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ORIGINAL ARTICLE

A single-dose placebo- and moxifloxacin-controlled study of the effects of temsirolimus on cardiac repolarization in healthy adults

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Abstract
Purpose Temsirolimus, a selective inhibitor of mammalian target of rapamycin, is an approved treatment for patients with advanced renal cell carcinoma (RCC). This study assessed the effect of intravenous (i.v.) temsirolimus 25 mg, the recommended dose for patients with RCC, on the corrected QT (QTc) interval.
Methods This 3-period crossover study enrolled healthy subjects. In periods 1 and 2, subjects received i.v. placebo either alone or with open-label oral moxifloxacin. In period 3, subjects received a single dose of temsirolimus 25 mg. The primary statistical objective was to estimate the effect of temsirolimus compared with placebo on change from time-matched baseline QTc at the end of infusion (0.5 h). Assay sensitivity was evaluated by the effect of moxifloxacin on change from time-matched baseline QTc compared with placebo.
Results In total, 58 subjects were enrolled. Temsirolimus had no effect on QTc interval in the primary analysis. At 11 of 12 secondary time points, the upper bound for the temsirolimus QTc 90% confidence intervals for the time-matched change from baseline difference from placebo was <10 ms, with no evidence of QTc trends or relationship to concentrations of temsirolimus or its major metabolite, sirolimus. Moxifloxacin, the positive control, produced a significant increase in the QTc interval compared with placebo 0.5–4 h post-dose ($P < 0.0001$). No subject had a QTc interval exceeding 450 ms or a change from baseline of >30 ms.
Conclusions Therapeutic exposure to temsirolimus is not associated with clinically significant changes in QTc intervals in healthy adults.

Keywords Temsirolimus · Cardiac arrhythmia · Drug safety · mTOR · Renal cell carcinoma · Targeted therapy

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Introduction

Electrocardiographic (ECG) characterization of the effects on the QT interval is an increasingly important safety and regulatory concern in the development of new pharmacologic agents [1–3]. The QT interval is a measure of the duration of ventricular depolarization and repolarization and provides a surrogate end point for ventricular tachycardia. Prolongation of the QT interval increases the risk of cardiac arrhythmias, including torsade de pointes, which can lead to ventricular fibrillation and sudden death.

Manifestations of torsade de pointes include palpitations and symptoms of impaired cerebral circulation (e.g., dizziness, syncope, or seizure). Several factors can affect the QT interval, such as heart rate (HR), age, gender, electrolyte disturbances, and foods. Certain cardiac and non-cardiac drugs are also known to delay cardiac repolarization and prolong the QT interval. Consequently, all therapeutic agents in clinical development are required to undergo assessment of their effects on the QT interval as an integral component of cardiac safety evaluation.

Temsirolimus is a novel targeted therapy that selectively inhibits mammalian target of rapamycin (mTOR), a serine/threonine kinase that regulates cell growth and proliferation by controlling protein translation [4]. mTOR phosphorylates the eukaryotic translation initiation factor 4E-binding protein-1 (eIF-4BP1) and ribosomal S6 protein (p70S6K), resulting in the translation of cell cycle regulatory proteins (e.g., D-type cyclins, c-myc, and ornithine decarboxylase). mTOR also upregulates hypoxia-inducible factor (HIF) and HIF target genes, including the pro-angiogenic factor vascular endothelial growth factor (VEGF). Temsirolimus inhibits mTOR, resulting in antitumor and antiangiogenic activity. Pharmacologically, temsirolimus is intrinsically active and is metabolized by cytochrome P450 (CYP) 3A4 and 3A5, producing the active metabolite sirolimus [5].

Temsirolimus is approved worldwide for the treatment of advanced renal cell carcinoma (RCC) and, in Europe, for the treatment of relapsed/refractory mantle cell lymphoma (MCL). In a large, global, randomized phase 3 clinical trial, patients with previously untreated advanced RCC and poor prognostic factors who received once-weekly intravenous (i.v.) infusions of temsirolimus 25 mg experienced significantly longer overall survival ($P = 0.008$) and progression-free survival ($P < 0.001$) than did patients who received interferon alfa [6].

The present study was conducted to directly assess the effects of a single dose of i.v. temsirolimus 25 mg on the QT interval. According to the International Conference for Harmonization (ICH) E14 guidance, an ideal, thorough QT study to detect drug-induced QT prolongation should include placebo and a positive control drug with known effects on cardiac repolarization (e.g., moxifloxacin), as well as evaluation at supratherapeutic dose levels [7, 8]. Unfortunately, such study designs are often infeasible in patients with advanced cancer owing to their non-therapeutic nature, cumbersome logistics, and the need for a positive control drug. As a result, it is common for QT assessments of anticancer agents to be designed as single-dose studies in healthy subjects. Because i.v. temsirolimus 25 mg has an acceptable safety and tolerability profile in healthy subjects when administered as a single dose [9, 10], it was feasible to conduct this placebo- and moxifloxacin-controlled study at the RCC therapeutic dose in a non-cancer population. The 25 mg dose was previously defined as the highest dose considered to be feasible and ethical for testing in healthy subjects, thus a supratherapeutic dose, as recommended by the ICH for a thorough QT study, was not included in this study.

