ARTICLE

Concentration-QTc Modeling of Ozanimod's Major Active Metabolites in Adult Healthy Subjects

Emily Briggs[1], Sunny Chapel[1], Peijin Zhang[2], Maria Palmisano[2] and Jonathan Q. Tran[2,*]

Ozanimod, approved by regulatory agencies in multiple countries for the treatment of adults with relapsing multiple sclerosis, is a sphingosine 1-phosphate (S1P) receptor modulator, which binds with high affinity selectively to S1P receptor subtypes 1 and 5. The relationships between plasma concentrations of ozanimod and its major active metabolites, CC112273 and CC1084037, and the QTc interval (C-QTc) from a phase I multiple-dose study in healthy subjects were analyzed using nonlinear mixed effects modeling. QTc was modeled linearly as the sum of a sex-related fixed effect, baseline, and concentration-related random effects that incorporated interindividual and residual variability. Common linear, power, and maximum effect (E_{max}) functions were assessed for characterizing the relationship of QTc with concentrations. Model goodness-of-fit and performance were evaluated by standard diagnostic tools, including a visual predictive check. The placebo-corrected change from baseline in QTc (ΔΔQTc) was estimated based on the developed C-QTc model using a nonparametric bootstrapping approach. QTc was better derived using a study-specific population formula (QTcP). Among the investigated functions, an E_{max} function most adequately described the relationship of QTcP with concentrations. Separate models for individual analytes characterized the C-QTcP relationship better than combined analytes models. Attributing QT prolongation independently to CC1084037 or CC112273, the upper bound of the 95% confidence interval of the predicted ΔΔQTcP was ~ 4 msec at the plateau of the E_{max} curves. Therefore, ΔΔQTcP is predicted to remain below 10 msec at the supratherapeutic concentrations of the major active metabolites.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Concentration C-QTc modeling has been used to assess the QTc interval prolongation risk of new drugs. However, examples or publications of this application for drugs' major active metabolites are limited.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Do ozanimod's major active metabolites, CC112273 and CC1084037, prolong the QTc interval? Is the maximum effect (E_{max}) model appropriate for C-QTc model development?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ Ozanimod's major active metabolites do not prolong the QTc interval at supratherapeutic concentrations. The E_{max} model was most appropriate when compared with linear or power models.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?
☑ This paper illustrates the value of C-QTc analysis in supplementing the QTc prolongation risk assessments for drugs' major active metabolite(s).

Ozanimod is a sphingosine 1-phosphate (S1P) receptor modulator, which binds with high affinity selectively to S1P receptor subtypes 1 ($S1P_1$) and 5 ($S1P_5$). Ozanimod blocks the capacity of lymphocytes to egress from lymph nodes, reducing the number of lymphocytes in peripheral blood.[1] Ozanimod is approved by regulatory agencies in multiple countries for the treatment of relapsing forms of multiple sclerosis (MS) in adults. The recommended maintenance dosage is 0.92 mg orally q.d. The 7-day initiation regimen consisting of 0.23 mg q.d. on days 1–4 and 0.46 mg on days 5–7 is required upon treatment initiation to mitigate the transient decrease in heart rate (HR).[2,3] The mechanism by which ozanimod exerts therapeutic effects in MS is unknown, but may involve the reduction of lymphocyte migration into the central nervous system. Ozanimod is also being developed for patients with moderately to severely active inflammatory bowel disease, including ulcerative colitis and Crohn's disease.[4,5]

Ozanimod is extensively metabolized to form a number of circulating active metabolites, including two major active metabolites, CC112273 and CC1084037, with similar activity and selectivity for $S1P_1$ and $S1P_5$.[6] Following multiple dosing, ~ 94% of circulating total active drug exposure was represented by ozanimod (6%), CC112273 (73%), and

[1]Ann Arbor Pharmacometrics Group, Ann Arbor, Michigan, USA; [2]Clinical Pharmacology and Pharmacometrics and Research and Early Development, Bristol Myers Squibb Company, Princeton, New Jersey, USA. *Correspondence: Jonathan Q. Tran (dr.jtran@gmail.com)
Received: August 21, 2020; accepted: November 22, 2020. doi:10.1002/psp4.12580

CC1084037 (15%). Exposures of CC112273 and CC1084037 were highly correlated.[7] The median times to maximum plasma concentration (T_{max}) for ozanimod, CC112273, and CC1084037 were ~ 8, 10, and 16 hours, respectively. The mean terminal elimination half-life ($t_{1/2}$) for ozanimod was ~ 20–22 hours, whereas the mean $t_{1/2}$ for CC112273 and CC1084037 were similar at ~ 10 days.

The proarrhythmic risk of ozanimod was previously evaluated in a thorough QT (TQT) study.[8] Following a 14-day titration regimen of q.d. oral doses of ozanimod 0.23 mg for 4 days, 0.46 mg for 3 days, 0.92 mg for 3 days, and 1.84 mg for 4 days in healthy subjects, no evidence of clinically significant QTc prolongation was observed, as demonstrated by the upper boundary of the 90% confidence interval (CI; 2-sided) for the time-matched, placebo-corrected, baseline-adjusted mean QTc (ΔΔQTc) being below the 10 msec threshold for both ozanimod 0.92 and 1.84 mg. The TQT study was conducted early during the clinical development and before the human mass balance study, which identified the active metabolites CC112273 and CC1084037. Based on the long $t_{1/2}$ of both major active metabolites, ozanimod dosing duration in the TQT study was not adequate to achieve the anticipated steady-state or therapeutic concentrations for CC112273 or CC1084037.

