	6.79 (1.52)	6.78 (1.52)	−6.78 (1.52)	6.77 (1.52)	6.79 (1.52)
P1002, millisecond	−17.7 (18.5)	−17.9 (18.3)	−17.7 (18.4)	−17.7 (18.5)	−17.7 (18.5)
Slope, millisecond/ng/mL	0.00121 (28.4)	0.00117 (29.5)	0.00126 (27.2)	0.00114 (30.2)	0.00119 (29.6)
E_{D1}, hour	—	1.09 (66.3)	—	—	—
E_{D2}, hour/ng/mL	—	—	0.000188 (101)	—	—
E_{L1}	—	—	—	−0.0724 (31.6)	—
E_{L2}	—	—	—	—	−0.00000406 (300)
ω_{int}	0.0382 (13.2)	0.0382 (13.2)	0.0381 (13.2)	0.0383 (13.1)	0.0382 (13.2)
ω_{slope}, millisecond	0.00178 (35.7)	0.00180 (35.6)	0.00180 (35.8)	0.00178 (35.3)	0.00177 (35.7)
OFV	11,964.43	11,963.83	11,963.41	11,956.59	11,964.33

A_1 and A_2, amplitude 1 and 2 in the circadian rhythm function; E_D, a phase delay parameter; E_L, a period lengthening parameter; OFV, objective function value; P1002, mean baseline QTcF difference for Study B8001002 relative to Study B8001001; $QTcF_0$, mean QTcF response in the absence of drug and co-variate effects at Day 1 predose (baseline; time=0); RSE, relative standard error; SEXF, mean baseline QTcF difference for females relative to males; ϕ_1 and ϕ_2, phase 1 and 2 in the circadian rhythm function; σ, residual random variability; ω_{int}, intersubject variability for baseline QTcF; ω_{slope}, intersubject variability for slope.
[a]RSE values for intersubject variability terms (ω_{int} and ω_{slope}) were calculated based on the variance.
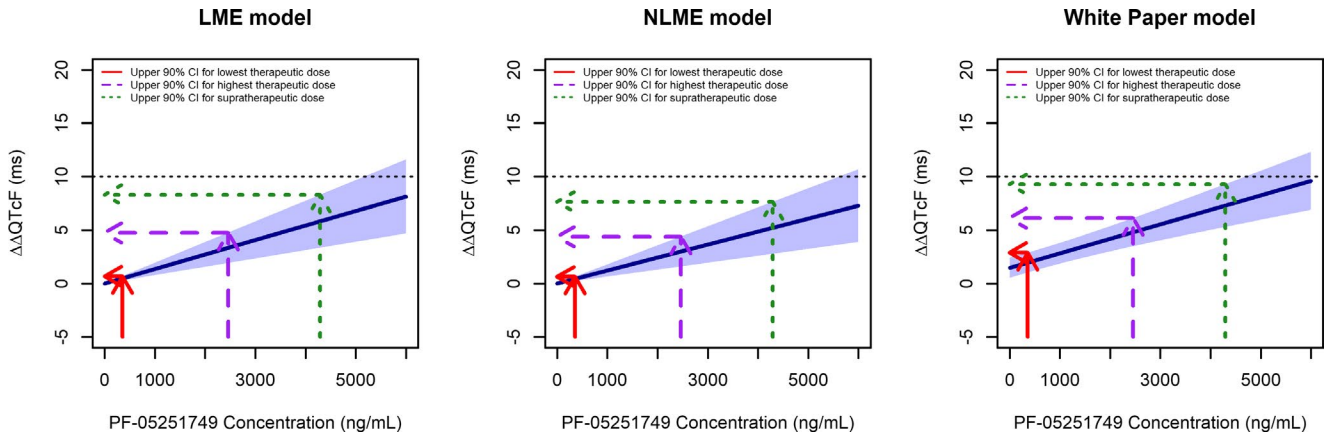

Figure 1 Model-derived ΔΔQTcF vs. PF-05251749 concentration plot across different types of models. Blue line and shaded regions represent mean prediction and 90% confidence intervals (CIs), red arrow is upper bound of 90% CI at mean steady-state maximum concentration (C_{max}) for lowest therapeutic dose (50 mg), purple dashed arrow is for highest therapeutic dose (400 mg), and green dotted arrow is for supratherapeutic dose (750 mg). LME, linear mixed effect; NLME, nonlinear mixed effect; QTcF, Fridericia corrected QT interval; ΔΔQTcF, placebo-corrected QTcF change from baseline.

the presence of a maximum 1–3 hours delay (–11.5 to –6.13% difference compared with true slope), whereas they were positively biased in the presence of a maximum 5 hours delay (17.6% difference). When the drug effect on the QTc circadian rhythm was properly captured using the NLME model, the bias in the slope estimate was not significant with <1% difference across different degrees of phase delay. Precision of the slope estimate (standard error) was similar between the LME and NLME models (data not shown).

