Clinical Pharmacology in Drug Development
2021, 10(3) 272–282

DOI: 10.1002/cpdd.862

Clinical and Model-Based Evaluation of the Effect of Glasdegib on Cardiac Repolarization From a Randomized Thorough QT Study

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Abstract

Glasdegib is a potent, selective oral inhibitor of the Hedgehog signaling pathway. This phase 1 double-blind thorough QT study (NCT03162900) evaluated the effects of glasdegib on QTc interval. The study enrolled 36 healthy volunteers to receive a single dose of 150 mg glasdegib (representing a therapeutic dose), 300 mg glasdegib (representing a supratherapeutic dose), 400 mg moxifloxacin (positive control), or placebo under fasted conditions. The study demonstrated that therapeutic and supratherapeutic doses of glasdegib had no significant effect on QTc interval; the upper bound of the 2-sided 90% confidence intervals (CIs) for all time-matched least-squares mean differences in QT interval corrected using Fridericia's formula (QTcF) between glasdegib and placebo was below the prespecified criterion of 20 milliseconds (Food and Drug Administration correspondence reviewed and accepted). Based on an exposure–response analysis, glasdegib was determined not to have a meaningful effect on heart rate (change in RR interval). The mean (90%CI) model-derived baseline and placebo-adjusted QTcF at the average maximum observed concentration values corresponding to therapeutic and supratherapeutic glasdegib doses was 7.3 milliseconds (6.5-8.2 milliseconds) and 13.7 milliseconds (12.0-15.5 milliseconds), respectively. Together these results demonstrated that following therapeutic and supratherapeutic glasdegib dosing, the change in QTc from baseline was well below the 20-millisecond threshold of clinical concern in oncology.

Keywords

cardiac repolarization, exposure-response, glasdegib, safety, QTc

Glasdegib is a selective, once-daily, oral small-molecule inhibitor of Smoothened, a key protein in the Hedgehog (Hh) pathway. Aberrant Hh signaling has been identified in many solid tumor types and in hematologic malignancies. As an inhibitor of the Hh signaling pathway, glasdegib may act as an inhibitor of leukemic stem cells.[1] Glasdegib is approved by the US Food and Drug Administration (FDA) in combination with low-dose cytarabine (LDAC) for the treatment of newly diagnosed acute myeloid leukemia (AML) in adult patients who are ≥75 years old or who have comorbidities that preclude use of intensive induction chemotherapy.[2]

In preclinical evaluation using an in vitro assay in human embryonic kidney 293 cells, glasdegib demonstrated the ability to inhibit the human ether-à-go-go-related gene (*hERG*) potassium channels in a concentration-dependent manner, suggesting the potential to affect the cardiovascular system.[3] In the first-in-patient dose-escalation study, following single-agent glasdegib treatment (5 to 600 mg once daily), some patients with advanced hematologic malignancies experienced QT corrected for heart rate (QTc) of >500 milliseconds following multiple dosing at the 2 highest evaluated doses of 400 mg once daily (maximum tolerated dose) and 600 mg once daily (maximum administered dose).[4] Based on phase 1 clinical evidence of consistent downregulation of the Hh pathway at ≥100 mg once daily, clinical efficacy signals, and the safety and tolerability profile, as well as to provide an additional exposure margin for possible

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Submitted for publication 18 February 2020; accepted 6 July 2020.

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drug-drug interactions (DDIs) with the potential to increase glasdegib exposure, a 100-mg oral once-daily dose was chosen for further clinical evaluation.[4,5] In a phase 2 study, 100 mg glasdegib once daily was administered in combination with chemotherapy backbone to patients with AML or high-risk myelodysplastic syndromes (MDSs).[6] At the 100-mg once-daily glasdegib dose in combination therapy, QT interval values of >500 milliseconds, corrected using Fridericia's formula (QTcF), or changes in QTcF > 60 milliseconds from baseline were noted in a few instances in the setting of multiple confounders, such as underlying disease and concomitant medications (grade 3 QTcF changes were also observed in patients treated with chemotherapy alone).[6] Consequently, a phase 1 study (study B1371023) in healthy volunteers was designed and conducted to estimate the effect of glasdegib on cardiac repolarization, specifically on the QTc interval.

In this thorough QT (TQT) study, single oral doses of glasdegib 150 and 300 mg were selected to achieve maximum observed plasma concentrations (C_{max}) and exposures representative of steady-state therapeutic (approximately 100 mg once-daily) and supratherapeutic (approximately 200 mg once-daily) doses. The higher single doses used in this study aimed to account for the accumulation of glasdegib following repeated daily dosing.[4] The choice of the supratherapeutic dose was based on available pharmacokinetic (PK) data, the known DDI potential with a strong cytochrome P450 (CYP)3A4/5 inhibitor (ketoconazole), which increased mean C_{max} of glasdegib by 40%, cumulative safety information, and last, the intent to allow for adequate coverage (~100% increase in C_{max}) above and beyond the anticipated maximum exposures in clinical situations.[5] Placebo and positive control (moxifloxacin) treatments were also included in this randomized TQT study, in line with International Council for Harmonisation (ICH) E14 guidance.[7] Although it is difficult to determine whether a mean change in QTc interval can be considered inconsequential, based on the ICH E14 guidelines, the threshold of regulatory concern is that the upper bound of the 1-sided 95% confidence interval (CI) around the largest time-matched mean effect on QTc be <10 milliseconds. In addition, the guidelines indicate that any drug causing mean QTc prolongation >20 milliseconds has a substantially increased likelihood of causing cardiac arrythmias. The threshold of <20 milliseconds is widely accepted for anticancer drugs, given that the potential therapeutic benefit of these agents is frequently deemed to outweigh the risk of cardiac events.[8-10] A model-based population PK/pharmacodynamic (PD) analysis using the clinical data from this TQT study was subsequently performed to characterize the exposure-response (E-R) relationship between glasdegib plasma concentrations and QTc in healthy volunteers. This analysis used a prespecified linear mixed-effects (LME) model recently recommended for use in analyzing electrocardiogram (ECG) concentration data,[11,12] as it allows for the characterization of QTc change from baseline (ΔQTc) under both placebo and active (glasdegib) treatment conditions, as well as model-based prediction of the placebo-adjusted change from baseline in QTc (ΔΔQTc).