Methods

Study population

Eligible subjects were healthy men or women (non-childbearing potential) aged 18–50 years, with a body mass index between 18 and 30 kg/m^2 and body weight of at least 50 kg. The investigator determined health status on the basis of medical history, physical examination, clinical laboratory test results, vital signs, and 12-lead electrocardiogram (ECG). Subjects were excluded if they had any cardiovascular, hepatic, renal, respiratory, gastrointestinal, endocrine, immunologic, dermatologic, hematologic, neurologic, or psychiatric disease, or any surgical or medical condition that may interfere with the distribution, metabolism, or excretion of temsirolimus or moxifloxacin. Other exclusion criteria included risk factors for torsades de pointes (including heart failure, hypokalemia, hypomagnesaemia, or hypocalcaemia); a history of long QT syndrome, syncope, seizure, or the unexplained cardiac-related death of a family member under 30 years of age; any clinically important deviation from normal limits (physical examination, vital signs, 12-lead ECG, or clinical laboratory test results); or QTc duration $\geq$470 ms in women based on the machine read tracing at screening or on day 2.

Study design and treatments

This was a single-dose, single-blind with respect to temsirolimus, crossover, placebo- and moxifloxacin-controlled inpatient study. The protocol was reviewed and approved by the Independent Investigational Review Board of SeaView Research, Inc, in Plantation, Florida (IRB #: IRB00003563). Written informed consent was obtained from all subjects at the time of enrollment, and the study was conducted in accordance with the Declaration of Helsinki and its amendments.

Subjects participated in 3 sequential study periods based on a partially randomized dose sequence. Owing to the prolonged half-life of sirolimus (mean, 54.6 h) [11], the sequence of treatment was randomized only with respect to moxifloxacin and placebo (periods 1 and 2); temsirolimus was administered in period 3 for all subjects. Before each period, a run-in (with ECG collection) on day 1 provided time-matched baseline ECG tracings for post-dose comparisons.

In periods 1 and 2 on study day 1, subjects were given a single-blind placebo i.v. infusion either alone or with

open-label oral moxifloxacin 400 mg. In period 3 on study day 1, subjects were administered single-blind temsirolimus 25 mg i.v. infusion approximately 30 min after receiving pretreatment with i.v. diphenhydramine 25 mg. Because clinical use of temsirolimus routinely includes diphenhydramine pretreatment, only the combined effect on QTc was assessed. Each period was separated by a washout interval of at least 4 days to minimize potential carryover effects of moxifloxacin, which has a half-life of 12 h and is known to induce a 7- to 12-ms prolongation of QTc [12].

Bioanalytical and pharmacokinetic analyses

Blood samples were collected within 2 h before administration and at 0.25, 0.5 (immediately before the end of infusion), 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 36, and 48 h after administration in periods 1, 2, and 3 to measure the concentrations of temsirolimus and sirolimus. To determine the concentrations of moxifloxacin, additional blood samples (5 mL) were obtained at 2 and 24 h after administration in study periods 1 and 2 only.

The whole blood samples were analyzed for temsirolimus and sirolimus simultaneously using a validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method with internal standard [9, 13]. The collective range of quantitation of these assays ranged from 0.25 to 2,500 ng/mL. Moxifloxacin concentrations were analyzed in plasma samples using a validated LC/MS/MS method; the lower limit of quantitation was 40 ng/mL.

The whole blood concentration data for temsirolimus and sirolimus were analyzed for each subject in study period 3 using a non-compartmental method [14]. The whole blood peak concentration (C_{max}) and time to C_{max} (t_{max}) were determined directly from the observed concentration–time points. The terminal elimination phase disposition rate constant (λ_z) was estimated by a log-linear regression of the terminal monoexponential portion of the whole blood concentration–time curve. The terminal phase elimination half-life ($t_{½}$) was calculated as the quotient of $0.693/\lambda_z$. The area under the concentration–time curve (AUC_T), truncated at the last observed whole blood concentration at time T (C_T), was calculated using the trapezoidal rule during the ascending portion of the curve and the log-trapezoidal rule during the descending portion of the curve. Total AUC then was estimated as $AUC = AUC_T + C_T/\lambda_z$. No formal pharmacokinetic (PK) analysis of moxifloxacin concentrations was performed.

ECG and QTc assessment

Triplicate 12-lead ECG tracings (taken 1–2 min apart) were digitally captured at the same time points as the post-dose PK collections, and the intervals were averaged at each time point. The ECGs were performed at a speed of 25 mm/s and included rhythm, HR, and PR, QRS, QT, and QTc intervals. ECG data were manually over-read by a centralized third-party reviewer.