Figure 4 summarizes the estimation errors for the period-lengthening scenario. The overall interpretation of the results was similar to the phase-delay scenario; bias in the slope estimate was dependent on the degrees of period

lengthening for the LME model, whereas it was consistently small with a <2.71% difference for the NLME model across different degrees of period lengthening. Bias of the LME model was worse than the phase-delay case, with –38.1 to –27.2% difference in the presence of a maximum 1.1-fold to 1.3-fold period lengthening. The negative bias was reduced in the presence of a maximum 1.5-fold period lengthening (–5.68% difference). Precision of the slope estimate (standard error) was similar between the LME and NLME models (data not shown).

Model performance for the LME and NLME models was further evaluated by assessing the false-negative rate in detecting QTc liability when the underlying true signal was
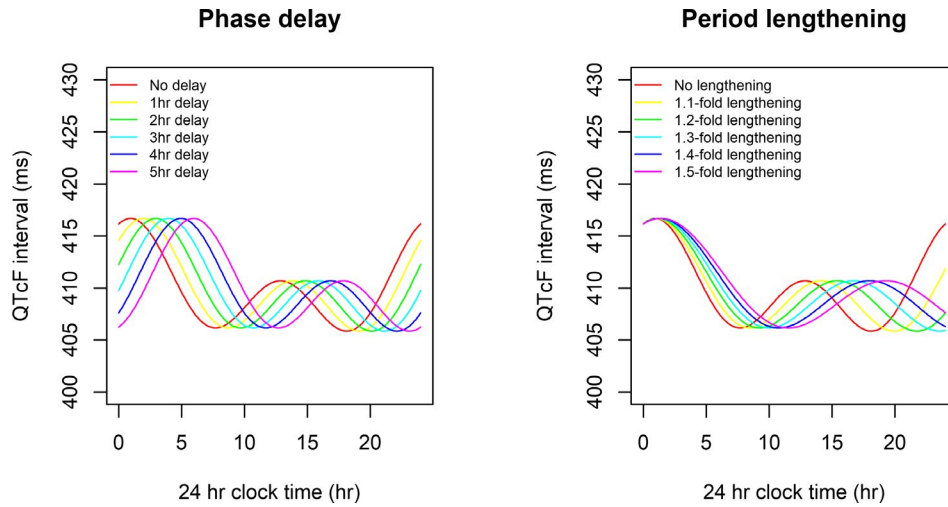
Figure 2 Two scenarios of drug-induced QTcF circadian rhythm change. hr, hour; QTcF, Fridericia corrected QT interval.
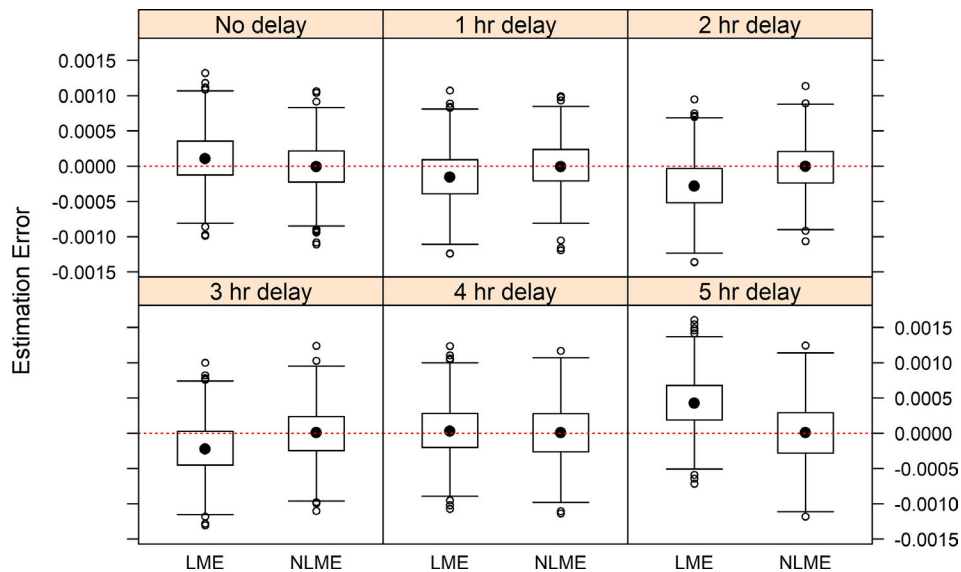