Methods

Study Design and Treatments

Study B1371023 (ClincalTrials.gov, NCT03162900) was a phase 1 single-dose, single-center, randomized, double-blind, placebo- and moxifloxacin-controlled, crossover TQT study in healthy volunteers. The subjects, investigator, and site personnel involved in the study were blinded to study treatments (except open-label moxifloxacin), and the sponsor was unblinded. Subjects were randomized to 1 of 4 treatment sequences. Each treatment sequence consisted of 4 treatment periods (placebo, moxifloxacin 400 mg, glasdegib 150 mg, and glasdegib 300 mg) as described in Figure 1, with a washout period ≥6 days between successive study treatment doses. Following an overnight fast (≥10 hours), subjects received oral treatment at ~8:00 AM on day 1 of each period. Subjects were required to refrain from lying down, eating, or drinking beverages other than water during the first 4 hours after dosing.

The primary objective of the study was to estimate the effect of glasdegib on QT/QTc relative to time-matched placebo. Other objectives included: (1) evaluate study sensitivity by assessing the effect of moxifloxacin on QTc interval, (2) assess the safety and tolerability of single doses of glasdegib in healthy adult volunteers, and (3) evaluate the PK of glasdegib and the relationship between QTc and plasma concentrations.

This study was conducted at the Pfizer Clinical Research Unit, Belgium, in compliance with the ethical principles originating in or derived from the Declaration of Helsinki and in compliance with all ICH Good Clinical Practice guidelines. The final study protocol and informed consent documentation were approved by the institutional review board, the Comite d'Ethique Hospitalo-Facultaire Erasme-ULB, Brussels, Belgium. All subjects provided written informed consent prior to participating and before any screening procedures were initiated.

Subjects

Eligible subjects included healthy women of nonchildbearing potential and men aged 18 to 55 years, with a body mass index of 17.5 to 30.5 kg/m^2, body weight

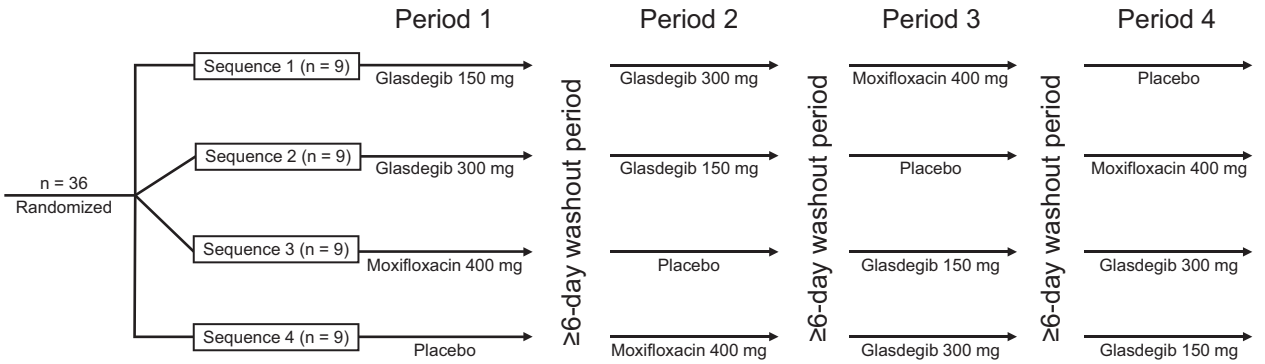

Figure 1. Trial design and randomization scheme. Healthy subjects received each of the 4 treatments (glasdegib 100 mg, glasdegib 300 mg, moxifloxacin 400 mg, and placebo) in the order randomly assigned by their treatment sequence.

>50 kg, and no known history of QTc prolongation, cardiovascular disease, or ECG abnormalities.

Safety and Electrocardiogram Assessments

Safety evaluations included monitoring adverse events (AEs) and serious AEs (SAEs), safety laboratory tests, physical examinations, vital signs, and 12-lead ECGs. Using the semiautomated method, triplicate 12-lead (with a 10-second rhythm strip) measurements in the supine position were collected ~2 minutes apart to determine the mean QTc interval. ECG assessment for all treatments occurred –1, –0.5, and 0 hours predose and 0.5, 1, 1.5, 2, 3, 4, 6, and 24 hours postdose and were collected prior to blood draws. Baseline ECG values were determined by averaging the mean of the triplicates collected –1, –0.5, and 0 hours predose.

Pharmacokinetic Evaluation

Blood samples for PK analysis were collected 0, 0.5, 1, 1.5, 2, 3, 4, 6, 24, 72, 96, and 120 hours postdose using collection tubes with dipotassium ethylenediaminetetraacetic acid anticoagulant. All study treatments had identical sample collection times.

Moxifloxacin samples were planned to be analyzed only if deemed necessary (ie, if no positive QTc signal was observed); based on the observed results, the analysis of moxifloxacin was not required. Analysis of placebo PK samples was also not performed.