Baselines for ECG intervals were derived as the average of triplicates from time-matched ECGs on day 1 of the corresponding period. The day 1, 0-h average was used as the time-matched average for the day 1, 24- and 48-h tracings; the day 1, 12-h average was used as the time-matched average for the day 1, 36-h tracings.

Primary end point

The main focus was the comparison of QT interval corrected for HR for temsirolimus with that of placebo. The primary statistical objective was to estimate the effect on QTc at the 0.5-h time point following the start of the i.v. infusion using the QTc based on Fridericia's correction (QTcF), on Bazett's correction (QTcB), and on a population-specific correction (QTcN). The QTcN was computed using the slope of a linear regression model fitted to the average QT across the tracings and average cardiac cycle duration (RR interval) and HR across the tracings interval of all data collected from all subjects on baseline observations of each treatment period. Exploratory analyses were carried out to assess the appropriateness of each correction method, including plots of QTcF, QTcN, and QTcB versus HR and RR interval, and Pearson correlation coefficient between corrected QT and RR interval.

The baseline-adjusted QTc was statistically analyzed using a repeated-measures mixed analysis of covariance (ANCOVA) model with sequence, treatment, time, and time by treatment interaction as fixed effects; subject as a random effect; and time-matched baseline as a covariate. One-sided 90% confidence intervals (CIs) were computed using the repeated-measures mixed ANCOVA model for the baseline-adjusted difference in QTc at the 0.5-h time point following the start of the i.v. infusion between the active treatment and placebo. If the 90% CIs for the active treatment fell entirely below 10 ms, this would be considered to have no effect on the QTc interval.

As a secondary end point, the average of the tracings' change from baseline QTc interval using QTcF, QTcB, and QTcN at all post-dose sampling times was statistically analyzed as a secondary end point. One-sided 90% CIs for the baseline-adjusted difference in QTc at all post-dose time points between the active treatment and placebo were computed. In addition, secondary analyses were performed on uncorrected QT (QT_{raw}) and HR data using the same ANCOVA model used for the primary analysis. Categorical analyses summarized QTc intervals >450 ms, >470 ms, and

>500 ms, as well as baseline-adjusted QTc intervals >30 ms or >60 ms.

QTc assay sensitivity

To evaluate assay sensitivity, the mixed ANCOVA model was used to statistically compare baseline-adjusted QTcF, QTcB, and QTcN between the positive control, moxifloxacin, and placebo at the 5% significance level. This was done at 0.25, 0.5, 1, 1.5, 2, and 4 h after dose administration, which contains the reported t_{max} for moxifloxacin of 1 h. The study was considered appropriate to assess the QTc effect of temsirolimus if the lower limit of the 90% CIs were >0 ms.

Pharmacokinetic/pharmacodynamic analysis

Relationships between temsirolimus administration and QTcF, QTcB, and QTcN intervals were examined graphically. Relationships were described using a mixed-effects model with HR as covariate and NONMEM. In addition, models were also examined wherein the QTcN was selected as the dependent variable. As appropriate, linear, log-linear, or maximal estimated value (E_{max}) models were used to describe the relationship between QT and whole blood concentrations of temsirolimus.

The models for QTcN are described as follows:

$$\text{Linear model}: \ \text{QTcN} = E_0 + \text{Slope} \times C$$

$$E_{max}\text{model}: \ \text{QTcN} = E_0 + E_{max} \times C/(C + \text{EC}_{50}),$$

wherein E_0 is the baseline estimate for QTcN, Slope is the slope of the relationship between QTcN and drug concentration C, EC_{50} is the drug concentration at which half-maximal effect is observed, and E_{max} is the maximum cardiovascular effect.

Determination of population sample size

A minimum of 48 subjects was required to complete the study to provide at least 80% power, so that the upper bound of the 95% one-sided CI for the largest time-matched mean effect of temsirolimus on the QTc interval excluded 10 ms. More than 48 patients were to be enrolled to allow for potential discontinuations.

Results

Demography and subject participation

The study enrolled 58 healthy males aged 18–50 years (median 36 years). Thirty-eight (66%) subjects were white and 18 (31%) were black. Median weight was 81 kg (range, 60–108 kg), and median body mass index (BMI) was 26.7 kg/m^2 (range, 20.3–30.5 kg/m^2). Thirty subjects were randomized to receive the moxifloxacin–placebo–temsirolimus sequence, and 28 subjects received the placebo–moxifloxacin–temsirolimus sequence. Demographic characteristics were similar between the 2 groups.

Fifty-seven (98%) subjects completed all 3 periods and received temsirolimus. One subject in the moxifloxacin–placebo–temsirolimus group discontinued from the study on day 6 after receiving moxifloxacin and placebo. An additional subject received less than the planned dose of temsirolimus when he reported weakness, shortness of breath, and confusion after 12 min of the temsirolimus infusion in period 3, and the infusion was stopped. There were no associated ECG changes and the symptoms resolved in less than 2 h. This subject continued on study and was included in the safety and QTc analyses, but not in the temsirolimus PK analysis.

