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ARTICLE

A phase I trial assessing the safety, pharmacokinetics, cerebrospinal fluid penetrance, and food effect of BTK inhibitor tolebrutinib in healthy volunteers

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Abstract

Tolebrutinib is an oral, brain-penetrant, covalent Bruton's tyrosine kinase inhibitor in development to treat multiple sclerosis at 60 mg/day with food. A phase I trial was conducted in healthy volunteers to assess the safety and pharmacokinetics of tolebrutinib at oral doses higher than 60 mg with food and during fasting, and to determine cerebrospinal fluid (CSF) exposure after a single dose of 60 or 120 mg with food. The trial included double-blind, placebo-controlled single ascending dose (120, 240, and 300 mg; fed and fasted) and multiple ascending dose (120, 180, and 240 mg) arms. Additional open-label cohorts received a single 60 mg dose with a high-fat meal and during fasting using a crossover design or a single 60 or 120 mg dose with food and lumbar puncture to obtain CSF. Tolebrutinib was rapidly absorbed and converted to an active metabolite (designated "M2"), both of which had a terminal half-life of ~5 h. Tolebrutinib and M2 exposures increased following administration with food versus fasting, and plasma levels were generally dose proportional. For up to 4 h (the last measurement timepoint) after a 60 mg dose, CSF concentrations of tolebrutinib exceeded its in vitro cellular potency (half-maximal inhibitory concentration [IC_{50}]) for microglia, and tolebrutinib and M2 surpassed their biochemical IC_{50}. Tolebrutinib was well-tolerated, and treatment-emergent adverse events were generally mild. Concentration-QTc modeling showed no effects on QT/QTc intervals for any tolebrutinib dose or fed status. In conclusion, tolebrutinib has an acceptable safety profile at suprathera-peutic doses and achieved bioactive CSF exposures at the phase III dose.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Bruton's tyrosine kinase (BTK) is an important signaling enzyme expressed on B lymphocytes and myeloid cells, including microglia. These cell types are

considered key drivers of multiple sclerosis (MS) pathophysiology. Tolebrutinib is an oral, brain-penetrant, covalent BTK inhibitor currently being evaluated for the treatment of MS in four phase III trials at 60 mg once daily with food.

WHAT QUESTION DID THIS STUDY ADDRESS?

This phase I trial in healthy volunteers evaluated the safety and pharmacokinetics of tolebrutinib taken with food and during fasting and at higher doses than those assessed in a first-in-human trial. Additional aims were to examine cerebrospinal fluid (CSF) penetrance for the 60 mg dose taken with food and to determine QT interval prolongation risk up to the predicted highest clinical exposure levels through modeling of pharmacokinetic and electrocardiogram data.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Tolebrutinib was well-tolerated and safe up to 300 mg single doses and up to 240 mg once daily for 14 days. Exposures for tolebrutinib and its active metabolite M2 were enhanced when taken with food and generally followed dose proportionality. Tolebrutinib 60 mg reached CSF levels exceeding the cellular potency (half-maximal inhibitory concentration) for microglia. No evidence of QT interval prolongation was found for tolebrutinib and M2 exposures more than fivefold higher than those at the phase III 60 mg dose.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

These results confirm that tolebrutinib crosses the blood–brain barrier at the 60 mg dose being examined in phase III trials for MS and support tolebrutinib's safety at supratherapeutic doses, thus covering possible scenarios of high clinical exposure.

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, demyelinating, and degenerative disease of the central nervous system (CNS) that affects ~2.8 million people worldwide, and is the main cause of nontraumatic neurological disability in young and middle-aged adults.[1,2] Whereas multiple treatments are available to address transient increases in MS-related disability, disease progression remains a reality for approximately one-half of actively treated patients with relapsing forms of MS,[3] and there are currently limited therapeutic options for progressive MS, including non-relapsing secondary progressive MS.

Bruton's tyrosine kinase (BTK) has important roles in signaling cascades of B lymphocytes and myeloid cells, including CNS-resident microglia.[4,5] These cell types are considered key drivers of MS pathophysiology.[6,7] BTK is critical for the activation, differentiation, and maturation of B cells, and additionally regulates the activation of innate immune cells, including microglia, macrophages, mast cells, basophils, and neutrophils.[5,8–10] Accordingly, a brain-penetrant BTK inhibitor may target both adaptive and innate immune dysfunction in MS, potentially mitigating neuroinflammation and neurodegeneration.

Tolebrutinib is an oral, brain-penetrant, covalent BTK inhibitor in development for the treatment of MS (for a recent review of tolebrutinib, including its pharmacology, see Krämer et al.[11]). Tolebrutinib potently inhibits BTK activity with an in vitro half-maximal inhibitory concentration (IC_{50}) of 10 nM for B cells and 0.7 nM for microglia.[12] Following absorption, tolebrutinib is rapidly and extensively converted to several metabolites, including an active metabolite 3-hydroxy-tolebrutinib (designated "M2") through single hydroxylation of tolebrutinib's piperidine moiety (see Figure S1 for chemical structures of tolebrutinib and M2).[13] The first-in-human phase I trial established the plasma pharmacokinetics of tolebrutinib in the 5–120 mg dose range and also demonstrated cerebrospinal fluid (CSF) exposure at levels exceeding the in vitro IC_{50} for B cells and microglia after a single 120 mg dose under fasting conditions.[12] In a phase IIb dose-finding trial, tolebrutinib 60 mg/day over 12 weeks reduced the number of new contrast-enhancing T1 and new/enlarging T2 brain lesions by

greater than or equal to 85% in participants with relapsing MS.[14] The phase III trials investigating tolebrutinib in MS are ongoing, including two trials for relapsing MS (NCT04410991 and NCT04410978) and one trial each for non-relapsing secondary progressive MS (NCT04411641) and primary progressive MS (NCT04458051).