In this work, modeling was performed using data from a phase I multiple-dose study conducted after the TQT study to characterize the relationships between plasma concentrations of ozanimod and its major active metabolites, CC112273 and CC1084037, and the QTc interval (C-QTc) in healthy subjects. Once established, the C-QTc model was used to predict mean drug-related QTc changes at the anticipated clinically relevant CC112273 and CC1084037 concentrations in patients with MS.

METHODS

Study design and treatment

This was a randomized, double-blind, placebo-controlled, multiple-dose study in healthy adult subjects. All eligible subjects were admitted to the clinical research unit on day –2 and were domiciled in the clinical research unit until day 31. Subjects were randomized 1:1 to ozanimod or placebo. Subjects received q.d. oral doses of either placebo for 30 days or ozanimod 0.23 mg on days 1–4, 0.46 mg on days 5–7, 0.92 mg on days 8–10, and 1.84 mg on days 11–30. The study protocol and informed consent were reviewed and approved by an institutional review board (IntegReview, Austin, TX). All subjects provided written informed consent before study entry. The study was conducted in accordance with the ethical principles of Good Clinical Practice and the Declaration of Helsinki.

Electrocardiogram and pharmacokinetic collections

Electrocardiograms (ECGs) were recorded continuously over 24 hours on days –1 (baseline), 1 (0.23 mg), 5 (0.46 mg), 8 (0.92 mg), and 28 (1.84 mg) using a 12-lead digital Holter recorder (M12R; Global Instrumentation LLC, Manlius, NY). All Holter data were transmitted to the ECG core laboratory (Bioclinica, Princeton, NJ) over the internet through a secure transfer program. The 12-lead ECGs were extracted automatically in triplicates at the following nominal timepoints on days –1, 1, 5, 8, and 28 just before pharmacokinetic (PK) sample collections (except on day –1 with no PK samples): prior to dosing (0 hour) and at 1, 2, 4, 6, 8, 10, 12, 14, 16, and 24 hours after dosing. The subject was rested in the supine position for the first 5 minutes before extractions and then 10 minutes during extractions. The extracted 12-lead ECGs were analyzed by the cardiologist who was blinded to subject, treatment, and visit identifiers for intervals, diagnostic findings, precise QT interval (QT/QTc determination), T wave morphology, and the presence of pathological U waves. The arithmetic mean of triplicate measurements per timepoint was used in the data analysis.

Various correction methods were assessed for their ability to remove the correlation of QT with HR, including QTc Bazett, QTc Fridericia, and population-based (QTcP). The QTcP was calculated as:

$$\mathrm{QTcP}_{ij} = \frac{\mathrm{QT}_{ij}}{\left(10^{-3} \cdot \mathrm{RR}_{ij}\right)^{\gamma}}$$

where QT_{ij} and RR_{ij} are the measured QT and RR intervals, respectively, for the ith subject at jth timepoint and γ is the correction factor. The 10^{-3} factor was included to correct units of measured RR_{ij}. The γ was estimated as the slope derived from linear regression of natural log-transformed QT_{ij} and RR_{ij} values from drug-free ECGs (placebo and day –1 for the ozanimod treatment group). ECGs recorded while patients were treated with ozanimod were excluded in the QTcP derivation so as not to correct for possible drug effects.

Exploratory assessments

The QT/QTc intervals and HR were assessed graphically for diurnal variation on each ECG day in subjects in the placebo group. If no clear trends were observed, then the fluctuations would be treated as a component of residual variability. Drug-free values of QTcP were evaluated as a function of RR interval to select the QTc end points for primary analysis. Exploratory plots were generated for paired values of ozanimod or metabolite concentrations and QTcP to identify any tendency of concentration-dependent QT prolongation. To aid visualization, a graphical smoothing method was applied to exploratory plots.

C-QTc model development

The QTc end point was modeled linearly as a sum of the baseline-related, sex-related, and concentration-related effects. Initially, single-analyte models with various functions for the concentration-related effect were developed separately, and the corresponding linear, maximum effect (E_{max}), and power models were expressed as:

$$\mathrm{QTc}_{ij} = \theta_{\mu} \cdot e^{\eta_{\mu,i}} + \theta_{s} \cdot \mathrm{Sex} + \left(\theta_{c} + \eta_{c,i}\right) \cdot C_{ij} + \varepsilon_{ij}$$

$$\mathrm{QTc}_{ij} = \theta_{\mu} \cdot e^{\eta_{\mu,i}} + \theta_{s} \cdot \mathrm{Sex} + \left(E_{max} + \eta_{c,i}\right) \cdot \left(\frac{C_{ij}}{\mathrm{EC}_{50} + C_{ij}}\right) + \varepsilon_{ij}$$

$$\mathrm{QTc}_{ij} = \theta_{\mu} \cdot e^{\eta_{\mu,i}} + \theta_{s} \cdot \mathrm{Sex} + \left(\theta_{c} \cdot e^{\eta_{c,i}}\right) \cdot \left(C_{ij}/C_{ref}\right)^{\delta} + \varepsilon_{ij}$$

where QTc_{ij} is the QTc at the *j*th time for the *i*th subject, θ_μ is the overall mean or baseline QTc (when other covariate contributions and random effects are 0), θ_S is the mean QTc difference for women (sex = 1) relative to men (sex = 0), θ_c is the mean change in QTc per unit concentration, C_{ij}, C_{ref} is a reference concentration, δ is the power term for the ratio of C_{ij}/C_{ref}, E_{max} is the maximum change in QTc associated with concentration, EC_{50} is the half-maximal effective concentration, $\eta_{\mu,i}$ is the interindividual variability for baseline QTc, $\eta_{c,i}$ is the interindividual variability for the slope or E_{max} parameter, and ε_{ij} is the residual variability (RV). Both additive and proportional error terms (θ_{add} and θ_{pro}, respectively) were evaluated for RV as

$$\varepsilon_{ij} = \sqrt{\theta_{pro}^2 \cdot F_{ij}^2 + \theta_{add}^2} \cdot \varepsilon$$

where F_{ij} is the predicted value of QTc_{ij} and ε has the zero mean value and the unity variance.