Safety

A total of 56 (97%) subjects reported adverse events (AEs): 21 (36%) subjects each while receiving placebo or moxifloxacin and 53 (93%) subjects while receiving temsirolimus. Two subjects experienced AEs during the prestudy period and 34 (59%) during the run-in period. No subjects experienced AEs associated with QT prolongation, including syncope, seizure, torsade de pointes, or ventricular flutter or fibrillation.

Treatment-emergent AEs (TEAEs) were reported by 47 (83%) subjects while receiving temsirolimus, by 18 (31%) subjects while receiving moxifloxacin, and by 16 (28%) subjects while receiving placebo. For temsirolimus, the most frequent TEAEs were acne (18 [32%] subjects), headache and stomatitis (16 [28%] subjects each), aphthous stomatitis (12 [21%] subjects), and rash (9 [16%] subjects). For moxifloxacin and placebo, the most common TEAEs were contact dermatitis (8 [14%] subjects] and application site reaction (5 [9%] subjects), respectively.

Of the 55 subjects who reported TEAEs, 37 (64%) experienced events that were mild and 17 (29%) had events that were moderate in severity. One severe TEAE (acne) was reported with temsirolimus. All TEAEs were resolved by the final study evaluation.

Temsirolimus pharmacokinetics

Whole blood samples for temsirolimus and its active metabolite, sirolimus, were available from 57 subjects. Mean concentration versus time profiles are shown in Fig. 1, and the mean PK parameters are shown in Table 1. Because of the abbreviated time course examined relative to the longer known sirolimus half-life, estimations for

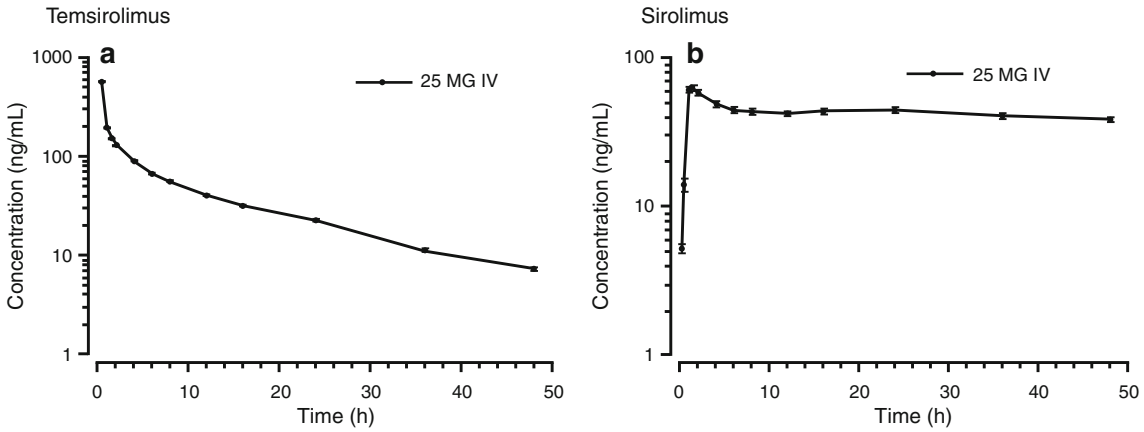

Fig. 1 Temsirolimus (**a**) and sirolimus (**b**) mean (standard error) concentration versus time profiles following a single 25 mg dose of intravenous temsirolimus (period 3). *IV* intravenous

Table 1 Mean pharmacokinetic parameters of temsirolimus and sirolimus following a single 25 mg intravenous dose of temsirolimus

Parameters	Mean ± SD (CV %) [geometric mean]
Temsirolimus	
C_{max}, ng/mL	588 ± 55 (9) [585]
Median t_{max}, h (range)	0.5 (0.3, 0.52)
$t_{½}$, h	14.2 ± 1.2 (8) [14.2]
AUC_T, ng h/mL	1,884 ± 221 (12) [1,872]
AUC, ng h/mL	2,037 ± 260 (13) [2,021]
Sirolimus	
C_{max}, ng/mL	64.0 ± 20.2 (32) [61.2]
Median t_{max}, h (range)	1.6 (1.1, 24.1)
AUC_T, ng h/mL	2,041 ± 639 (31) [1,957]

AUC area under the concentration–time curve, AUC_T area under the concentration–time curve to the last observable concentration at time *T*, C_{max} peak concentration, *CV* coefficient of variation, *SD* standard deviation, t_{max} time to peak concentration, $t_{1/2}$ terminal phase elimination half-life

Table 2 Change from baseline-adjusted QTcN for moxifloxacin versus placebo

Time, h	LSM difference	*P* value[a]	90% CI, ms
0.25	−0.309	0.791	−2.59, 1.98
0.5	5.273	<0.0001	2.99, 7.56
1	7.806	<0.0001	5.52, 10.09
1.5	7.030	<0.0001	4.74, 9.32
2	8.466	<0.0001	6.18, 10.75
4	8.561	<0.0001	6.28, 10.85
8	4.873	<0.0001	2.59, 7.16
12	5.540	<0.0001	3.25, 7.83
24	4.537	0.0001	2.25, 6.82
36	1.759	0.126	−0.50, 4.07
48	1.028	0.378	−1.26, 3.31

CI confidence interval, *LSM* least-squares mean, *QTcN* corrected QT interval based on a population-specific correction formula

[a] Mean difference is significant at $\alpha = 0.001$

some parameters for sirolimus (e.g., $t_{½}$ and AUC) were omitted from the analysis.