Glasdegib plasma concentrations were measured using a validated, sensitive, and specific high-performance liquid chromatography-tandem mass spectrometric method at Covance Bioanalytical Services (Shanghai, China).[13] Calibration curves were linear over the range of 3 to 3000 ng/mL for glasdegib in plasma, using weighted (1/concentration^2) linear regression. The lower limit of quantification (LLOQ) of glasdegib was 3 ng/mL. PK plasma samples were stored at −70°C and assayed within the 575 days of established frozen plasma stability. Interassay accuracy (percentage relative error) at 9, 100, 2250, and 15 000 (diluted 10-fold) ng/mL glasdegib in quality-controlled plasma samples ranged from −1.8% to 11.3%. Interassay precision (percentage coefficient of variation [%CV]) was ≤6.4% across quality-control levels.

Glasdegib PK parameters including C_{max}, time when C_{max} was reached (T_{max}), area under the plasma concentration-time profile (AUC) from time 0 to the time of the last quantifiable concentration, AUC from time 0 extrapolated to infinite time (AUC_{inf}), apparent oral clearance, apparent volume of distribution, and terminal half-life were calculated using noncompartmental analysis of plasma concentration-time data. Samples below the LLOQ were set to 0 for analysis.

Sample Size Determination

A sample size of 36 subjects (9 per sequence) provided at least 98% power to exclude the upper bound of 2-sided 90%CIs (equivalent to a 1-sided 95%CI) of a time-matched difference in QTcF between glasdegib and placebo of >20 milliseconds (FDA correspondence reviewed and accepted) at each point.[3] The overall study power for 8 postdose times following the day 1 dose was ≥85%. These calculations were based on the assumptions that the expected mean difference in QTcF between glasdegib and placebo would be no greater than 15 milliseconds at each time, and the intrasubject variability was 5.27 milliseconds, based on the mean of 13 previous Pfizer TQT studies (data on file).

Given a 1-sided significance of 0.05, 36 subjects provided ≥99% power to detect ≥5-millisecond difference in QTcF between moxifloxacin and placebo 3 hours postdose to demonstrate the assay sensitivity.[14-17]

Statistical Analysis

The PK parameter analysis population was defined as all subjects randomized and treated who had at least 1 of the glasdegib PK parameters of primary interest in at least 1 treatment period.

The ECG analysis population was defined as all subjects randomized and treated who had at least 1 postdose ECG measurement in at least 1 period. The average of triplicate ECG measurements was used in all statistical analyses. All statistical analysis was conducted in SAS version 9.1, TS2M3 (SAS Institute, Cary, North Carolina). The postdose QTcF intervals were analyzed with baseline as a covariate. Analysis of covariance (ANCOVA) was conducted using a mixed-effects model with sequence, period, treatment, time, and treatment-by-time interaction as fixed effects, subject within sequence as a random effect, and baseline QTcF as a covariate. The 2-sided 90%CI (equivalent to a 1-sided 95%CI) for time-matched change from placebo in QTcF at each time on day 1 was computed for each dose of glasdegib and moxifloxacin.

Given glasdegib's indication for use in oncology patients, the study was designed to exclude a large effect of glasdegib on the QTc interval. Absence of large effect of glasdegib on the QTc interval was to be concluded if the upper bounds of the CIs for all the time-matched mean differences between glasdegib and placebo were <20 milliseconds (FDA correspondence reviewed and accepted).[3] This study was deemed adequately sensitive to detect QT/QTc prolongation if the lower bound of the 2-sided 90%CI for the mean difference between moxifloxacin and placebo was >5 milliseconds 3 hours postdose. Categorical analysis of QTcF for absolute postdose maximum value and maximum increase from baseline was also generated.

Exposure-Response Analysis

Using the clinical data from the TQT study, further analyses were conducted to characterize the E-R relationship between glasdegib plasma concentration and QTc using a PK/PD model.

Characterization of the QTc-concentration relationship was performed in the following stepwise manner:

(1) The effect of glasdegib on heart rate (RR interval) was evaluated to support the assumption that the QT-RR relationship is the same regardless of the presence or absence of drug.
(2) The concordance in the time course of glasdegib plasma concentrations and QTcF (absence of hysteresis) was evaluated through assessment of the PK profiles along with the placebo-adjusted change from baseline in QTcF ($\Delta\Delta$QTcF) profiles over time, by dose level.
(3) The QT interval correction for RR was determined. Although QTcF was the primary end point for analysis, LME methods were also used to estimate a study population-specific correction factor (β) to allow for determination of study population-specific QTc (QTcS).
(4) QTcF, QTc using Bazett's formula, and the QTcS factors were evaluated for appropriateness (elimination of the QTc-RR relationship).
(5) The presence of a linear QTc-concentration relationship was verified to support the use of a linear PK/PD model.
(6) The relationship between baseline-adjusted QTc (ie, ΔQTcF, ΔQTcS) and glasdegib plasma concentration was evaluated initially with a prespecified LME model[11] in which the QTc and concentration data from both placebo and glasdegib treatment periods (therapeutic and supratherapeutic) were analyzed, with concentrations during placebo treatment set to 0. This base model to describe the dependent variable ΔQTc (QTc change from period-specific baseline at time = 0) included the following fixed-effect parameters: intercept, slope, and the effects of treatment (categorical), time (categorical), and baseline QTc (continuous) on the intercept (see Equation 1 at the end of this article). Characterizing the placebo response at each nominal time accounted for the effect of diurnal variation in QTc. Subject was included as a random effect on both the intercept and slope. A nonsignificant slope would indicate a lack of evidence of an effect of glasdegib concentration on the QTc interval.