The comparison of two hierarchical competing single-compound models was based on a likelihood ratio test using the difference in the objective function value (OFV) provided by NONMEM and the degrees of freedom that is equal to the difference in parameter numbers. If more than one single-compound model demonstrated statistical significance, then a simultaneous model was to be developed by incorporating the C-QTc relationships for the compounds in a stepwise manner based on the parsimony principle. For non-nested models, the Akaike Information Criterion (AIC) was used for model selection where ΔAIC was computed as the difference in AIC between a candidate model and the best model with the lowest value.

Standard diagnostic plots were used to evaluate the adequacy of data fitting. For the stability of fitted models, pairwise correlations between the parameter estimates were examined to ensure no absolute values ≥ 0.95. Additionally, the condition number of the correlation matrix of the parameter estimates (the ratio of the largest to smallest eigenvalues) was monitored to ensure it remained < 1,000. Observed QTc values were considered potential outliers if the absolute values of the corresponding conditional weighted residuals, population weighted residuals, or individual weighted residuals were ≥ 6. The influence of potential outliers was evaluated by comparing the estimates of fixed-effect parameters and RV in models with and without the outliers. All CIs were calculated as a two-sided CI.

The final model was assessed for its predictive performance using the technique of visual predictive check. Based on key elements of the study design, simulated data were generated using the estimates of model parameters assuming a multivariate normal distribution for the random effects. Means of the QTc were computed by nominal time and dose level. This procedure was replicated 1,000 times, and the resultant distribution of simulated means was compared with the distribution of observed means computed in a similar fashion. Uncertainty in the parameters was not incorporated when performing the replicates. The model was considered adequate if the 5th, 50th, and 95th percentiles of observed data were contained within the 90% CIs summarizing the corresponding distributions of simulated data.

The data fitting was performed using the first-order conditional estimation with interaction method in NONMEM, version 7.3 software (ICON Development Solutions, Ellicott City, MD). The dataset assembly and postfitting processing were performed using R software, version 3.1.2. Simulations were performed using NONMEM and R.

RESULTS

The C-QTc analysis included 54 subjects (28 subjects in the ozanimod group and 26 subjects in the placebo group) with 2,881 ECG measurements. Of the 54 subjects, 31 subjects (57.4%) were men, 23 subjects (42.6%) were women, 27 subjects (50%) were white, 22 subjects (40.7%) were black, 1 subject (1.9%) was Asian, and 4 subjects (7.4%) were another race.

Prior to C-QTc model development, QTcP observations were summarized by treatment group and study day (**Table S1**). The overall difference between mean QTcP of all placebo ECGs and all treatment ECGs was 4.3 msec. As the dose increased, there was no clear linear trend in mean QTcP, suggesting a possible maximum effect on QTcP.

The QTcP observations were further summarized for the ozanimod treatment group by time postdose (**Table S2**). In general, the mean QTcP observations were higher from 8–24 hours than 0–6 hours corresponding to an increase in concentrations.

Additionally, an exploratory graphical assessment was performed to determine if HR and QTcP was substantially influenced by ozanimod or metabolite exposure over 28 days, including dose titration. The trend lines were relatively flat, particularly in comparison with placebo data (at zero concentrations), suggesting no clear relationship of ΔHR with concentration (**Figure S1**). The trend lines suggest that a linear model may not be appropriate given the plateau of QTcP at higher concentrations (**Figure S2**). The effects of HR with time were also assessed (**Figure S3**). There were some fluctuations of HR and QTcP within a day; however, these effects were out-of-phase and considered as residual variability rather than modeled as a diurnal effect.

The relationships of QTc Bazett, QTc Fridericia, and QTcP and HR (represented by RR interval) are shown in **Figure S4** for drug-free data (placebo and day −1 for the ozanimod treatment group). Bazett's correction method did not adequately remove the correlation between QT and RR. Fridericia's correction method resulted in a more accurate correction for HR than Bazett's correction. However, the population correction most appropriately accounted for the QT relationship with HR, and therefore was selected for C-QTc analyses. Correlations among ozanimod, CC112273, and CC1084037 concentrations were drawn to illustrate challenges in paired QTcP modeling (**Figure S5**).

The analyses began by developing the best single-analyte model using paired QTcP and plasma concentration data for ozanimod, CC112273, and CC1084037. For each analyte, the reference model included only baseline-related and sex-related effects on QTcP. Linear, E_{max}, and power functions of concentration were sequentially added to the reference model and evaluated. The effects of ozanimod, CC112273, and CC1084037 concentrations on QTcP and

the relationships were most adequately described by E_{max} functions given the model fit, OFV, and diagnostic plots (**Figures 1 and 2**). **Table 1** presents parameter estimates and precision for the single-analyte C-QTc models with E_{max} functions.

The C-QTc model with an E_{max} function of CC1084037 concentration had the lowest AIC, whereas the ΔAIC for its CC112273 counterpart was merely 5. By contrast, the C-QTc model with an E_{max} function of ozanimod concentration was associated with a large ΔAIC of ≥ 12. Adding an E_{max} function of CC112273 or ozanimod concentration in the C-QTc model with the E_{max} function of CC1084037 concentration produced successful minimization and covariance steps and further reduced the OFV by 44.1 and 23.8, respectively. However, the addition of a second E_{max} function to the CC1084037 E_{max} model caused the sign of the CC1084037 E_{max} parameter to change to a negative value and the E_{max} parameter for the other analyte to be positive. The condition number, representing model stability increased by over 100 points, suggests that the paired C-QTc model was ill-conditioned. Furthermore, a model including an E_{max} function of ozanimod concentration and an E_{max} function of CC112273 concentration was also evaluated and decreased the OFV by 35.0 from the C-QTc model with the E_{max} function of CC112273 alone, but also produced a negative E_{max} for CC112273. The significant changes of the E_{max} parameter estimates and increased condition number suggests that the model is unable to precisely estimate the individual contributions to QT prolongation with highly correlated analytes and minimal changes in QTcP.