Moxifloxacin plasma concentrations

Observations of moxifloxacin concentrations were available from 58 subjects. Data (not shown) indicated that sufficient concentrations were observed to establish the suitability of the treatment to serve as a positive control for QT interval prolongation in this study.

QT assay sensitivity

After a single oral dose of moxifloxacin, a drug with a known cardiac repolarizing effect, the mean changes from baseline-adjusted moxifloxacin QTcN compared with placebo were significantly greater than zero for all time points between 0.5 and 4 h (Table 2). In addition, the lower limit of all 90% CIs was >5 ms at 1, 2, and 4 h post-dose. Findings were similar when QTcB and QTcF corrections were used, for which the lower limits of 90% CIs were increased >5 ms from 0.5 to 8 h after administration compared with placebo. Therefore, the study was deemed adequately sensitive to detect effects of temsirolimus on QT interval prolongation.

Heart rate and QT corrections

Up to 12 h following temsirolimus administration, HR was not significantly different from HR following placebo administration. However, at later time points (16, 24, 36,

and 48 h), statistically significant increases in HR were observed with temsirolimus compared with placebo ($P \leq 0.01$); the maximum mean increase was 9.1 (90% CI, 7.5, 10.7) beats per minute (bpm), which occurred at 48 h post-dose. With moxifloxacin, HR was significantly increased compared with placebo at 0.5, 1, and 8 h post-dose ($P < 0.05$); the maximum mean increase was 3.3 bpm (90% CI, 1.7, 4.9), which was observed at the 1-h time point. No changes from baseline HR of >15 bpm were observed at any time during the study.

Because the QT interval is highly dependent on HR, 3 different correction formulas, QTcF, QTcB, and QTcN, were applied to the ECG data. The correction factor for QTcN was as follows: $QT + 0.1371 \times (1 - RR)$, in which RR corresponds to the time duration of one cardiac cycle. Correlation coefficients for QTcB, QTcF, and QTcN were calculated to assess the relationship of each correction between HR and RR (Fig. 2). The correlations suggest that there is a slight association between QTcB and QTcF versus HR and RR intervals. QTcN was deemed to be the most appropriate correction for HR as it was the only correction method for which the Pearson correlation coefficient was not significantly different from zero.

Primary end point

Statistical analysis showed that, at the primary end point of 0.5 h, change from baseline QTcN following temsirolimus administration was not significantly different from that with placebo (Table 3). The 90% CI for the time-matched change from baseline difference from placebo was −2.58 to 1.99 ms, the upper bound being <10 ms. This finding confirms that temsirolimus concentration does not produce a direct pharmacodynamic effect on the QT interval following i.v. administration of temsirolimus at the recommended RCC dose of 25 mg.

At 11 of 12 secondary time points, the upper bound for the temsirolimus QTcN 90% CIs for the time-matched change from baseline difference versus placebo was <10 ms. At the 36-h time point, the 90% CI for the time-matched change from baseline difference from placebo was 5.77 to 10.34 ms. This increase in QTcN was not associated