    Interindividual variability was included for the mean population parameters of both intercept and slope using an additive error model for each individual. Residual variability was also modeled as an additive error.
(7) Evaluation of model adequacy (goodness of fit) was completed through various diagnostic plots. Model predictive performance was assessed through visual predictive checks (VPCs).
(8) The model-derived difference in baseline-corrected QTc (ie, $\Delta\Delta$QTcF, $\Delta\Delta$QTcS) was computed across relevant glasdegib concentrations using the final PK/PD model. This provided the mean and 2-sided 90%CI for $\Delta\Delta$QTc at concentrations of interest (eg, C_{max} at therapeutic dose (100 mg once daily) and supratherapeutic dose (200 mg once daily) based on prior observed data in the patient population).[6,18]

Data manipulation, post-processing, and graphics were conducted using R Studio (version 3.4.1). Estimation was conducted using the nlme library (version 3.1-131).

Results

Subject Disposition and Baseline Characteristics

A total of 36 subjects were enrolled and randomized. All subjects were male, with a mean age of

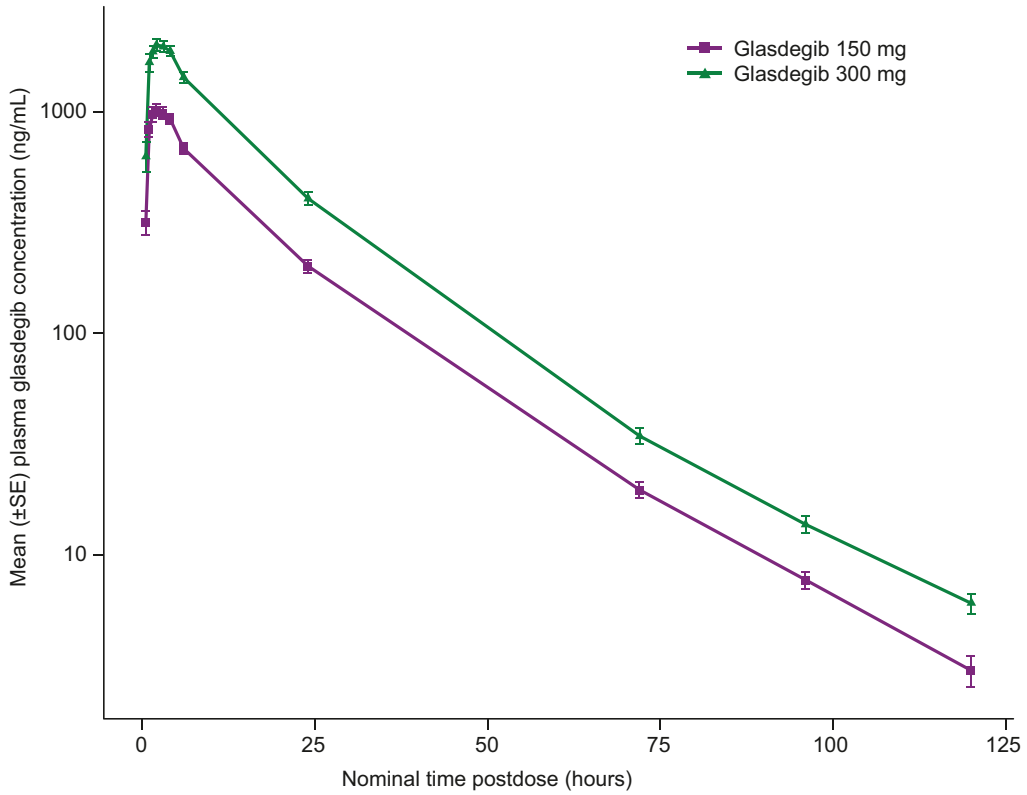

Figure 2. Arithmetic mean ± standard error plasma glasdegib concentration-time profiles following single oral doses of 150 and 300 mg. Values below the limit of quantitation were set to zero for calculation of summary statistics.

36.2 years, and 89% were white (Supplementary Table S1). One subject was discontinued from the study, following placebo and moxifloxacin dosing periods only because of an AE of alanine transferase elevation.

Pharmacokinetics

Median glasdegib plasma concentration-time profiles following single oral doses of 150 and 300 mg are presented in Figure 2, demonstrating a median T_{max} of 2 hours postdose with a range of 1 to 4 and 1 to 6 hours, respectively. The geometric mean AUC_{inf} and C_{max} increased proportionally with dose, and variability (%CV) ranged from 30% to 35% for AUC_{inf} and from 27% to 29% for C_{max}. Glasdegib PK parameters by dose are summarized descriptively in Table 1.

Electrocardiogram

ANCOVA (baseline as a covariate) statistical analysis of QTcF during moxifloxacin treatment compared with placebo demonstrated adequate sensitivity to assess the effect of glasdegib on the QTcF interval (Figure 3 and Table 2). None of the subjects met categorical criteria of absolute QTcF interval ≥480 seconds or increase from baseline in QTcF interval ≥30 milliseconds after receiving any treatment. The upper bound of the 2-sided 90%CIs (equivalent to 1-sided 95%CI) for all time-matched least-squares mean differences in QTcF between glasdegib 300 mg (supratherapeutic plasma exposure) and placebo were less than the predefined cutoff of 20 milliseconds; the highest 90%CI upper bound for the largest, placebo- and baseline-adjusted

Table 1. Descriptive Summary of Plasma Glasdegib Pharmacokinetic Parameters

Parameter, Units[a]	Glasdegib 150 mg	Glasdegib 300 mg
n	35	35
AUC_{inf}, ng·h/mL	16 180 ± 5733.9	32 420 ± 9865.5
AUC_{last}, ng·h/mL	16 070 ± 5728.5	32 300 ± 9837.4
C_{max}, ng/mL	1119 ± 387.6	2298 ± 651.0
T_{max}, h	2.00 (1.00-4.02)	2.00 (1.00-6.00)
CL/F, L/h	10.37 ± 3.58	10.09 ± 3.05
$t_{1/2}$, h	15.83 ± 2.06	15.52 ± 1.77
AUC_{extrap}, %	0.6660 ± 0.30	0.4113 ± 0.19

AUC_{extrap}%, percentage of AUC_{inf} that was extrapolated; AUC_{inf}, area under the plasma concentration-time profile from time 0 extrapolated to infinite time; AUC_{last}, area under the plasma concentration-time profile from time 0 to the time of the last quantifiable concentration; CL/F, apparent oral clearance; C_{max}, maximum observed concentration; SD, standard deviation; $t_{1/2}$, terminal half-life; T_{max}, time when maximum observed concentration was reached.