Changes from baseline in QTcP with ozanimod administration were predicted based on the individual CC1084037 and CC112273 E_{max} models. A nonparametric bootstrapping approach was used to estimate parameter uncertainty for E_{max}. For CC1084037, the median E_{max} was 1.69 msec, and the 95% CI was calculated as (0.25–4.09 msec; **Figure 3**). This result was fairly consistent with the final model E_{max} estimate (95% CI) of 1.39 (–1.12 to 3.90) msec. For CC112273, the median E_{max} (95% CI) was 1.77 (0.14–4.04) msec (**Figure 4**); the final model E_{max} estimate (95% CI) was 1.26 (–1.43 to 3.95) msec.

Based on E_{max} model parameters and the 95% CI estimate for E_{max}, a ΔΔQTcP – CC1084037 concentration curve was constructed with the associated 95% CI (**Figure 5**). Superimposed on this E_{max} curve was an anticipated mean maximum concentration at steady-state ($C_{max,ss}$) for CC1084037 of 3747 pmol/L following ozanimod 0.92 mg q.d. in patients with MS (data on file). This plot shows that

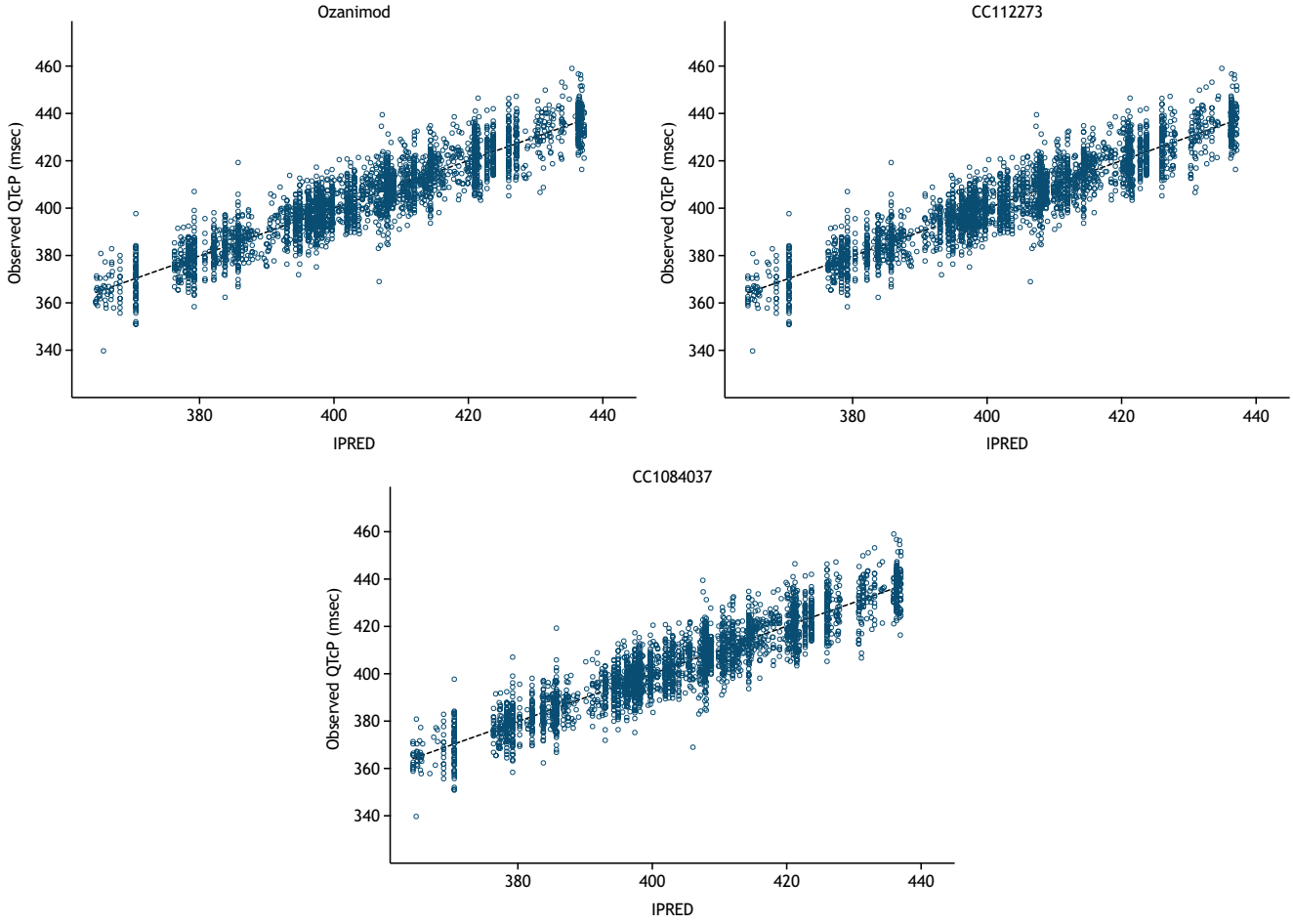


Figure 1 Concordance plots of model predicted and observed QTcP for E_{max} with ozanimod CC112273, and CC1084037 concentrations on QTcP. E_{max}, maximum effect; IPRED, individual predicted QTcP.