Figure 3 Box and whisker plots of the estimation errors for slope parameters in both LME and NLME models across different phase-delay scenarios. Boxes denote the 25th and 75th percentiles, and the closed circles denote the median. Whiskers represent the 5th and 95th percentiles. hr, hour; LME, linear mixed effect; NLME, nonlinear mixed effect.

positive. The results for both phase delay and period lengthening are summarized in **Figure 5**. As expected from the bias observed in the slope investigation, the false-negative rate for the LME model was dependent on the degrees of phase delay and period lengthening: as high as 13.3% for the phase-delay scenario and as high as 70.0% for the period-lengthening scenario. On the other hand, the false-negative rate for the NLME model was consistently small across different scenarios of circadian rhythm change, with < 2.71% for phase delay and < 6.33% for period lengthening.

No QTc prolongation case in the presence of QTc circadian rhythm change
When the underlying truth was no QTc prolongation (true slope = 0), model performance was summarized assessing false-positive slope rates as shown in **Figure 6**. Consistent

with the generally negative bias in slope estimates seen in **Figures 3 and 4**, the false-positive slope rate for the LME model was generally small for most of the QTc circadian rhythm change scenarios except that the rate was relatively high in the presence of a 5-hour phase delay (11.7%). For the NLME model, the false-positive slope rate was consistently small, with 2.90–3.81% for the phase-delay scenario and 2.77–4.63% for the period-lengthening scenario. Even with the false-positive slope, the magnitude of the slope was small such that the false-positive rate for QTc liability was zero across all of the circadian rhythm change scenarios.

DISCUSSION

Circadian variations in QT intervals have been previously characterized by a cosine[14,15] or a multioscillator function
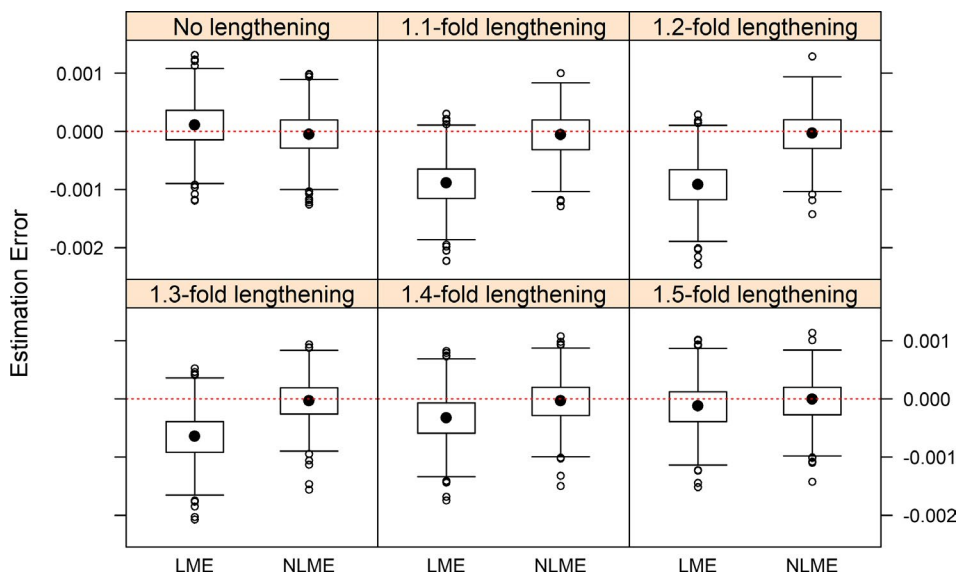
Figure 4 Box and whisker plots of the estimation errors for slope parameters in both LME and NLME models across different period lengthening scenarios. Boxes denote the 25th and 75th percentiles and the closed circles denote the median. Whiskers represent the 5th and 95th percentiles. LME, linear mixed effect; NLME, nonlinear mixed effect.
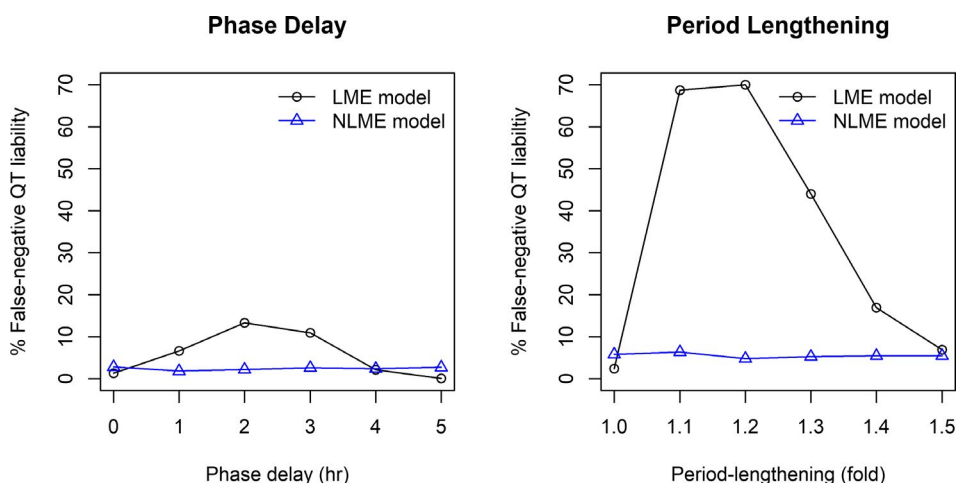