[a]Arithmetic mean ± SD for all except median (range) for T_{max}.

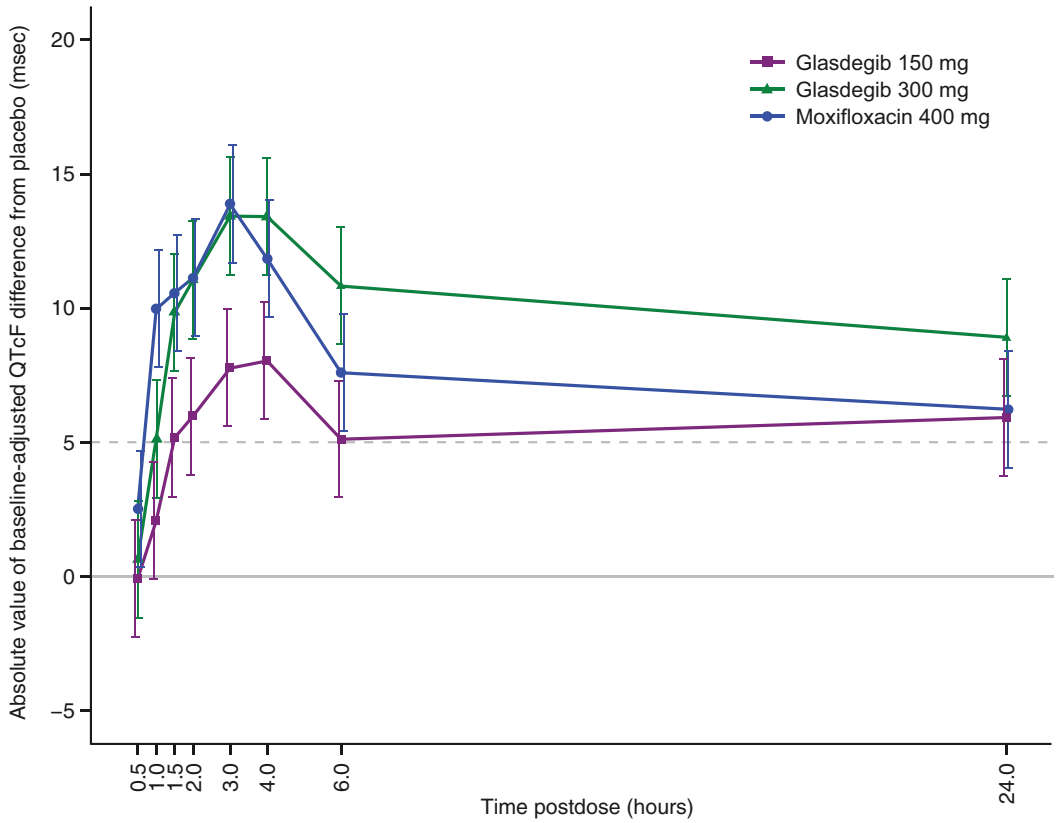

Figure 3. Plot of estimated LS mean treatment differences versus placebo of QTcF with 90% confidence intervals by treatment over time postdose. The 5-millisecond horizontal line represents the sensitivity criteria. LS mean, least-squares mean; QTcF, QT interval corrected for heart rate using Fridericia's formula.

QTcF change was 15.61 milliseconds 3 hours postdose (Table 2).

Similarly, for glasdegib 150-mg treatment (therapeutic plasma exposure), the highest 90%CI upper bound for the largest and placebo- and baseline-adjusted QTcF change was 10.22 milliseconds 4 hours postdose (Table 2). Therefore, the absence of a large effect of glasdegib on the QTcF interval was demonstrated in this study at both therapeutic and supratherapeutic C_{max}.

Safety

No deaths, SAEs, medication errors, or discontinuations from the study or study treatment because of AEs were reported during the study. There were 25 treatment-emergent AEs (TEAEs) with 20 treatment-related AEs (TRAEs) following administration of glasdegib 150 mg, 26 TEAEs with 20 TRAEs following administration of glasdegib 300 mg, 27 TEAEs with 23 TRAEs following administration of moxifloxacin 400 mg, and 32 TEAEs with 27 TRAEs reported for placebo. All the AEs reported were considered mild to moderate in severity. Alanine transaminase $>3 \times$ the upper limit of normal observed in 1 subject was reported as an AE; this subject did not receive glasdegib treatment.

Exposure-Response Analysis

Data from all 35 subjects who received glasdegib were included in the analysis. Singlet ECG readings as well as the average of the triplicates were included, resulting in 9 time-matched ECG-PK pairs per treatment arm at nominal times between 0 and 24 hours postdose in the analysis data set. No ECG readings or PK sample results were missing.

Plots and results from the LME model of RR change from baseline (ΔRR) versus concentration indicated a relationship of negligible magnitude between ΔRR and glasdegib concentration (Supplementary Figure S1), and it was concluded that fixed correction factors (ie, β) were adequate to eliminate the underlying RR effect on the QT intervals, regardless of the concentration of glasdegib. No PK/PD hysteresis, indicating a lag between plasma concentration and effect on QTc, was observed.