The purposes of the present phase I trial were to examine the safety and pharmacokinetics of tolebrutinib at higher doses than those studied in the first-in-human trial and under fed and fasting conditions and, second, to determine CSF exposure for the 60 mg dose currently being investigated in the phase III trials and for a higher 120 mg dose. QT interval prolongation risk was determined through modeling of pharmacokinetic and electrocardiography (ECG) data.[15,16] The double-blind, placebo-controlled component of the phase I trial involved three single ascending doses (SADs) of tolebrutinib given in a single-sequence, fasting-then-fed crossover design and three multiple ascending doses (MADs) administered daily for 14 days under fed conditions. Additional cohorts received open-label tolebrutinib, either at the 60 mg dose under fasting and fed conditions in a crossover design to assess the effect of food on pharmacokinetics and safety, or at 60 or 120 mg under fed conditions to assess CSF exposure.

METHODS

Study design

This was a phase I single-center trial in healthy adult volunteers (see Table S1 for the full list of selection criteria). The trial included two distinct parts: part 1, which comprised three sub-parts (SAD, food effect [FE], and CSF pharmacokinetics), and part 2 (MAD; Figure 1). Part 1-SAD examined three doses of tolebrutinib (120, 240, and 300 mg) in a double-blind, placebo-controlled manner. Doses were administered under fasting conditions and then again 72 h later (i.e., day 4) under fed conditions (high-fat meal; 800–1000 Kcal and ≥50% fat content) in three cohorts ($n = 12$ active and 3 placebo for 120 mg; $n = 8$ active and 2 placebo for 240 and 300 mg). For each cohort, two sentinel participants (1 active and 1 placebo) were treated 24 h prior to other participants in the cohort. Dose escalation was determined by a safety monitoring committee who reviewed safety and pharmacokinetic data after completion of each dose cohort. In part 1-FE, open-label tolebrutinib 60 mg was administered under fasting and fed (high-fat meal) conditions in crossover with 72 h between doses ($n = 7$ fasting-then-fed; $n = 7$ fed-then-fasting). In part 1-CSF pharmacokinetics, open-label tolebrutinib 60 mg ($n = 7$) or 120 mg ($n = 4$) was administered once under fed (moderate-fat meal; ~680 Kcal and 43% fat content) conditions with CSF sampled twice over 4 h via lumbar puncture. Part 2-MAD examined three doses (120, 180, and 240 mg) in a double-blind, placebo-controlled manner. Doses were administered once daily for 14 days under fed conditions (moderate-fat meal) in three cohorts, each comprising 12 participants ($n = 9$ active and 3 placebo), with dose escalation determined by a safety monitoring committee. Sentinel dosing was not used in part 2 because no accumulation was expected,[12] and part 1 results from higher dosing groups were available prior to treatment initiation in the MAD cohorts. Tolebrutinib was provided as 60 mg film-coated tablets, also used in the phase III trial program.

This trial was conducted in accordance with Good Clinical Practice and the ethical principles of the Declaration of Helsinki. All participants provided written informed consent before beginning any study procedures. The protocols, amendments, and participant-informed consents received appropriate institutional review board approval prior to initiation.

Safety assessments

Safety assessments included reporting of adverse events (AEs), physical examinations, clinical laboratory testing, and measurement of vital signs, including standard 12-lead ECG in all cohorts as well as 24-h Holter monitoring in part 1-SAD and part 1-FE cohorts for concentration-QT interval analysis, as described below. Both ECG sources were used to identify potentially clinically significant ECG abnormalities.

AEs, including serious AEs (SAEs), were recorded from the time of informed consent until study completion. The treatment-emergence period was defined as the time period between the first administration of tolebrutinib or placebo to either the first dose of the next treatment period (for the first treatment period of part 1-SAD and part 1-FE) or the end of study visit. AE severity was assessed with the Common Terminology Criteria (version 5) for Adverse Events. Safety and tolerability were assessed in all participants who had received at least one dose of tolebrutinib or placebo. Safety data were summarized with descriptive statistics.

Pharmacokinetics

Pharmacokinetic analysis included data from all participants who received at least one dose of tolebrutinib and had at least one primary pharmacokinetic parameter evaluable by noncompartmental methods using Phoenix WinNonlin version 6.3 (Certara LP). For part 1-SAD and part 1-FE,