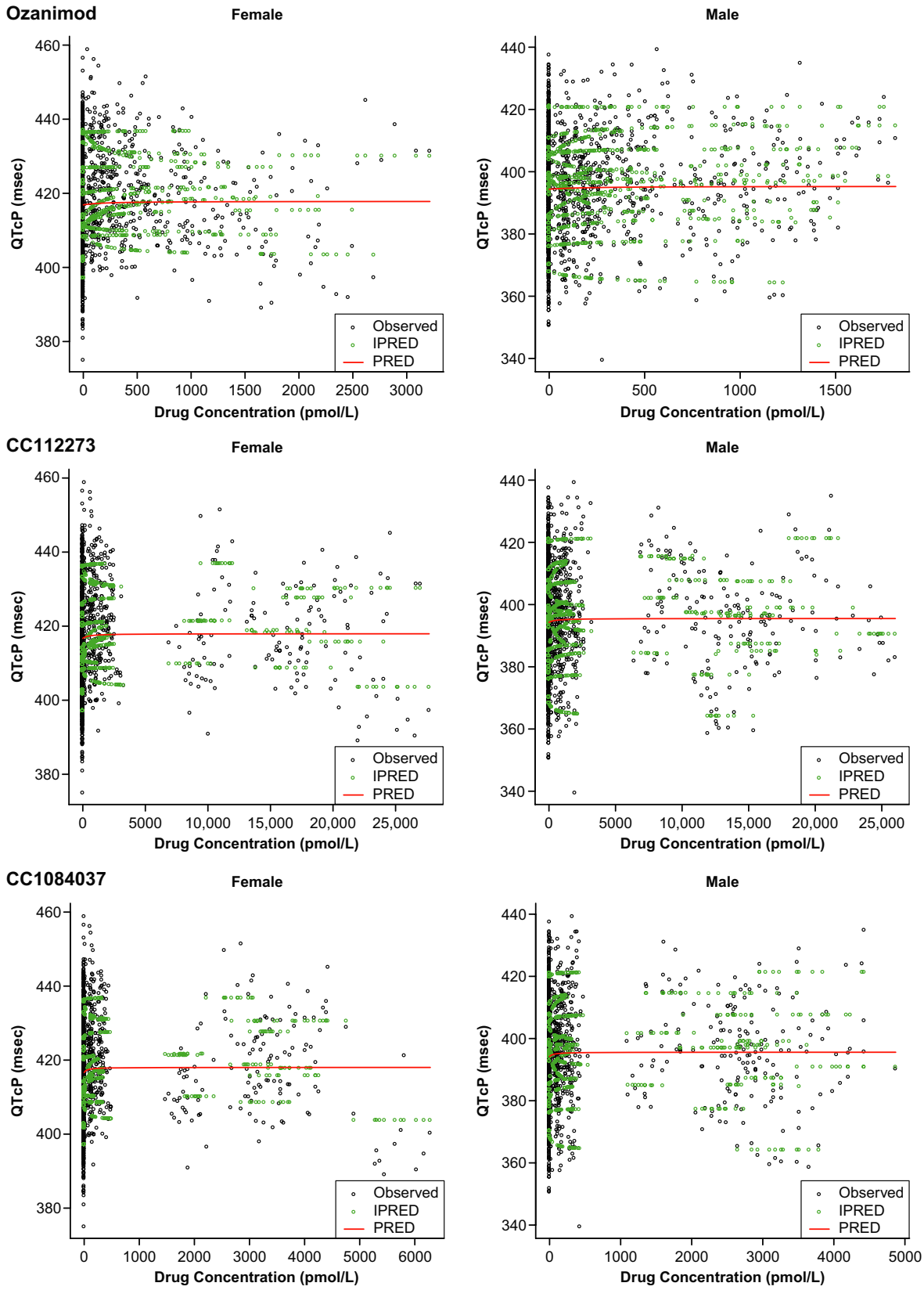


Figure 2 Diagnostic plots of E_{max} with ozanimod, CC112273, or CC1084037 concentrations on QTcP by sex. E_{max}, maximum effect; IPRED, individual predicted QTcP; PRED, population predicted QTcP; QTcP, QTc population formula.

Table 1 Parameter estimates and precision of the single-analyte C-QTc models with E_{max} functions of concentrations

	Estimate	SE	Estimate	SE	Estimate	SE
Parameter	**Ozanimod**		**CC112273**		**CC1084037**	
Baseline, msec	394	2.41	394	2.4	394	2.38
Sex,[a] msec	22.5	3.46	22.4	3.43	22.4	3.40
E_{max}, msec	0.972	1.64	1.26	1.37	1.39	1.28
EC_{50}, pmol/L	195	133	482	284	57.4	40.6
RV-proportional, unitless	−0.0176	0.00603	−0.0179	0.00612	−0.0182	0.0051
RV-additive, msec	2.54	6.93	2.12	8.58	1.63	9.44
IIV on baseline, ω^2	0.00102	0.000165	0.00101	0.000162	0.001	0.000161
IIV on E_{max}, ω^2	54	25.0	40.5	14.5	36.1	13.1
OFV	14,846.278		14,834.582		14,830.006	
CN	693		686		486	