For determination of QTcS, the study population-specific QTc factor (β) and corresponding 95%CI were estimated to be 0.250 (0.220-0.281). On assessment of

Table 2. ANCOVA Statistical Summary Comparison of QTcF Between Moxifloxacin, Glasdegib, and Placebo Postdose

		Moxifloxacin 400 mg, N = 36		Glasdegib 150 mg, N = 35		Glasdegib 300 mg, N = 35	
Nominal Time Postdose (h)	Placebo N = 36 LS Mean, Milliseconds	LS Mean, Milliseconds	Difference (Moxifloxacin-Placebo), Milliseconds (90%CI)	LS Mean, Milliseconds	Difference (Glasdegib-Placebo), Milliseconds (90%CI)	LS Mean, Milliseconds	Difference (Glasdegib-Placebo), Milliseconds (90%CI)
0.5	406.53	409.05	2.53 (0.36-4.69)	406.43	−0.09 (−2.28 to 2.09)	407.18	0.65 (−1.53 to 2.83)
1	405.79	415.74	9.96 (7.79-12.12)	407.86	2.07 (−0.11 to 4.25)	410.92	5.14 (2.96-7.32)
1.5	404.91	415.48	10.57 (8.41-12.74)	410.08	5.17 (2.99-7.35)	414.75	9.84 (7.66-12.02)
2	404.60	415.74	11.13 (8.97-13.30)	410.58	5.98 (3.80-8.16)	415.66	11.06 (8.88-13.24)
3	404.29	418.16	13.87 (11.70-16.03)	412.06	7.76 (5.58-9.95)	417.72	13.43 (11.25-15.61)
4	405.19	417.03	11.84 (9.67-14.00)	413.23	8.03 (5.85-10.22)	418.60	13.41 (11.23-15.59)
6	402.27	409.86	7.59 (5.43-9.76)	407.38	5.11 (2.93-7.29)	413.08	10.82 (8.64-13.00)
24	405.05	411.29	6.23 (4.07-8.40)	410.98	5.92 (3.74-8.10)	413.96	8.91 (6.73-11.09)

ANCOVA, analysis of covariance; CI, confidence interval; LS mean, least-squares mean; QTcF, QT interval corrected for heart rate using Fridericia's formula.
Baseline was defined as the mean of the 3 average triplicate measurements taken at 3 times (–1 hour, –0.5 hour, and 0 hours) before dosing within each period and used as a covariate in the model.

Table 3. Final Model Results and Model-Derived Predictions for QTcF and QTcS

		Model-Derived ΔΔQTc, Milliseconds (90%CI)	
QTc Parameter	Slope Estimate From Final Model, ΔQTc (Milliseconds) per ng/mL (95%CI)	Therapeutic Steady-State C_{max} (1137 ng/mL)	Supratherapeutic Steady-State C_{max} (2445 ng/mL)
QTcF	0.005 (0.004-0.006)	7.34 (6.46-8.22)	13.72 (11.95-15.49)
QTcS	0.004 (0.003-0.005)	6.55 (5.68-7.43)	11.89 (10.14-13.64)

CI, confidence interval; C_{max}, maximum plasma concentration; QTc, QT interval corrected for heart rate; QTcF, QTc using Fridericia's formula; QTcS, study population-specific QTc; ΔQTc, QTc change from baseline; ΔΔQTc, placebo-adjusted QTc change from baseline.

all correction factors, Fridericia's method did not completely remove the relationship between RR and QTc, with a slope estimate of –0.003 (95%CI, −0.042 to −0.019); see Supplementary Figure S2. QTcS correction fully eliminated the relationship between QT and RR, with a slope estimate of 0.001 (95%CI, −0.011 to 0.013). Although Fridericia's correction did not completely eliminate the QTc-RR relationship, the slope estimate for the correction was quite small, and given that QTcF has been demonstrated to be an adequate correction factor in other analyses, QTcF was used in the primary E-R analysis, with additional analysis using QTcS provided.[19]

The mean slope estimate describing the ΔQTcF-concentration relationship in this study was 0.005 ms/ng/mL, with the 95%CI around the slope estimate excluding 0, indicating a positive concentration-dependent effect of glasdegib plasma concentration on the length of QTcF (Table 3, Supplementary Table S2). Change in ΔQTcS was similar, with a slightly smaller slope. Because the study population was composed solely of healthy male subjects of a narrow age range, screening of intrinsic and extrinsic factors (covariates) was not performed; the final model was the same as the base model.

Diagnostic plots to assess model adequacy, and goodness of fit did not show any evidence of model misspecification. Scatterplots of observed versus model-predicted (population and individual) dependent variable (ΔQTc) values did not demonstrate any evidence of over- or underprediction of ΔQTcF (Supplementary Figure S3); plots of standardized residuals did not display any systematic trends (ie, residuals were randomly scattered about 0; Supplementary Figure S4), and quantile-quantile plots (not shown) and boxplots (Supplementary Figure S5) of standardized residuals supported the assumption of a normally distributed residual error, which was independent of time and treatment.

Adequate predictive performance of the final models was demonstrated through VPCs; the percentiles calculated from the observed data were generally

contained within the 95%CIs estimated from the simulated data at the 2.5th, 50th (median), and 97.5th percentiles (Figure 4A).

The model-derived predicted mean (90%CI) estimates for ΔΔQTcF using the final LME model at the average steady-state C_{max} following once-daily dosing of glasdegib 100 mg (therapeutic) and once-daily dosing of 200 mg (supratherapeutic) are shown in Table 3 and Figure 4B. At the supratherapeutic dose, the mean predicted increase in ΔΔQTcF was 13.72 milliseconds, with the upper 90%CI <20 milliseconds.