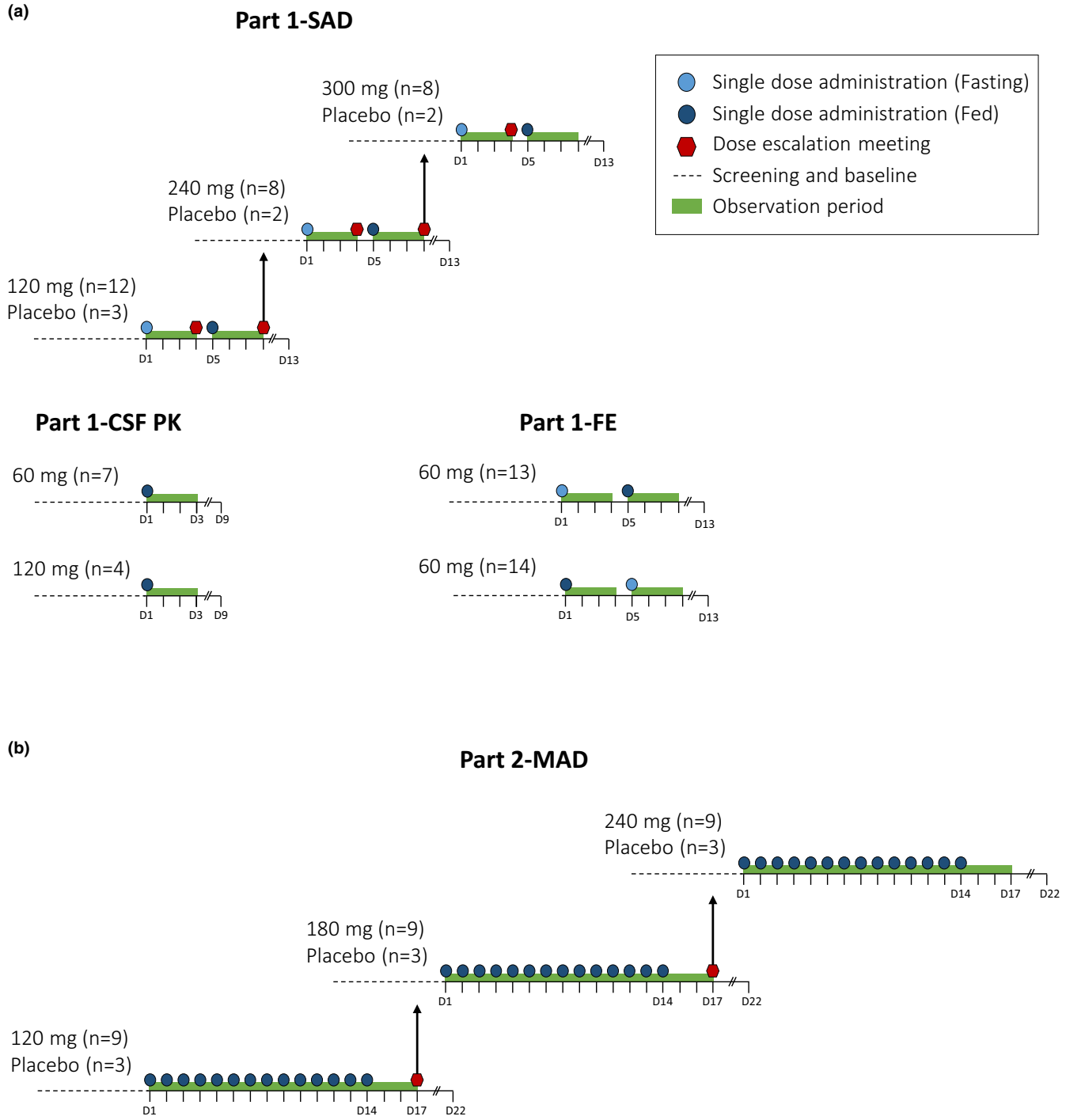

FIGURE 1 Study design. (a) Part 1 SAD, CSF pharmacokinetics, and FE studies. (b) Part 2 MAD study. CSF, cerebrospinal fluid; FE, food effect; MAD, multiple ascending dose; PK, pharmacokinetics; SAD, single ascending dose.

blood samples were obtained predose and at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 12, 24, and 48 h postdose. For part 1-CSF pharmacokinetics, blood samples were obtained at the same time schedule as in part 1-SAD and part 1-FE, and CSF samples were obtained via lumbar puncture twice at either 1 and 3 or 2 and 4-h postdose. In part 2-MAD, blood samples were obtained at the same time schedule as part 1 on days 1 and 14, with additional trough samples acquired on days 5, 8, 11, and 13, as well as 24 and 48 h after the final dose (i.e., days 15 and 16).

The concentrations of tolebrutinib and M2 were quantified by a specialist laboratory (Charles River Laboratories) using a validated liquid chromatography with tandem mass spectroscopy method with a lower limit of quantification of 10 pg/mL for tolebrutinib and 5 pg/mL for M2.

Analyses to determine the effect of fed status, dose proportionality, and accumulation were performed for tolebrutinib, M2, and combined tolebrutinib and M2 exposures, and involved modeling of the maximum plasma concentration observed (C_{max}) and the area under the plasma concentration versus time curve (AUC). These analyses included all available pharmacokinetic data from participants without relevant protocol deviations. Combined tolebrutinib and M2 exposures were calculated as the sum of the values for tolebrutinib and M2 divided by their respective molecular weights (455 and 471 g/mol, respectively). To quantify the effect of fed status for each dose, estimates and 90% confidence intervals (CIs) for the ratio of the geometric means for fed and fasting conditions were obtained by computing point estimates and 90% CIs for the difference between the arithmetic means for the fed and fasted food conditions using a linear model, and then converting to ratios by the antilog transformation. To test for dose proportionality in part 1-SAD and part 1-FE for each food condition and, separately, in part 2-MAD on day 1 and day 14, pharmacokinetic parameters for tolebrutinib and M2 were analyzed using a power model (pharmacokinetic parameter = α*dose$^{\beta}$) with the log-transformed value of the pharmacokinetic parameter as the dependent variable and log-transformed dose level as the independent variable.[17] To evaluate tolebrutinib and M2 accumulation and time dependency for each of the dose groups and the pooled cohort in part 2-MAD, a linear model of log-transformed accumulation ratio (day 14/day 1) was built. The steady-state was assessed graphically for part 2-MAD.