Figure 5 False-negative study rate for clinically meaningful QT prolongation case (upper bound of 90% confidence interval for ΔΔQTcF ≥ 10 milliseconds) in both LME and NLME models across different scenarios of QTcF circadian rhythm change. hr, hour; LME, linear mixed effect; NLME, nonlinear mixed effect; QTcF, Fridericia corrected QT interval; ΔΔQTcF, placebo-corrected QTcF change from baseline.

including ≥2 cosine functions.[16,17] In the present study, a potential PF-05251749 effect on QTc circadian rhythm change was evaluated simultaneously with QTc prolongation using the NLME model based on a multioscillator function. Two cosine functions best described the placebo data in this analysis, and the circadian parameter estimates (**Table 2**) were similar to the previously reported QTc circadian parameters based on two cosine functions.[16] Also, the fitted shape of the placebo QTc circadian rhythm (red line in **Figure 2**) was similar to the previously reported QTc circadian pattern, with a large drop from midnight to 9 AM, a large increase from 4 PM to midnight, and relatively flat between 9 AM and 4 PM.[18] When evaluated with the proposed

NLME model, the QTc rhythm change parameters were not supported in the data set. Both LME and NLME model results indicated that a 10-millisecond placebo-adjusted change from baseline, which is a regulatory threshold, can be excluded. The upper bound of the two-sided 90% CI for ΔΔQTcF at the steady-state mean C_{max} of the suprathera-peutic dose was 8.28 milliseconds for the LME model and 7.64 milliseconds for the NLME model.

The hypothesized drug-induced QTc circadian rhythm change for PF-05251749 was not detected in the current study data set. The PF-05251749 effect on a physiological circadian rhythm was previously evaluated using clock gene data from a phase I multiple-ascending dose study. When
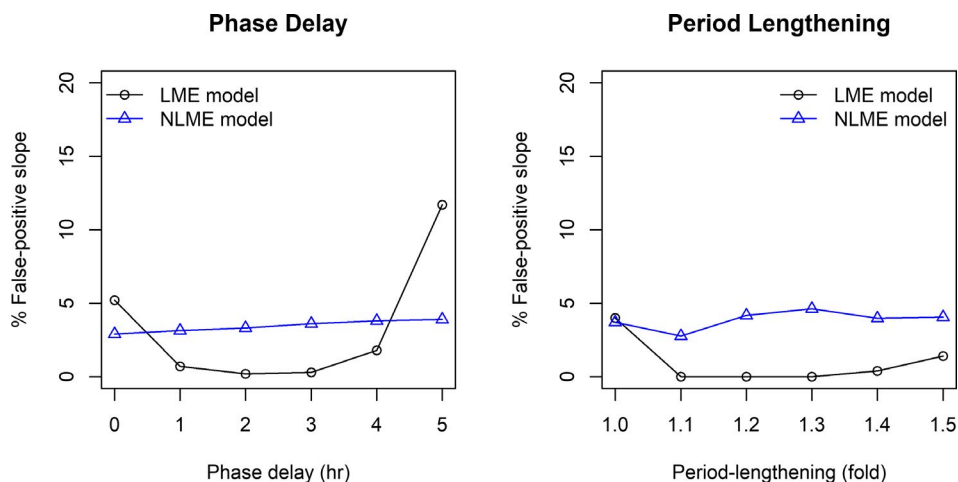
Figure 6 False-positive slope rate for no QT prolongation case in both LME and NLME models across different scenarios of QTcF circadian rhythm change. hr, hour; LME, linear mixed effect; NLME, nonlinear mixed effect; QTcF, Fridericia corrected QT interval.