Discussion

This report of a TQT study performed with glasdegib in healthy volunteers demonstrates that although glasdegib had an effect on cardiac repolarization, it was below the threshold of clinical concern in the context of an oncology setting. This conclusion is based on the results of the primary statistical analysis in the TQT study, as the upper bound of the 2-sided 90%CIs for all time-matched least-squares mean differences in QTcF between glasdegib (therapeutic and supratherapeutic exposures) and placebo was <20 milliseconds. Although a positive effect on the QTcF interval was observed in the TQT study, this increase did not reach the prespecified threshold of clinical concern (20 milliseconds, FDA reviewed and accepted) generally accepted for oncology therapies, even at the supratherapeutic dose. The design of the TQT study included the advantages of double blinding and full randomization, statistical powering, robust PK/QT monitoring following dosing to capture the time course around the C_{max} as well as at later times, and finally, exclusion of comorbidities and concomitant medications, which may act as confounders in the cancer treatment setting. Furthermore, the study was determined to be adequately sensitive to assess the effect of glasdegib on the QTcF interval by evaluating the effect of a positive control (moxifloxacin) relative to a time-matched placebo control at the historical T_{max} for moxifloxacin. Categorical analyses showed that no subjects in the TQT study had absolute QTcF values ≥480 milliseconds postdose or a ≥30-millisecond increase from baseline in QTcF. These findings were in contrast to grade 3 QTcF changes observed in patients with AML and high-risk MDS receiving glasdegib + LDAC.[6] However, cases of QTcF prolongation were also reported in patients receiving LDAC alone, making conclusions about the effect of glasdegib on QTc difficult and confirming the impact of confounders in the patient setting. In contrast, the TQT study, conducted in a more controlled setting with exclusion of major confounders, adequate controls (placebo, moxifloxacin), and robust statistical

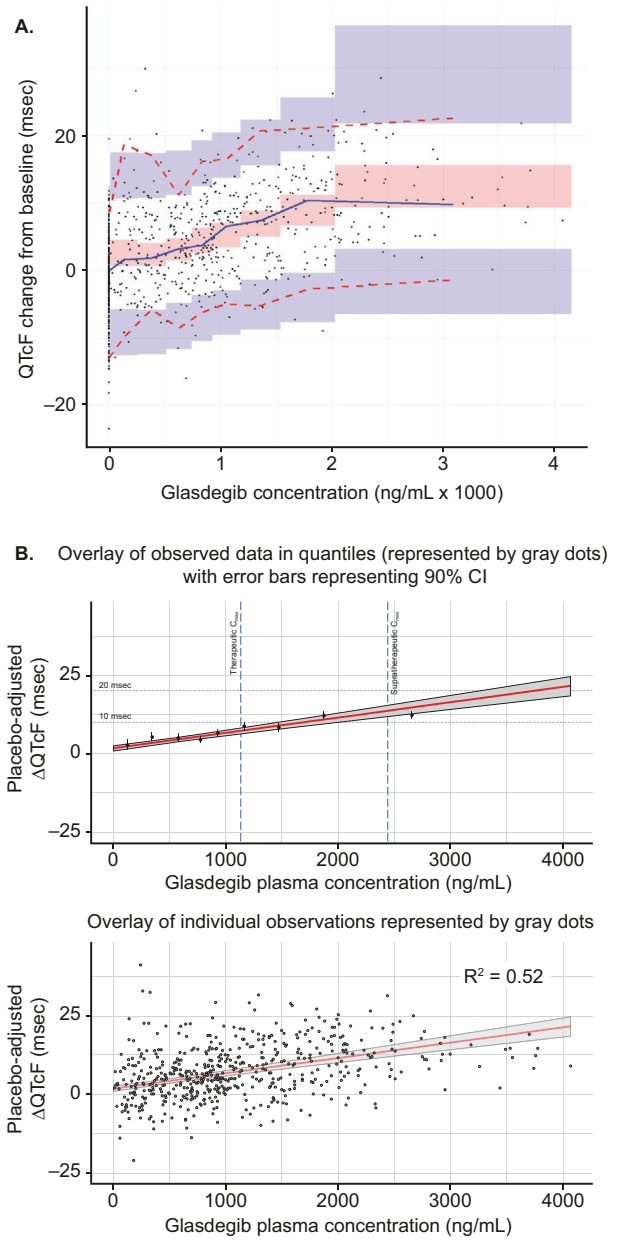

Figure 4. (A) Visual predictive check of the final ΔQTcF model. The red lines represent the 2.5th and 97.5th observed percentiles, and the blue line represents the median observed percentile. The shaded areas are the 95%CI of the 2.5th, 50th, and 97.5th percentiles estimated from the simulated data (1000 simulations). The black points represent the observed data overlaid on the plot. (B) Plot of observed and model-predicted placebo-adjusted change from baseline in QTcF versus glasdegib concentration. The red line represents mean model prediction with 90%CI in gray with blue outline. CI, confidence interval; C_{max}, maximum observed concentration; QTcF, QT interval corrected for heart rate using Fridericia's formula; ΔQTcF, change from baseline in QTcF.

rigor, facilitated the isolation of the true effects of glasdegib on cardiac repolarization. Furthermore, the E-R analysis to evaluate the relationship between ΔQTc and glasdegib concentration was consistent with these above-mentioned results from the primary statistical analysis in the TQT study, supporting the conclusion that glasdegib treatment does not have a large effect on the QTcF at clinically relevant exposures or at those ~2-fold higher.