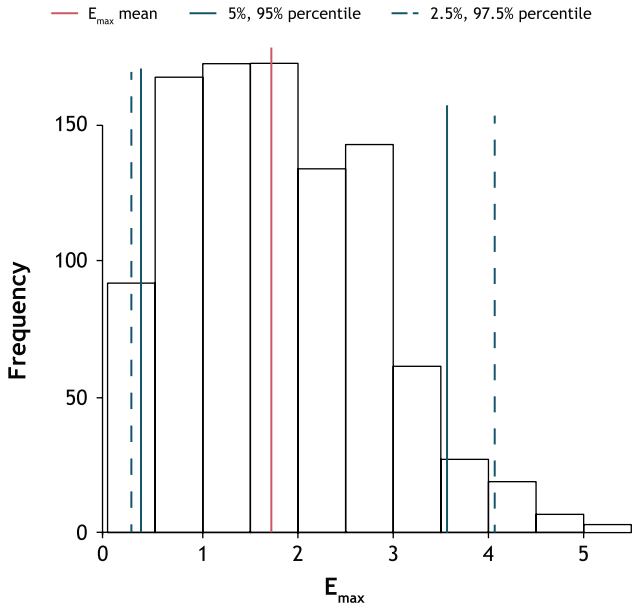
CN, condition number; C-QTc, concentration-QTc interval; E_{max}, maximum effect; EC_{50}, half-maximal effective plasma concentration; IIV, interindividual variability; RV, residual variability; OFV, objective function value; SE, standard error.
[a]Sex, difference in QTcP for women relative to men.

the E_{max} curve begins to plateau at a CC1084037 concentration of ~ 200 pmol/L, which is nearly 20-fold below the anticipated mean CC1084037 $C_{max,ss}$ for ozanimod therapeutic dose. CC1084037 concentrations are predicted to be associated with a ΔΔQTcP estimate with upper 95% CI of ~ 4 msec. Because concentrations are on the plateau of the E_{max} curve at the therapeutic dose of ozanimod 0.92 mg, the upper bound of the 95% CI predicted for ΔΔQTcP is not expected to exceed 4 msec at CC1084037 concentrations associated with ozanimod supratherapeutic dose.

Similarly, a ΔΔQTcP – concentration curve was also constructed for CC112273 (**Figure 6**). Superimposed on this E_{max} curve was the anticipated $C_{max,ss}$ for CC112273 of 19,413 pmol/L following ozanimod 0.92 mg q.d. in patients with MS (data on file). The plateau of the ΔΔQTcP – CC112273 concentration curve (~ 2,000 pmol/L) is nearly 10-fold below the anticipated mean CC112273 $C_{max,ss}$ for the ozanimod therapeutic dose. The upper 95% CI for the ΔΔQTcP – CC112273 concentration curve was ~ 4 msec; therefore, ΔΔQTcP is not expected to exceed 4 msec at CC112273 concentrations associated with ozanimod supratherapeutic dose.

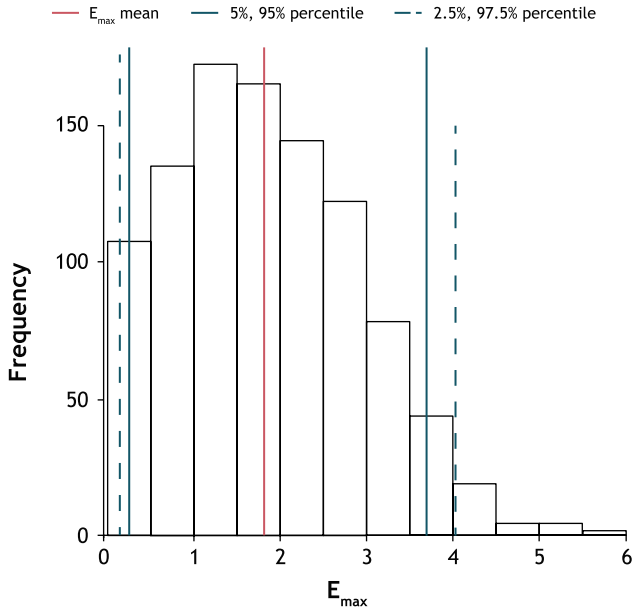
DISCUSSION

A dedicated TQT study was previously conducted to examine the effects of therapeutic and supratherapeutic doses of ozanimod on cardiac repolarization according to the E14 Guidance of the International Conference on Harmonisation.[9] The TQT study was designed based on the PK characteristics of ozanimod and was conducted early during the clinical development and before the identification of the major active metabolites, CC112273 and CC1084037. Per E14 guidance, the duration of dosing should be sufficient to characterize the effects of the parent and its active metabolites at relevant concentrations. Although ozanimod dosing duration in the TQT study was sufficient for ozanimod with the $t_{1/2}$ of 20–22 hours, it was not adequate for its major active metabolites due to the long $t_{1/2}$ of ~ 10 days. The identification of major active metabolites during late drug development has posed major challenges, including the refusal-to-file by the US Food and Drug Administration regarding ozanimod's New Drug Application.[10] To provide adequate Clinical Pharmacology characterization of the major active metabolites for the New Drug Application resubmission and to avoid conducting another TQT study, we collected intensive paired ECG and concentration data strategically designed from a phase I multiple-dose study in healthy adult subjects and performed to serve multiple purposes: (1) to characterize the PK properties of CC112273 and CC1084037, (2) to evaluate the drug interaction, and (3) to collect intensive paired concentration-ECGs data for C-QTc analysis for ozanimod



	0%	2.5%	5%	25%	50%	75%	95%	97.5%	100%
E_{max}	0.01	0.25	0.36	0.97	1.69	2.54	3.57	4.09	5.40

Figure 3 Histogram of E_{max} values estimated from nonparametric bootstrapping (N = 1,000) with CC1084037 E_{max} model. E_{max}, maximum effect.



	0%	2.5%	5%	25%	50%	75%	95%	97.5%	100%
E_{max}	0.02	0.14	0.23	1.02	1.77	2.59	3.72	4.04	5.57

Figure 4 Histogram of E_{max} values estimated from nonparametric bootstrapping (N = 1,000) with CC112273 E_{max} model. E_{max}, maximum effect.