daily leukocyte messenger RNA levels of multiple periodic genes were investigated, the genes showed dose-dependent phase delays by day 7, with the largest drug effect on the *PER3* gene of a mean 9.2 hours (AM dosing) and 19.9 hours (PM dosing) delay at the top dose.[19] The gold standard circadian rhythm biomarker, dim light melatonin onset (DLMO), also showed a maximum phase delay of 2.1 hours (AM dosing) and 2.3 hours (PM dosing) by day 15.[20] The discrepancy between DLMO and the *PER3* gene phase shift is the result of the censoring of the DLMO end point limited by the study design. Based on these findings, the drug-induced phase delays on the QTc circadian rhythm were tested only at the steady state (day ≥ 7) for NLME models 2 and 3, but a circadian rhythm change parameter was still not supported, with a very large relative standard error and insignificant OFV drop. Undetectable QTc circadian rhythm change may be attributed to limited information coming from sparse steady-state ECG sampling times in this study (0, 0.5, 1.5, 3, 5, and 24 hours on days 7 and 14). As a result, within the current study setting, acute QTc circadian rhythm change was not detected.

The CQTc modeling methodology has been extensively studied in recent decades, and the International Conference on Harmonization (ICH) "E14 Questions & Answers (R3)" document was revised to accept CQTc modeling as a primary analysis to evaluate the QT prolongation risk of new drugs.[21] A recently published white paper proposed a pre-specified LME model for CQTc analysis, such that if basic assumptions are met in the exploratory graphical checks, the proposed LME model can be used.[12] In the present analysis, an LME model based on QTcF was used, which is different from the white paper model using ΔQTcF as a dependent variable. By using an LME model based on QTcF, the slope parameter can be comparable with the NLME model slope, and ΔΔQTcF estimates can be easily calculated from the slope estimate. Because the model used nonbaseline corrected value (QTcF) as a dependent variable, more covariates than in the white paper model were included in the intercept to describe the baseline characteristics. Other than that, the basic model structure was analogous to the white paper model. The performance of the white paper model was also investigated, and as expected from the similar model structure, the ΔΔQTcF estimates for PF-05251749 were similar to the QTcF-based LME model (**Figure 1**). The simulation results for bias in the slope estimate under the circadian rhythm modification scenarios were almost identical to the QTcF-based LME model (results not shown).

However, fitting the prespecified LME model without taking the drug pharmacology into account could lead to a misinterpretation of the QT liability as seen in the simulation results, especially for a drug with circadian rhythm modification potential. The LME model is commonly used to quantify the concentration-QTc relationship of a drug, but it handles circadian rhythm with a fixed factor effect of nominal time and may not capture the effects of variation within the rhythmicity. When the LME model was fitted to the CQTc data set including circadian rhythm changes, the estimated CQTc slope was either positively or negatively biased depending on the degree of circadian rhythm change. This bias in slope could induce significantly high type I or type II errors. These potential errors were highlighted in the drug-induced QTc circadian rhythm change scenarios (1–5 hours of phase delay or 1.1–1.5 fold of period lengthening), and the false-negative QT liability rate was as high as 70% for period lengthening case. An interaction term between nominal time and dose was also tested within the LME model to investigate if it could account for the drug-induced circadian rhythm change. However, those LME model runs were terminated because of a singularity error, most likely a result of the large number of parameters to be estimated (number of time points × number of doses). On the other hand, the proposed NLME model was able to simultaneously address both circadian rhythm change and QTc prolongation with one or two additional parameters and assuming a functional form of the circadian rhythm.