For part 1-CSF pharmacokinetics, CSF-to-unbound plasma concentration ratios for tolebrutinib and M2 were calculated for individual participants, then averaged. The percentages of tolebrutinib and M2 unbound to plasma proteins were determined to be 11.8% for both compounds using in vitro equilibrium dialysis.

Electrocardiography

Continuous ECG recordings were performed 24 h before and 24 h following dosing for both observation periods for part 1-SAD and part 1-FE using an internationally recognized Holter monitor with adequate resolution for ECG extraction. ECG recordings were transferred by the study site to an ECG core laboratory for further analysis. From the ECG recordings, three 10-s segments were extracted using Antares (AMPS-Montichiari) validated software from a 5-min window immediately preceding each blood sampling event for postdose measurement or nominal time-matched windows for predose measurements. ECG intervals (QT, QT corrected using Fridericia's formula [QTcF], QT corrected using Bazett's formula [QTcB], and heart rate) were acquired semi-automatically and then averaged across the three 10-s segments, giving one value for each parameter at each nominal timepoint.

Statistical analyses of ECG data were performed using SAS software (version 9.4; SAS Institute) and included data from all randomized participants provided they had at least one change from baseline in ECG assessment and additionally, for tolebrutinib groups, at least one measured pharmacokinetic concentration. By convention, the pharmacokinetic concentrations in the placebo group were set to zero. Baseline was the mean of the predose triplicate ECG assessment. Concentration-QTc modeling was performed as per recommended guidelines[15,16] using pooled data from part 1-SAD and part 1-FE, for each food condition separately. The main ECG parameter for this analysis was change from baseline in QTcF and the pharmacokinetic parameters were plasma concentrations of tolebrutinib or M2. The relationships between the change from baseline in QTcF and tolebrutinib or M2 concentrations were first explored graphically to investigate any potential delayed or sustained effects and to define the type of modeling to be used. A linear mixed effect model was used with fixed terms for population intercept, treatment intercept (1 for active and 0 for placebo) and nominal time intercept, with the concentration (slope) and the centered baseline QTcF as covariates, and with random terms for individual participant deviation from the population intercept and slope using SAS Proc Mixed. Estimates of each effect of the model and the corresponding 90% CIs were provided. Predictions of placebo subtracted change from baseline in QTcF ($\Delta\Delta$QTcF) at the geometric mean C_{max} for each dose were also calculated from the model. Absolute QTcF values and change from baseline were summarized with descriptive statistics.

RESULTS

Study population

Between August 2020 and June 2021, the 96 healthy individuals were enrolled, randomized, and treated (for demographic details see Tables S2 and S3). Of these, 35 participants received tolebrutinib or placebo in part 1-SAD, 11 received tolebrutinib in part 1-CSF pharmacokinetics, 14 received tolebrutinib in part 1-FE, and 36 received tolebrutinib or placebo in part 2-MAD. Two of the 14 participants from part 1-FE did not complete the study period, one due to withdrawn consent during the treatment period and the other due to a post-treatment AE of coronavirus disease 2019 (COVID-19). Two participants in part 1-CSF pharmacokinetics from the 60 mg arm were excluded from pharmacokinetic analyses due to exceptionally low measured

plasma and CSF concentrations of tolebrutinib and M2 (plasma concentrations were 0.3% to 3.3% of the mean for other participants), which was confirmed by re-assay. One participant in part 1-CSF pharmacokinetics from the 60 mg arm had a missing CSF sample at 4-h postdose. Three of the 36 participants from part 2-MAD discontinued the study prematurely due to withdrawn consent, including two participants who discontinued during the treatment period on days 4 or 6 and one participant who discontinued on day 15 after the treatment period.

Safety and tolerability

Tolebrutinib was well-tolerated at all dose levels regardless of fed status, with no SAEs, severe treatment-emergent AEs (TEAEs), or TEAEs leading to treatment discontinuation reported in part 1 or part 2 of the study. In part 1, the only TEAEs that were reported in multiple participants who received tolebrutinib were back pain and TEAEs related to the lumbar puncture procedure in part 1-CSF pharmacokinetics (i.e., post lumbar puncture syndrome and procedural pain; Table 1). In part 2, the only TEAE that was reported in more than one participant in any of the tolebrutinib dose groups was headache, occurring in two (7.4%) participants (Table 2). All TEAEs were of grade 1 (mild) severity except for the TEAEs of procedural pain and post lumbar puncture syndrome that were of grade 2 (moderate) severity in three participants (2 in the 60 mg dose arm and 1 in the 120 mg dose arm).

There were no unexpected safety findings associated with tolebrutinib treatment based on treatment-emergent

TABLE 1 Incidence of TEAEs by MedDRA preferred term in part 1 SAD, CSF PK, and FE studies.