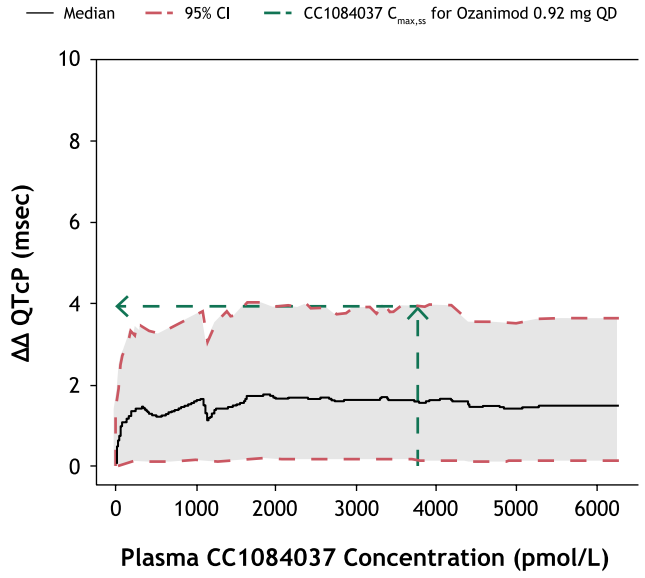


Figure 5 Estimated relationship of ΔΔQTcP with CC1084037 concentration following ozanimod administration using the 95% CI for E_{max}. ΔΔQTcP, placebo-corrected change from baseline in QTcP; $C_{max,ss}$, maximum plasma concentration at steady state; CI, confidence interval; E_{max}, maximum effect; QTcP, QTc population formula.

and its major active metabolites.[7] Ozanimod was titrated to 1.84 mg over 30 days (0.23 mg q.d. on days 1–4, 0.46 mg q.d. on days 5–7, 0.92 mg q.d. on days 8–10, and 1.84 mg q.d. on days 11–30) in this study to achieve the observed steady-state exposure of CC112273 and CC1084037 in patients with MS receiving the maintenance dose of 0.92 mg q.d. Results show that ozanimod dose regimen and duration in this phase I study were adequate to achieve the $C_{max,ss}$ for the major active metabolites associated with the therapeutic dose of 0.92 mg q.d. in patients with MS. Furthermore, ECG and PK data collected in this phase I study also occurred around the T_{max} of ozanimod, CC112273, and CC1084037 of ~ 8, 10, and 16 hours, respectively.

Different C-QTc models including linear, E_{max}, and power models were evaluated. The E_{max} model was considered the best approach for all three analytes based on the model fit, OFV, and diagnostic plots. The drop in OFV indicated a statistically significant relationship of QTcP with concentration for all three analytes. However, the magnitude of the effect as measured by E_{max} was small (< 2 msec) and poorly estimated (standard error ≥ estimate).

For linear mixed effect models of C-QT analysis, it is recommended that parent and metabolite concentrations not be analyzed separately because either parent or metabolite could result in biased parameter estimates, inflated point estimates, and CIs for predicted values.[11] Because all three analytes showed statistically significant effects on the QTc interval, the CC1084037 E_{max} model with the lowest AIC was used to further evaluate if the C-QTc relationship could be better characterized by adding the effect of ozanimod or CC112273. Despite a statistically significant decrease in the OFV, the addition of a second E_{max} function resulted in a negative E_{max} and a greater half-maximal effective concentration for the CC1084037 dependent effect. Such changes in parameter estimates of the reference model were pharmacologically implausible, reflecting little value in describing an insignificant C-QTc relationship using two correlated analytes. Furthermore, a model, including an E_{max} function of ozanimod concentration and an E_{max} function of CC112273 concentration, was also evaluated for completeness and decreased the OFV by 35.0 from the C-QTc model with the E_{max} function of CC112273 alone, but also produced a negative E_{max} for CC112273. Thus, model predictions were based on the individual CC1084037 and CC112273 E_{max} models. In addition to the high correlation between analytes, it should be noted that the PK properties of the parent ozanimod and its major active metabolites, CC112273 and CC1084037, are significantly different (i.e., different T_{max} and $t_{1/2}$) and therefore may account for challenge in C-QTc modeling of parent and metabolites together.

The upper bound of the 95% CI of the predicted ΔΔQTcP was ~ 4 msec at the plateau of the ΔΔQTcP – concentration E_{max} curves for both major active metabolites. Although this study did not achieve supratherapeutic concentrations of CC112273 and CC1084037, the E_{max} curves showed that the plateaus were reached at concentrations ~ 10-fold and 20-fold below the anticipated mean $C_{max,ss}$ for CC112273 and CC1084037, respectively, associated with the ozanimod therapeutic dose in patients with MS. Therefore, ΔΔQTcP is predicted to remain below 10 msec at supratherapeutic concentrations of the major active metabolites. Collectively, results from this C-QT analysis and the previous TQT study demonstrate that ozanimod treatment does not prolong the QTc interval. These results also align with the relationship between the hERG potency and clinical exposure data. Published literature has shown that drugs with a margin of > 30 between hERG half