Normal QTc circadian rhythm can be disrupted via drug-associated changes[22] or disease status.[19] Delaying

the circadian rhythm by temporarily lengthening the period has been reported for an inhibitor of CK1 enzymes.[23,24] In the current simulation analysis, one-directional circadian rhythm change scenarios, either drug-induced phase delay or drug-induced period lengthening, were tested to simplify the simulation scheme (**Figure 2**). The phase-delay scenario was fitted with an additive effect to the phase parameter (φ), and ≥ 98.7% convergence rate was achieved with the first-order conditional estimation with interaction method. The period-lengthening scenario was able to be described by a multiplicative effect on both phase and period parameters, but the first-order conditional estimation with interaction method had numerical difficulties, and therefore the stochastic approximation expectation maximization method was used. With the stochastic approximation expectation maximization run, the convergence rates were still low (64.3–99.9%) compared with phase-delay scenarios. Because a robust inference was the main interest and prespecification of the model is desired for CQTc analysis, modifying the NLME model for a higher convergence rate was not considered. However, it should be noted that when significant period lengthening is expected, standardization of the NLME model could be even more challenging, and a data-driven model development approach would need to be considered.

The are several limitations of this modeling and simulation. First, day −1 baseline ECGs are not available, which could limit detection of circadian rhythm change in the QTc intervals. However, day −1 baseline is not typically acquired in early phase I studies, and placebo data can be used for QTc circadian rhythm detection.[12,25] In addition, the simulation study showed that the NLME model produced acceptable results in the absence of day −1 baseline (**Figures 3 and 4**). Second, direct drug effects on both circadian rhythm and QTc prolongation were assumed, but the time delay between drug exposure and QTc circadian rhythm change may also happen. Third, sensitivities of the simulation results to different parameter sets such as EC50 have not been fully evaluated. Fourth, the simulation comparison was one-sided, i.e., simulation was performed based on the NLME model, and the performance of the prespecified LME model was compared with the true model. Fifth, only two different, one-directional ways of QTc circadian rhythm change were assessed, either phase delay or period lengthening. However, changes in circadian rhythm could be multidirectional, such as phase delay along with amplitude increase.[26] The current study suggested two simple mathematical ways to describe the possible QTc circadian rhythm change, but as more data are gathered with drugs that have the potential to modulate circadian rhythm, the appropriate functional form of rhythmical changes in the QTc interval should be reevaluated.

In conclusion, prespecification of the CQTc model should be reconsidered if circadian rhythm change is expected based on the pharmacology of the drug. Misspecification of the rhythmic change could induce significant type I and type II errors in detecting QT prolongation risk. Developing a more biologically based NLME model in addition to fitting the prespecified LME model enables simultaneous evaluation of drug effect on QTc prolongation as well as circadian rhythm modulation.