	Part 1-SAD				Part 1-CSF PK		Part 1-FE	
		Tolebrutinib			Tolebrutinib		Tolebrutinib[a]	
Preferred term, *n* (%)	Placebo (*n* = 7)	120 mg (*n* = 12)	240 mg (*n* = 8)	300 mg (*n* = 8)	60 mg (*n* = 7)	120 mg (*n* = 4)	Fasting (*n* = 13)	Fed (*n* = 14)
Participants with any TEAE	0	0	1 (12.5)[b]	0	6 (85.7)	1 (25.0)	0	2 (14.3)
Post lumbar puncture syndrome	0	0	0	0	4 (57.1)	1 (25.0)	0	0
Procedural pain	0	0	0	0	4 (57.1)	1 (25.0)	0	0
Back pain	0	0	1 (12.5)[b]	0	1 (14.3)	0	0	0
COVID-19	0	0	0	0	0	0	0	1 (7.1)
Diarrhea	0	0	0	0	1 (14.3)	0	0	0
Dizziness	0	0	0	0	1 (14.3)	0	0	0
Nausea	0	0	0	0	1 (14.3)	0	0	0
Presyncope	0	0	0	0	1 (14.3)	0	0	0
Initial insomnia	0	0	0	0	0	0	0	1 (7.1)

Abbreviations: COVID-19, coronavirus disease 2019; CSF, cerebrospinal fluid; FE, food effect; MedDRA, Medical Dictionary for Regulatory Activities; PK, pharmacokinetics; SAD, single ascending dose; TEAE, treatment-emergent adverse event.

[a]All participants received tolebrutinib 60 mg in part 1-FE.

[b]TEAE occurred during the fed period.

TABLE 2 Incidence of TEAEs by MedDRA preferred term in the part 2-MAD study.

		Tolebrutinib		
Preferred term, *n* (%)	Placebo (*n* = 9)	120 mg (*n* = 9)	180 mg (*n* = 9)	240 mg (*n* = 9)
Participants with any TEAE	1 (11.1)	1 (11.1)	2 (22.2)	2 (22.2)
Abdominal rigidity	0	1 (11.1)	0	0
Constipation	0	0	0	1 (11.1)
Eye irritation	0	0	0	1 (11.1)
Headache	0	0	1 (11.1)	1 (11.1)
Pruritus	1 (11.1)	0	0	0
Rash	0	0	1 (11.1)	0

Abbreviations: MAD, multiple ascending dose; MedDRA, Medical Dictionary for Regulatory Activities; TEAE, treatment-emergent adverse event.

changes in laboratory values, except for low neutrophil counts in two participants each in part 1-SAD (both in the 300 mg arm), part 1-FE (one participant each during the fed and fasted conditions), and part 2-MAD (both in the 240 mg arm). Of these six participants, three had low neutrophil counts at baseline. For the two participants with abnormal neutrophil counts in part 2-MAD, neutrophil levels returned to baseline values within the study period while tolebrutinib dosing continued.

QTcF interval 450–480 ms was detected in three participants: one participant in the 60 mg arm in part 1-CSF pharmacokinetics, one participant who received 60 mg under fed conditions in part 1-FE, and one participant in the 180 mg arm in part 2-MAD. A separate participant who received 60 mg under fed conditions in part 1-FE had a QTcF interval increase from baseline of greater than 60 ms. There was no instance of QTcF interval greater than 480 ms, QTcB greater than 450 ms, or QTcB increase from baseline of greater than 60 ms.

Pharmacokinetics

Following single oral administration, tolebrutinib was rapidly absorbed with a median time to maximum concentration (T_{max}) under fasting conditions of 0.63–1.00 h, which was slightly delayed under fed conditions (1.38–2.00 h; Table 3, Figure 2). The mean terminal half-life ($t_{1/2z}$) of tolebrutinib ranged from 3.2 to 7.8 h, with no apparent effect of dose or food status. Plasma pharmacokinetic parameters for M2 were similar to those for tolebrutinib (Table S4).

Determination of dose proportionality with a power model showed that tolebrutinib plasma C_{max} and AUC increased more than dose-proportionally between 60 and 300 mg under the fasting condition (Table S5). This effect was primarily due to large pharmacokinetic increases between the 240 and 300 mg doses. In contrast, approximately dose-proportionate pharmacokinetic increases were observed for tolebrutinib when taken with food as well as for M2 for both fed and fasted conditions (Table S5).

Compared with the fasting condition, tolebrutinib AUC was 1.5 to 2.2-fold higher when taken with food for the 60, 120, and 240 mg doses (Table S6). There was no effect of fed status on tolebrutinib AUC for the 300 mg dose or for tolebrutinib C_{max} at any dose. AUC for M2 was similarly enhanced when tolebrutinib was taken with food, although the effect was weaker than for the parent compound (Table S6).

Multiple-dose pharmacokinetic parameters for tolebrutinib (Table 4) and M2 (Table S7) on days 1 and 14 in part 2-MAD were similar to the values for single dose administration in part 1. For both tolebrutinib and M2, C_{max} and AUC_{tau} increased with no notable deviation from dose proportionality and there was no accumulation after repeated dosing, with mean accumulation ratios for the pooled dose cohort close to 1 for tolebrutinib (0.97 [90% CI 0.73–1.29] for C_{max} and 1.09 [90% CI 0.91–1.30] for AUC_{tau}) and M2 (0.97 [90% CI 0.79–1.18] for C_{max} and 1.07 [90% CI 0.93–1.24] for AUC_{tau}).