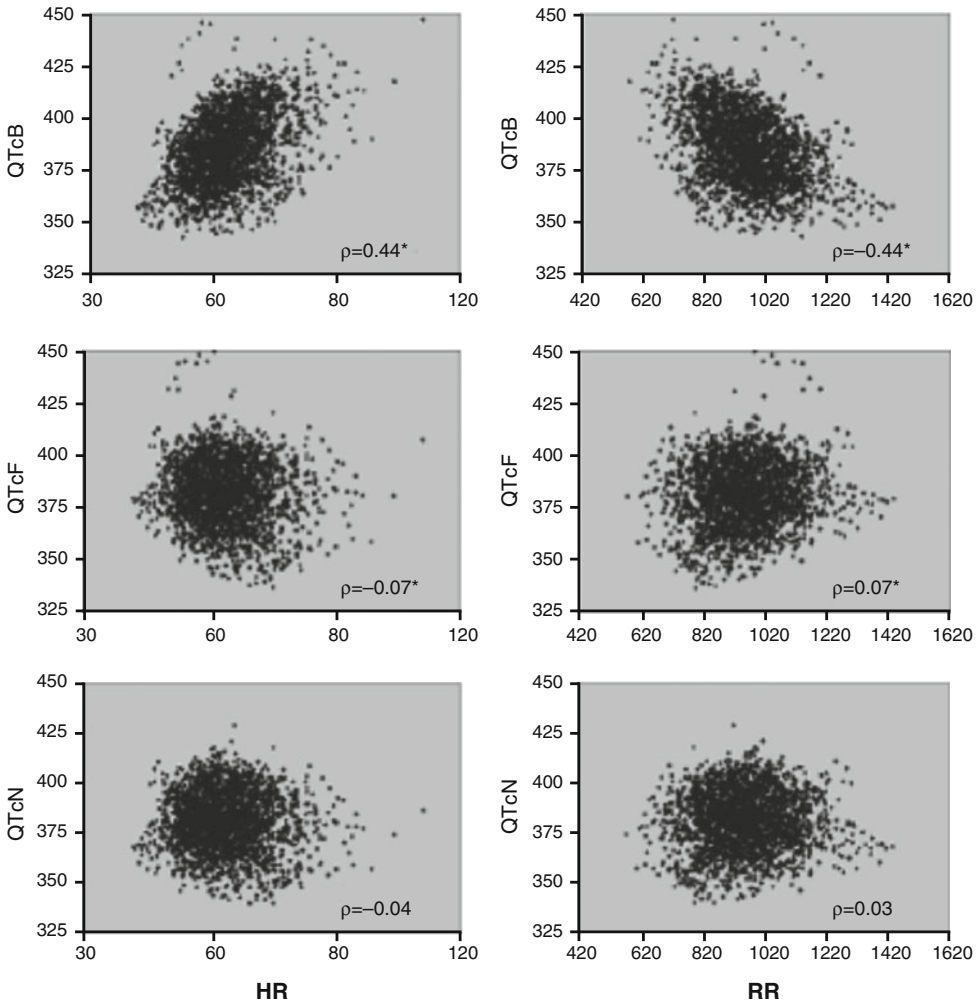

Fig. 2 Baseline QTcB, QTcF and QTcN interval versus heart rate (HR) and cardiac cycle duration (RR) with Pearson correlation coefficients. *Reject H_0: $\rho = 0.0$ at $\alpha = 0.05$. *QTcF* corrected QT interval based on Fridericia's correction, *QTcB* corrected QT interval based on Bazett's correction, *QTcN* corrected *QT* interval based on a population-specific correction formula

Table 3 90% confidence intervals for change from baseline QTc for the difference between intravenous temsirolimus 25 mg and placebo

Time point (h)	QTcN (ms)	QTcB (ms)	QTcF (ms)
0.25	−4.62, −0.05	−5.73, 0.17	−4.53, 0.08
0.5[a]	−2.58, 1.99	−4.22, 1.67	−2.31, 2.29
1	3.60, 8.18	3.38, 9.28	3.82, 8.42
1.5	2.18, 6.75	1.49, 7.39	2.38, 6.98
2	1.85, 6.43	1.27, 7.19	1.73, 6.34
4	2.15, 6.72	2.77, 8.66	2.03, 6.64
6	3.27, 7.84	3.84, 9.74	3.55, 8.15
8	0.81, 5.39	1.49, 7.40	0.96, 5.58
12	3.89, 8.46	4.47, 10.37[b]	4.25, 8.86
16	2.25, 6.82	4.84, 10.74[b]	2.27, 6.87
24	2.20, 6.77	3.66, 9.57	2.01, 6.61
36	5.77, 10.34[b]	10.09, 15.99[b]	6.82, 11.42[b]
48	2.94, 7.51	11.62, 17.53[b]	3.18, 7.78

QTc corrected QT interval, *QTcB* Bazett's correction for QT interval, *QTcF* Fridericia's correction for QT interval, *QTcN* corrected QT interval based on a population-specific correction formula

[a] Time point for primary analysis

[b] Intervals wherein upper limit is >10 ms

with t_{max} for temsirolimus (median, 0.48 h) or sirolimus (median, 1.58 h). Furthermore, at all other time points, the upper bound of the 90% CI was <10 ms, indicating that this was an isolated event and not associated with a trend in QTcN.

Similarly, examination of QTcB and QTcF indicated no effect of temsirolimus on QTc. The 90% CI for the time-matched change from baseline difference from placebo for QTcB was −4.22 to 1.67 and −2.31 to 2.29 ms for QTcF, the upper bound being <10 ms for both intervals. At 12, 16, 36, and 48 h following the start of the i.v. infusion, the upper limits of the 90% CI for the QTcB time-matched change from baseline difference from placebo were >10 ms. In addition, at 36 h, the upper limits of the 90% CI for the QTcF time-matched change from baseline difference from placebo were greater than 10 ms. However, these secondary end points did not provide the most appropriate QT correction for HR, suggesting that the small effects on QTc may be due to changes in HR or to the same variable or variables contributing to changes in QTcN.