1. Melo, M.C. *et al*. Sleep and circadian alterations in people at risk for bipolar disorder: a systematic review. *J. Psychiatr. Res.* **83**, 211–219 (2016).
2. Lewy, A.J., Lefler, B.J., Emens, J.S. & Bauer, V.K. The circadian basis of winter depression. *Proc. Natl. Acad. Sci. USA.* **103**, 7414–7419 (2006).
3. Lewy, A.J. *et al*. Winter depression: integrating mood, circadian rhythms, and the sleep/wake and light/dark cycles into a bio-psycho-social-environmental model. *Sleep Med. Clin.* **4**, 285–299 (2009).
4. McCarthy, M.J. & Welsh, D.K. Cellular circadian clocks in mood disorders. *J. Biol. Rhythms.* **27**, 339–352 (2012).
5. Mairesse, J. *et al*. Chronic agomelatine treatment corrects the abnormalities in the circadian rhythm of motor activity and sleep/wake cycle induced by prenatal restraint stress in adult rats. *Int. J. Neuropsychopharmacol.* **16**, 323–338 (2013).
6. Kaladchibachi, S.A., Doble, B., Anthopoulos, N., Woodgett, J.R. & Manoukian, A.S. Glycogen synthase kinase 3, circadian rhythms, and bipolar disorder: a molecular link in the therapeutic action of lithium. *J. Circadian. Rhythms.* **5**, 3 (2007).
7. Cermakian, N. & Boivin, D.B. A molecular perspective of human circadian rhythm disorders. *Brain Res. Rev.* **42**, 204–20 (2003).
8. Lee, C., Etchegaray, J.P., Cagampang, F.R., Loudon, A.S. & Reppert, S.M. Posttranslational mechanisms regulate the mammalian circadian clock. *Cell* **107**, 855–867 (2001).
9. Xu, Y. *et al*. Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. *Nature* **434**, 640–644 (2005).
10. Isojima, Y. *et al*. CKIepsilon/delta-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proc. Natl. Acad. Sci. USA* **106**, 15744–15749 (2009).
11. Schroder, E.A. *et al*. The cardiomyocyte molecular clock regulates the circadian expression of Kcnh2 and contributes to ventricular repolarization. *Heart Rhythm.* **12**, 1306–1314 (2015).
12. Garnett, C. *et al*. Scientific white paper on concentration-QTc modeling. *J. Pharmacokinet. Pharmacodyn.* **45**, 383–397 (2018).
13. Vink, A.S., Clur, S.B., Wilde, A.A.M. & Blom, N.A. Effect of age and gender on the QTc-interval in healthy individuals and patients with long-QT syndrome. *Trends Cardiovasc. Med.* **28**, 64–75 (2018).
14. Chain, A.S., Krudys, K.M., Danhof, M. & Della Pasqua, O. Assessing the probability of drug-induced QTc-interval prolongation during clinical drug development. *Clin. Pharmacol. Ther.* **90**, 867–875 (2011).
15. Kervezee, L. *et al*. Levofloxacin-induced QTc prolongation depends on the time of drug administration. *CPT Pharmacometrics Syst. Pharmacol.* **5**, 466–474 (2016).
16. Grosjean, P. & Urien, S. Moxifloxacin versus placebo modeling of the QT interval. *J. Pharmacokinet. Pharmacodyn.* **39**, 205–215 (2012).
17. Piotrovsky, V. Pharmacokinetic-pharmacodynamic modeling in the data analysis and interpretation of drug-induced QT/QTc prolongation. *AAPS J.* **7**, E609–E624 (2005).

18. Smetana, P., Batchvarov, V.N., Hnatkova, K., Camm, A.J. & Malik, M. Sex differences in repolarization homogeneity and its circadian pattern. *Am. J. Physiol. Heart Circ. Physiol.* **282**(5), H1889–H1897 (2002).

19. Ong, J.J., Sarma, J.S., Venkataraman, K., Levin, S.R. & Singh, B.N. Circadian rhythmicity of heart rate and QTc interval in diabetic autonomic neuropathy: implications for the mechanism of sudden death. *Am. Heart J.* **125**, 744–752 (1993).

20. Gaudreault, F. *et al.* Prospective application and validation of a systems pharmacology model to guide the clinical development of PF-05251749: a casein kinase 1 (CK1) inhibitor. Abstracts for the Ninth American Conference on Pharmacometrics (ACoP9). *J. Pharmacokinet. Pharmacodyn.* **45**, 3–134 (2018).

21. ICH E14 guideline: the clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Questions & Answers (R3) <https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E14/E14_Q_As_R3__Step4.pdf>.

22. Antimisiaris, M., Sarma, J.S., Schoenbaum, M.P., Sharma, P.P., Venkataraman, K. & Singh, B.N. Effects of amiodarone on the circadian rhythm and power spectral changes of heart rate and QT interval: significance for the control of sudden cardiac death. *Am. Heart J.* **128**(5), 884–891 (1994).

23. Badura, L. *et al.* An inhibitor of casein kinase I epsilon induces phase delays in circadian rhythms under free-running and entrained conditions. *J. Pharmacol. Exp. Ther.* **322**, 730–738 (2007).

24. Meng, Q.J. *et al.* Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. *Proc. Natl. Acad. Sci. USA.* **107**, 15240–15245 (2010).

25. Huh, Y. & Hutmacher, M.M. Evaluating the use of linear mixed-effect models for inference of the concentration-QTc slope estimate as a surrogate for a biological QTc model. *CPT Pharmacometrics Syst. Pharmacol.* **4**(1), e00014 (2015).

26. Kim, J.K. *et al.* Modeling and validating chronic pharmacological manipulation of circadian rhythms. *CPT Pharmacometrics Syst. Pharmacol.* **2**, e57 (2013).