CSF penetration

CSF analyses included data from five participants in the 60 mg group and four participants in the 120 mg group. Due to the protocol-defined sampling schedule (see Methods), this yielded two at each timepoint, except at $t = 2$ h for the 60 mg group ($n = 3$). Tolebrutinib and M2 concentrations were detected in the CSF at all timepoints for the 60 and 120 mg doses, except for M2 at the 1-h timepoint for both participants in the 60 mg cohort and one participant in the 120 mg cohort. Maximal CSF concentrations were measured at 2 h postdose for tolebrutinib and at 3–4 h for M2, ~1–2 h after their respective T_{max} in plasma (Figure 3). For up to 4 h following administration of 60 and 120 mg doses, CSF concentrations of tolebrutinib and M2 surpassed their biochemical IC_{50} values (0.7 and 0.8 nM, respectively),[13] and tolebrutinib surpassed its in vitro IC_{50} value for microglia (0.7 nM).[12] M2's IC_{50} value for microglia is unknown. At 4 h postdose, mean CSF-to-unbound plasma concentration ratios were 0.77 (60 mg dose) and 0.98 (120 mg dose) for tolebrutinib and 0.21 (60 mg dose) and 0.45 (120 mg dose) for M2.

Concentration-QT interval modeling

The time profile plots of mean tolebrutinib concentration and mean change from baseline in QTcF for fed and fasted conditions are shown in Figure S2. Concentration-QTcF models did not identify a relationship between plasma tolebrutinib or M2 concentration and QTcF under fasting or fed conditions. The slopes of the regression lines from linear models of plasma concentration and QTcF corrected for placebo and baseline (ΔΔQTcF) were negative and nonsignificant for tolebrutinib (−0.022 [95% CI −0.084 to 0.039] for fasting and −0.030 [95% CI −0.085 to 0.026] for fed; Figure S3) and M2 (−0.012 [95% CI −0.021 to 0.002] for fasting and −0.010 [95% CI −0.032 to 0.011] for fed [data not shown]). For all dose groups, model-derived ΔΔQTcF values ranged from −0.17 to 4.33 ms at tolebrutinib C_{max} and −0.14 to 4.32 ms at M2 C_{max}, and the upper-boundaries of the

TABLE 3 Tolebrutinib pharmacokinetic parameters in part 1-SAD (120, 240, and 300 mg) and part 1-FE (60 mg) cohorts by dose and food status.

	Tolebrutinib							
	60 mg		120 mg		240 mg		300 mg	
	Fasting ($n=12$)	Fed ($n=13$)	Fasting ($n=12$)	Fed ($n=12$)	Fasting ($n=8$)	Fed ($n=8$)	Fasting ($n=8$)	Fed ($n=8$)
C_{max} (ng/mL)	13.0 ± 14.2 (9.31) [110]	13.2 ± 7.58 (11.5) [57.4]	45.5 ± 29.4 (39.2) [64.7]	32.6 ± 21.3 (27.8) [65.5]	86.4 ± 59.1 (65.4) [68.4]	94.1 ± 67.3 (77.1) [71.5]	153 ± 132 (118) [86.6]	93.2 ± 48.8 (83.5) [52.4]
T_{max} (h)	0.63 [0.50–1.00]	2.00 [0.50–6.00]	0.63 [0.50–0.75]	1.38 [0.50–4.00]	1.00 [0.50–1.50]	1.38 [1.00–4.00]	1.00 [0.75–2.00]	1.63 [1.25–6.03]
C_{last} (ng/mL)	0.04 ± 0.02 (0.03) [64.6]	0.04 ± 0.02 (0.04) [45.5]	0.04 ± 0.03 (0.03) [66.6]	0.02 ± 0.01 (0.02) [56.5]	0.04 ± 0.04 (0.03) [101]	0.03 ± 0.02 (0.03) [52.7]	0.03 ± 0.01 (0.03) [44.1]	0.05 ± 0.02 (0.05) [44.8]
T_{last} (h)	18.0 [12.0–48.0]	24.0 [12.0–24.0]	48.0 [24.0–48.0]	48.0 [24.0–48.0]	48.0 [24.0–48.1]	48.0 [48.0–48.0]	48.0 [48.0–48.0]	47.0 [46.8–47.0]
AUC_{last} (h*ng/mL)	20.7 ± 14.4 (16.8) [69.4]	45.5 ± 17.9 (41.7) [39.2]	63.4 ± 31.1 (57.8) [49.1]	103 ± 69.5 (89.2) [67.5]	133 ± 71.9 (112) [54.2]	214 ± 115 (193) [53.7]	288 ± 143 (261) [49.5]	305 ± 90.7 (292) [29.7]
AUC (h*ng/mL)	20.9 ± 14.4 (17.0) [69.0]	45.8 ± 17.9 (41.9) [39.2]	63.7 ± 31.1 (58.1) [48.8]	103 ± 69.4 (89.4) [67.3]	133 ± 71.8 (113) [53.8]	214 ± 115 (194) [53.7]	288 ± 143 (262) [49.5]	305 ± 90.7 (293) [29.7]
AUC_{ext} (%)	1.06 ± 0.616 (0.891) [58.1]	0.504 ± 0.280 (0.433) [55.5]	0.642 ± 0.609 (0.473) [94.7]	0.231 ± 0.242 (0.156) [105]	0.617 ± 0.906 (0.274) [147]	0.143 ± 0.0571 (0.133) [39.9]	0.130 ± 0.120 (0.0895) [92.4]	0.114 ± 0.0551 (0.102) [48.4]
$t_{1/2z}$ (h)	3.99 ± 2.38 (3.39) [59.5]	3.20 ± 0.653 (3.14) [20.4]	6.57 ± 2.39 (6.15) [36.3]	5.03 ± 2.38 (4.64) [47.3]	7.76 ± 3.33 (7.18) [42.9]	6.26 ± 1.43 (6.11) [22.8]	5.50 ± 1.62 (5.29) [29.4]	4.66 ± 0.808 (4.61) [17.3]
CL/F (L/h)	4400 ± 3420 (3520) [77.7]	1600 ± 915 (1430) [57.1]	2240 ± 931 (2060) [41.6]	1490 ± 621 (1340) [41.7]	2610 ± 1890 (2130) [72.5]	1340 ± 511 (1240) [38.0]	1250 ± 519 (1150) [41.6]	1070 ± 360 (1030) [33.5]
V_{ss}/F (L)	13,700 ± 8650 (11,100) [63.2]	6760 ± 3430 (6030) [50.8]	9240 ± 5870 (7610) [63.5]	6070 ± 2960 (5260) [48.9]	11,900 ± 11,700 (8180) [98.4]	5170 ± 2310 (4630) [44.6]	4510 ± 2620 (3760) [58.1]	5210 ± 2600 (4820) [49.9]