Pharmacokinetic/pharmacodynamic analysis

For both temsirolimus and sirolimus, analysis of the whole blood concentration versus effect relationship for QT (uncorrected for HR and using HR included as a covariate) failed to yield a model that would successfully converge. Subsequently, models with linear and E_{max} structures were examined with QTcN as the dependent outcome variable.

Data from the linear and E_{max} modeling suggested that there is no relationship between QTcN and temsirolimus or sirolimus concentrations. Results from the linear model indicated a distinct bias in the association of weighted residual and predicted values for QTcN, and consequently, with high objective function (OF) values for temsirolimus and sirolimus concentrations. These findings were due, in part, to poor estimations of the intercept values estimated in E_0, which was critical to successful determination of slope. Fixing the value of E_0 and alteration in the limits of initial estimates for theta and eta of E_0 failed to improve the association. The linear model therefore had poor predictive value because of the lack of any clear relationship between QTcN and drug concentration.

Results from the E_{max} model were associated with appreciably lower OF values for temsirolimus and sirolimus concentrations than those obtained with the linear model. The E_{max} model also displayed an apparent lack of bias in residual versus predicted values (Fig. 2). However, E_0 and EC_{50} values were associated with high interpatient variability and precision of these estimates could not be determined. As was seen with linear modeling, the poor estimations with the E_{max} models are thought due to the lack of a significant concentration–response relationship with QTcN. Therefore, the E_{max} model demonstrated no predictive value and should not be used to infer an exposure–response relationship (Fig. 3).

Discussion

In accordance with regulatory requirements, all new agents must undergo a clinical evaluation of the potential for effects on cardiac repolarization. Temsirolimus, an approved targeted therapy for patients with advanced RCC, was assessed for its effects on QT/QTc prolongation. In the primary analysis, a single 25 mg i.v. dose of temsirolimus had no effect on the QTc interval at the 0.5-h time point after administration (the t_{max} for temsirolimus). The upper bound of the 90% CI around the mean change in QTc was <10 ms at the t_{max} for temsirolimus (0.5 h after dose administration). At all other time points (with exception of the 36-h time point), the upper bound for the QTcN 90% CIs for the time-matched change from baseline difference from placebo was <10 ms. At the 36-h time point, the 90% CI for the time-matched change from baseline difference from placebo was 5.77–10.34 ms. This increase in QTcN was not associated with t_{max} for temsirolimus or with t_{max} for the active metabolite sirolimus. This change in QTcN was an isolated event and not associated with a trend in QTcN. Additionally, the plotted data provided no evidence to suggest a tendency for a time-protracted positive effect.

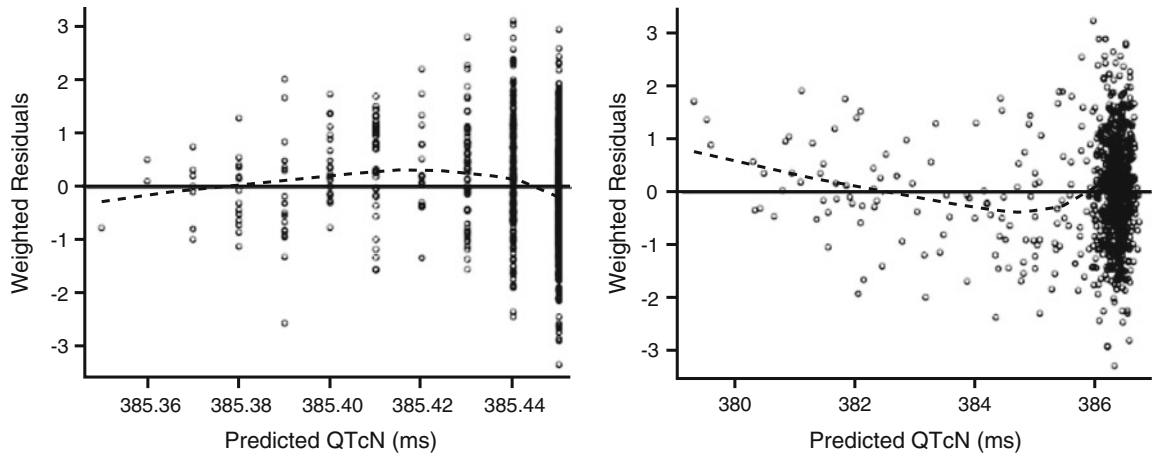

Fig. 3 Observed and predicted QTcN versus temsirolimus and sirolimus observed concentrations using the E_{max} model. *Open circles* represent derived individual observations, and *dashed lines* represent model predicted values *QTcN* corrected *QT* interval based on a population-specific correction formula

All of the 90% CIs beyond the 0.5-h time point excluded zero, which may suggest a small (<10 ms) but statistically significant effect of temsirolimus or sirolimus on cardiac repolarization. Alternatively, the findings could be related to the coadministration of temsirolimus with diphenhydramine, which is known to modestly prolong the QT interval, albeit at high doses [15]. Finally, the small change in QTcN could be related to the non-randomized study design or to an unspecified variable impacting the QT interval during period 3.