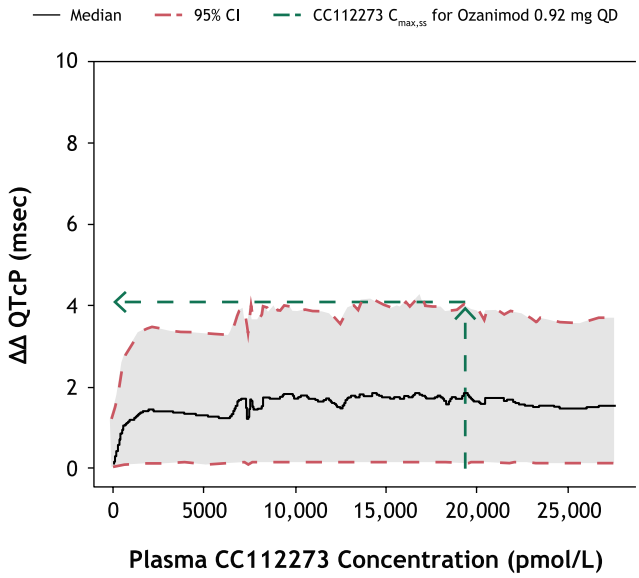


Figure 6 Estimated relationship of ΔΔQTcP with CC112273 concentration following ozanimod administration using the 95% CI for E_{max}. ΔΔQTcP, placebo-corrected change from baseline in QTcP; $C_{max,ss}$, maximum plasma concentration at steady state; CI, confidence interval; E_{max}, maximum effect; QTcP, QTc population formula.

maximum inhibitory concentration (IC_{50}) and free or unbound $C_{max,ss}$ are associated with low risk of QTc prolongation.[12] The hERG IC_{50} values for ozanimod, CC112273, and CC1084037 are 0.21, 0.60, and > 3.0 μM, respectively (data on file). Based on the plasma protein binding of 98.2%, 99.8%, and 99.3% for ozanimod, CC112273, and CC1084037, respectively,[7] the margins between the hERG IC_{50} and free $C_{max,ss}$ for ozanimod, CC112273, and CC1084037 are > 3000, suggesting a very low risk of QTc prolongation.

Attributing QT prolongation independently to either CC1084037 or CC112273, the upper bound of the 95% CI of the predicted ΔΔQTc was ~ 4 msec at the plateau of the E_{max} curves. Therefore, ΔΔQTcP is predicted to remain below 10 msec at supratherapeutic concentrations of the major active metabolites.

Supporting Information. Supplementary information accompanies this paper on the *CPT: Pharmacometrics & Systems Pharmacology* website (www.psp-journal.com).

Acknowledgments. The authors gratefully acknowledge H. Steve Kuan and Simon Zhou for their critical review of the C-QTc analysis.

Funding. This study was funded by Celgene Corporation. Support for third-party editorial assistance was provided by Peloton Advantage, LLC, an OPEN Health company, and was funded by Bristol Myers Squibb Company.

Conflict of Interest. S.C. and E.B. are employees of Ann Arbor Pharmacometrics Group. P.Z. and M.P. are employees of Bristol Myers Squibb. J.Q.T. is a former employee of Bristol Myers Squibb.

Author Contributions. E.B. and J.Q.T. wrote the manuscript. J.Q.T., P.Z., and M.P. designed the research. J.Q.T. and P.Z. performed the research. E.B., S.C., and J.Q.T. analyzed the data.

Data Sharing Statement. Celgene, a Bristol Myers Squibb company, is committed to responsible and transparent sharing of clinical trial data with patients, healthcare practitioners, and independent researchers for the purpose of improving scientific and medical knowledge as well as fostering innovative treatment approaches. Data requests may be submitted to Celgene, a Bristol-Myers Squibb Company, at https://vivli.org/ourmember/celgene/ and must include a description of the research proposal.

1. Tran, J.Q. *et al.* Results from the first-in-human study with ozanimod, a novel, selective sphingosine-1-phosphate receptor modulator. *J. Clin. Pharmacol.* **57**, 988–996 (2017).
2. Zeposia [package insert] (Celgene Corporation, Summit, NJ, 2020).
3. Zeposia [summary of product characteristics] (Celgene Distribution B.V., Utrecht, The Netherlands, 2020).
4. Sandborn, W.J. *et al.* Ozanimod induction and maintenance treatment for ulcerative colitis. *N. Engl. J. Med.* **374**, 1754–1762 (2016).
5. Feagan, B.G. *et al.* Ozanimod induction therapy for patients with moderate to severe Crohn's disease: a single-arm, phase 2, prospective observer-blinded endpoint study. *Lancet Gastroenterol. Hepatol.* **5**, 819–828 (2020).
6. Tran, J.Q., Zhang, P., Surapaneni, S., Selkirk, J., Yan, G. & Palmisano, M.Absorption, metabolism, and excretion, in vitro pharmacology, and clinical pharmacokinetics of ozanimod, a novel sphingosine 1-phosphate receptor agonist [abstract P993]. Triennial Joint Meeting of the European Committee for Treatment and Research in Multiple Sclerosis and Rehabilitation in Multiple Sclerosis September 11–13, 2019; Stockholm, Sweden.
7. Tran, J.Q. *et al.* Multiple-dose pharmacokinetics of ozanimod and its major active metabolites and the pharmacodynamic and pharmacokinetic interactions with Pseudoephedrine, a sympathomimetic agent, in healthy subjects. *Adv. Ther.* **37**, 4944–4958 (2020).
8. Tran, J.Q. *et al.* Cardiac safety of ozanimod, a novel sphingosine-1-phosphate receptor modulator: results of a thorough QT/QTc study. *Clin. Pharmacol. Drug Dev.* **7**, 263–276 (2018).
9. Guidance for industry E14 clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs <https://www.fda.gov/media/71372/download> (2005). Accessed May 19, 2020.
10. Summary Review: Zeposia (ozanimod) <https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/209899Orig1s000SumR.pdf> (2020). Accessed October 23, 2020.
11. Bonate, P.L. The effects of active metabolites on parameter estimation in linear mixed effect models of concentration-QT analyses. *J. Pharmacokinet. Pharmacodyn.* **40**, 101–115 (2013).
12. Pollard, C.E. *et al.* An analysis of the relationship between preclinical and clinical QT interval-related data. *Toxicol. Sci.* **159**, 94–101 (2017).