Note: Values are mean ± SD (geometric mean) [CV%] except for T_{last} and T_{max} which are shown as median [min–max].

Abbreviations: AUC, area under the plasma concentration versus time curve extrapolated to infinity; AUC_{ext}, percentage of extrapolation of AUC to infinity; AUC_{last}, area under the plasma concentration versus time curve $t=0$ to last measurement; CL/F, apparent total body clearance; C_{max}, maximum plasma concentration observed; C_{last}, plasma concentration at last measurement; CV, coefficient of variation; FE, food effect; SAD, single ascending dose; SD, standard deviation; $t_{1/2z}$, terminal half-life; T_{max}, time to reach C_{max}; T_{last}, time corresponding to the last concentration above the limit of quantification; V_{ss}/F, apparent volume distribution in steady-state.

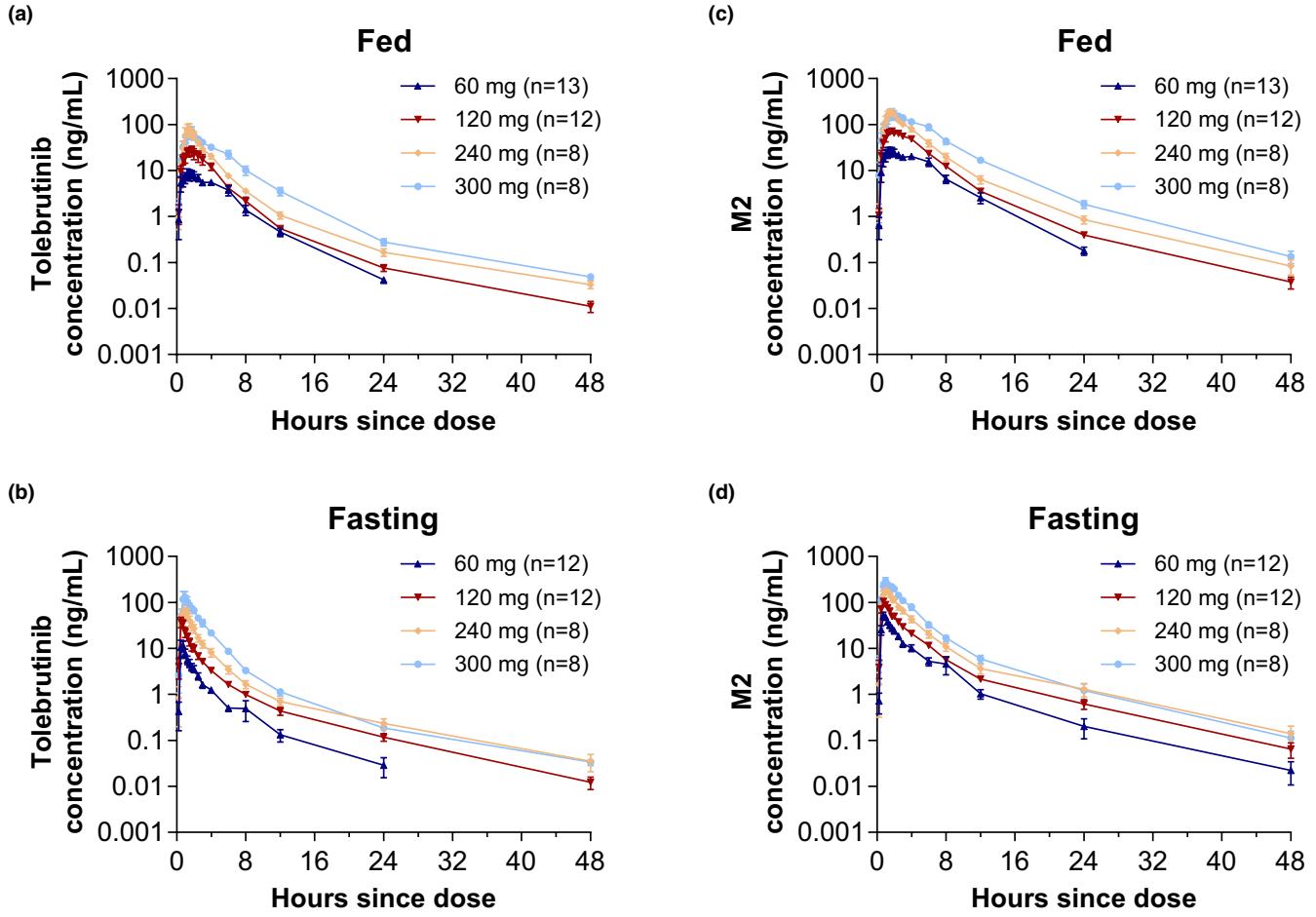

FIGURE 2 Mean plasma concentration of tolebrutinib (a, b) and M2 (c, d) over time after single oral administration of tolebrutinib 60–300 mg in fed (a, c) and fasting (b, d) state. For the fed condition, participants received a high-fat meal. Error bars indicate standard error.

corresponding two-sided 90% CIs were less than 8 ms (Tables S8 and S9).

DISCUSSION

This phase I trial investigated the safety and pharmacokinetics of tolebrutinib over a higher range of doses than those assessed in the first-in-human trial,[12] and included food effect assessment and determination of CSF penetrance for the 60 mg dose currently being examined in phase III trials for MS. Tolebrutinib was well-tolerated with single doses up to 300 mg and multiple once-daily doses up to 240 mg, with no effect on QT/QTc intervals. Tolebrutinib was rapidly absorbed and converted to its active metabolite M2. Exposures for tolebrutinib and M2 were generally dose-proportional and enhanced when tolebrutinib was taken with a high-fat meal. There was no accumulation of tolebrutinib or M2 after 14 days of repeated dosing, consistent with their short half-life and the once-daily dosing regimen. Effective CSF penetration was confirmed for the 60 mg dose, with tolebrutinib and M2 reaching pharmacologically relevant (i.e., bioactive) CSF levels.