The design of the study included appropriate positive and negative controls, consistent with the ICH E14 recommendation [7]. Moxifloxacin, the positive control, produced a significant increase in QTcN interval at 4 h post-dose (the reported C_{max}), thereby verifying that the conditions were adequately sensitive to detect effects of temsirolimus on cardiac repolarization.

Administration of i.v. temsirolimus 25 mg was well tolerated in healthy subjects when given as a single dose. The most common treatment-emergent AEs reported following temsirolimus dosing were acne, stomatitis, headache, and aphthous stomatitis; all were mild or moderate, with the exception of 1 case of severe acne. Following temsirolimus administration, no subject in this study had a QTc interval >450 ms and no subject had a change from baseline of >30 ms. No AEs suggestive of QTc interval prolongation were observed. Also, no cardiovascular AEs related to temsirolimus were reported.

Results of the PK analysis indicate that temsirolimus and sirolimus exposures were comparable to historic parameter values in healthy subjects and in patients with RCC following the 25 mg i.v. dose [9, 10, 16]. Analysis of the PK/pharmacodynamic (PD) relationship between temsirolimus or sirolimus concentrations in whole blood versus population-corrected QT (QTcN) did not exhibit any relevant relationship, irrespective of the model applied. Therefore, the PK/PD analysis supports the finding that the 25 mg i.v. dose of temsirolimus does not alter the QTc interval.

This study provides valuable information pertaining to the lack of effects of i.v. temsirolimus 25 mg on cardiac repolarization, but it has several limitations. First, potential effects of higher exposures on the QTc interval were not addressed and cannot be ruled out. Temsirolimus is a substrate of CYP3A4, and concomitant administration of a CYP3A4 inhibitor, such as ketoconazole, has no effect on temsirolimus C_{max} or AUC; however, a strong PK interaction leading to increased exposure of the primary metabolite sirolimus (C_{max}, 2.2-fold; AUC, 3.1-fold) was observed [17]. In our study, PK/PD modeling indicated no relationship between temsirolimus or sirolimus exposure and prolongation of the QTc interval, but vigilance is still warranted and a temsirolimus dose reduction to 12.5 mg should be considered if administered with CYP3A4 inhibitors. Also, a higher dose of temsirolimus (175 mg weekly for 3 weeks followed by 75 mg weekly) is administered in patients with relapsed and/or refractory MCL [18]; it is not known if our findings would translate to the elevated exposures in this population. However, in one trial of 69 patients with MCL receiving temsirolimus doses up to 175 mg, no patient with a normal QTcF at baseline had an increase in QTcF > 60 ms, and no patient had a QTcF interval > 500 ms [11].

A second limitation is that, despite the eligibility of both healthy men and women, all of the subjects enrolled in the study were men. Pharmacodynamic effects were not observed with temsirolimus, but the concentration-QT effects of some agents, such as quinidine, are more evident in women than in men [19]. Additionally, the study population comprised predominantly (97%) white and black subjects; thus, the effect in Asian subjects was not assessed.

Finally, only single doses of temsirolimus were administered and, therefore, the potential for cardiac accumulation of temsirolimus or sirolimus that might occur at steady-state levels, albeit anticipated to be modest, was not captured. In this study, PK parameters were closely comparable to values observed from previous pooled data reported with the administration of IV temsirolimus 25 mg in cancer patients [20, 21], in whom no evidence of treatment-emergent QTc prolongation or cardiac dysfunction was observed.

The absence of an effect of temsirolimus on the QTc interval is particularly noteworthy given that other targeted therapies approved for the treatment for advanced RCC have been found to be associated with cardiac toxicities. The multitargeted tyrosine kinase inhibitors that target the VEGF receptor pathway (sunitinib, sorafenib, and pazopanib) are an important part of standard therapy for RCC, but their potential for cardiac dysfunction, including heart failure, left ventricular arrhythmia, hypertension, myocardial infarction, and thromboembolism, requires careful monitoring [22–24] In patients with advanced cancer, sunitinib has a dose-dependent effect on the QTc interval [25]. Prolongation of the QTc interval has also occurred in patients with RCC treated with pazopanib [26]. A modest QTc prolongation was observed with sorafenib in patients with advanced cancer, but the magnitude of the effect was not thought to be clinically significant in this setting [27]. Knowledge of the differential cardiovascular safety profiles of the targeted therapies for advanced RCC is of particular importance for patients who are elderly or who have cardiovascular comorbidities, as well as for those in whom longer-term treatment or the sequential use of these agents following disease progression is anticipated.

In conclusion, the 25 mg i.v. dose of temsirolimus, which is the recommended dose for patients with advanced RCC, is not associated with changes in the QT interval that reach clinical or regulatory significance.

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Conflict of interest At the time of this study, all authors were employees of Wyeth Pharmaceuticals. In addition, J. Boni, C. Leister, and D. Sonnichsen were stockholders in Wyeth.

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