This E-R analysis used a prespecified LME model that has recently been recommended for use in analyzing ECG-concentration data such as this.[11] The LME model allowed for the characterization of ΔQTc under both placebo and active (glasdegib) treatment conditions, accounting for diurnal variation and allowing for model-based prediction of the ΔΔQTc through simulating ΔQTc under both placebo and treatment conditions. Further, such a PK/PD model allows for predicting the change in QTc that may be expected at various drug concentrations of clinical interest (including those following different doses/dosing regimens not included in the TQT study or in special populations or DDI scenarios, for example), along with associated CIs. Because of the nature of the healthy volunteer study, including the homogeneity of the subjects enrolled and the lack of impact of any tested covariates in the previous population PK/PD evaluation of glasdegib in cancer patients, intrinsic or extrinsic factors were not tested in this analysis of the QTc-concentration relationship.[19]

Previous DDI evaluation with a strong CYP3A4/5 inhibitor, ketoconazole, demonstrated a 40% mean increase in C_{max} of glasdegib.[5] At a glasdegib steady-state C_{max} representing such a 40% increase (1592 ng/mL), the upper bound of the 90%CI of model-predicted ΔΔQTcF is just above 10 milliseconds (mean, 9.5 milliseconds; 90%CI, 8.41-10.71 milliseconds). The TQT study was also designed to evaluate a more extreme worst-case scenario. At double the clinical dose, which is greater than the anticipated exposure even under DDI conditions, the upper bound of the model-predicted 90%CI for ΔΔQTcF (and ΔΔQTcS) is <20 milliseconds. The supratherapeutic concentrations obtained in this study and corresponding predicted ΔΔQTc were designed to provide additional exposure margins to account for potentially higher glasdegib exposures, which, theoretically, could be achieved in patients with organ impairment, based on the metabolic (CYP3A4/5) and renal excretion pathways involved in the elimination of glasdegib. Therefore, glasdegib treatment is not expected to lead to a ≥20-millisecond prolongation from baseline in QTcF under clinical conditions.

As expected, QTcS was the most appropriate correction factor, with a β estimated to be 0.250, close to the β of 0.333 used in Fridericia's correction. Modeling was performed using both QTcF and QTcS, because QTcF is considered the most clinically relevant factor and allows for QTc interval comparison across studies and compounds, as it does not depend on a particular population. The results from the ΔQTcF-concentration analysis were similar to those from the ΔQTcS-concentration analysis, and the overall conclusion from both models was the same: the upper bound of the 90%CI of the model-predicted ΔΔQTc in the worst-case-scenario supratherapeutic concentrations remained <20 milliseconds.

Conclusion

Absence of a large effect of glasdegib on the QTc interval was demonstrated at the therapeutic and supratherapeutic doses (the upper bounds of the 90%CIs were below 20 milliseconds for time-matched differences in baseline-corrected QTcF between treatment and placebo). Single oral doses of glasdegib at 150 and 300 mg were well tolerated, with an acceptable safety profile in healthy adult subjects.

From the E-R analysis using a prespecified LME model, glasdegib was determined not to have a meaningful effect on heart rate. At the mean therapeutic glasdegib C_{max} value previously observed in patients, the mean predicted increase in ΔΔQTcF was 7.34 milliseconds, with the upper bound of the 90%CI <10 milliseconds. At the mean supratherapeutic glasdegib C_{max} value observed in patients (ie, twice the therapeutic dose), the mean predicted increase in ΔΔQTcF was 13.72 milliseconds, with the upper bound of the 90%CI below the 20-millisecond threshold of clinical concern in oncology. Therefore, glasdegib treatment is not expected to lead to a large effect on the QTcF interval under clinical conditions.

Equation 1. The prespecified linear mixed-effects model for ΔQTc versus concentration

$$\Delta QTc_{ijk} = (\theta_j + \beta_{0k} + \eta_{0,i}) + \gamma(QTc_{ij0} - \overline{QTc_{ij0}}) + (\beta_1 + \eta_{1,i}) \cdot C_{ijk} + \varepsilon_{ijk}$$

where ΔQTc_{ijk} is the change from baseline in QTc for the i*th* subject in the *j*th treatment at the k*th* time relative to dosing; where θ_j is the treatment-specific intercept (any glasdegib versus placebo), β_{0k} is the population mean ΔQTc with placebo for time *k* (categorical fixed effect of time (diurnal variation); QTc_{ij0} is the subject and treatment-specific baseline QTc, $\overline{QTc_{ij0}}$ is the overall population mean of all baseline QTc values, and γ is the influence of the baseline QTc (centered on population baseline); $\beta 1$ is the slope that quantifies the relationship between ΔQTc and concentration; C_{ijk} is the concentration (and $C_{i0k} = 0$ for placebo); $\eta_{0,i}$ and $\eta_{1,i}$ are the subject-specific random effects (interindividual variability) for the intercept and slope, respectively,

each with a mean of 0 and variance of ω^2, and ε is the residual error, with a mean of 0 and variance of σ^2.

Acknowledgments

Medical writing support was provided by Gemma Shay, PhD, of Engage Scientific Solutions, and was funded by Pfizer.

Conflicts of Interest

The authors are employees of Pfizer, and own stock in Pfizer.

Funding

This study was funded by Pfizer.

Data Sharing

Upon request, and subject to certain criteria, conditions, and exceptions (see https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information), Pfizer will provide access to individual deidentified participant data from Pfizer-sponsored global interventional clinical studies conducted for medicines, vaccines, and medical devices: (1) for indications that have been approved in the United States and/or the European Union; or (2) in programs that have been terminated (ie, development for all indications has been discontinued). Pfizer will also consider requests for the protocol, data dictionary, and statistical analysis plan. Data may be requested from Pfizer trials 24 months after study completion. The deidentified participant data will be made available to researchers whose proposals meet the research criteria and other conditions, and for which an exception does not apply, via a secure portal. To gain access, data requestors must enter into a data access agreement with Pfizer.

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Supplemental Information

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