Irreversible covalent inhibition depends on concentration-time profiles for the parent compound in addition to any metabolites containing an active warhead. Of the metabolites previously identified through metabolic screening of radiolabeled tolebrutinib, M2 was the only one to retain the active warhead and preserve the ability to irreversibly and potently inhibit BTK in a biochemical assay and an in vitro Ramos cell assay, similar to tolebrutinib.[13] Nevertheless, the pharmacology of M2, including kinase selectivity, is incompletely characterized. This work shows that conversion of tolebrutinib to M2 is rapid and not influenced by food status or repeated dosing. Furthermore, M2 had a similar $t_{1/2}$ to that of tolebrutinib but with a two-to-six-fold higher C_{max} and AUC. These findings indicate that M2 likely makes a relevant contribution to the high levels of BTK occupancy after administration of tolebrutinib.[12] Accordingly, M2 is being measured in all clinical studies of tolebrutinib to assess sources of pharmacokinetic variability and appropriately evaluate pharmacokinetic-efficacy and -safety relationships.[13]

TABLE 4 Tolebrutinib pharmacokinetic parameters in the MAD cohort by dose and day.

	Day 1		
	120 mg (*n* = 9)	**180 mg (*n* = 9)**	**240 mg (*n* = 9)**
C_{max} (ng/mL)	37.7 ± 22.7 (32.0) [60.3]	46.0 ± 18.9 (41.3) [41.0]	81.2 ± 64.9 (58.6) [79.9]
T_{max} (h)	1.25 [0.50–4.0]	2.00 [0.50–4.0]	1.25 [0.50–3.0]
AUC_{tau} (h*ng/mL)	74.7 ± 23.7 (71.7) [31.7]	132 ± 56.2 (118) [42.7]	266 ± 182 (195) [68.5]
	Day 14		
	120 mg (*n* = 8)	**180 mg (*n* = 7)**	**240 mg (*n* = 7)**
C_{max} (ng/mL)	28.9 ± 16.4 (25.3) [56.7]	55.3 ± 32.8 (46.5) [59.3]	114 ± 74.3 (83.3) [65.4]
T_{max} (h)	2.50 [0.75–4.0]	2.50 [0.75–2.5]	1.75 [1.25–2.50]
AUC_{tau} (h*ng/mL)	88.1 ± 31.6 (83.3) [35.9]	144 ± 63.6 (132) [44.3]	331 ± 192 (244) [58.2]
$t_{1/2z}$ (h)	6.94 ± 1.03 (6.88) [14.8]	7.49 ± 2.27 (7.22) [30.3]	6.08 ± 1.96 (5.84) [32.3]

Note: Tolebrutinib was administered under fed conditions in part 2-MAD. Values are mean ± SD (geometric mean) [CV%] except for T_{max} which is shown as median [min–max].

Abbreviations: AUC_{tau}, area under the plasma concentration versus time curve for the dosing interval; C_{max}, maximum plasma concentration observed; CV, coefficient of variation; MAD, multiple ascending dose; SD, standard deviation; $t_{1/2z}$, terminal half-life; T_{max}, time to reach C_{max}.

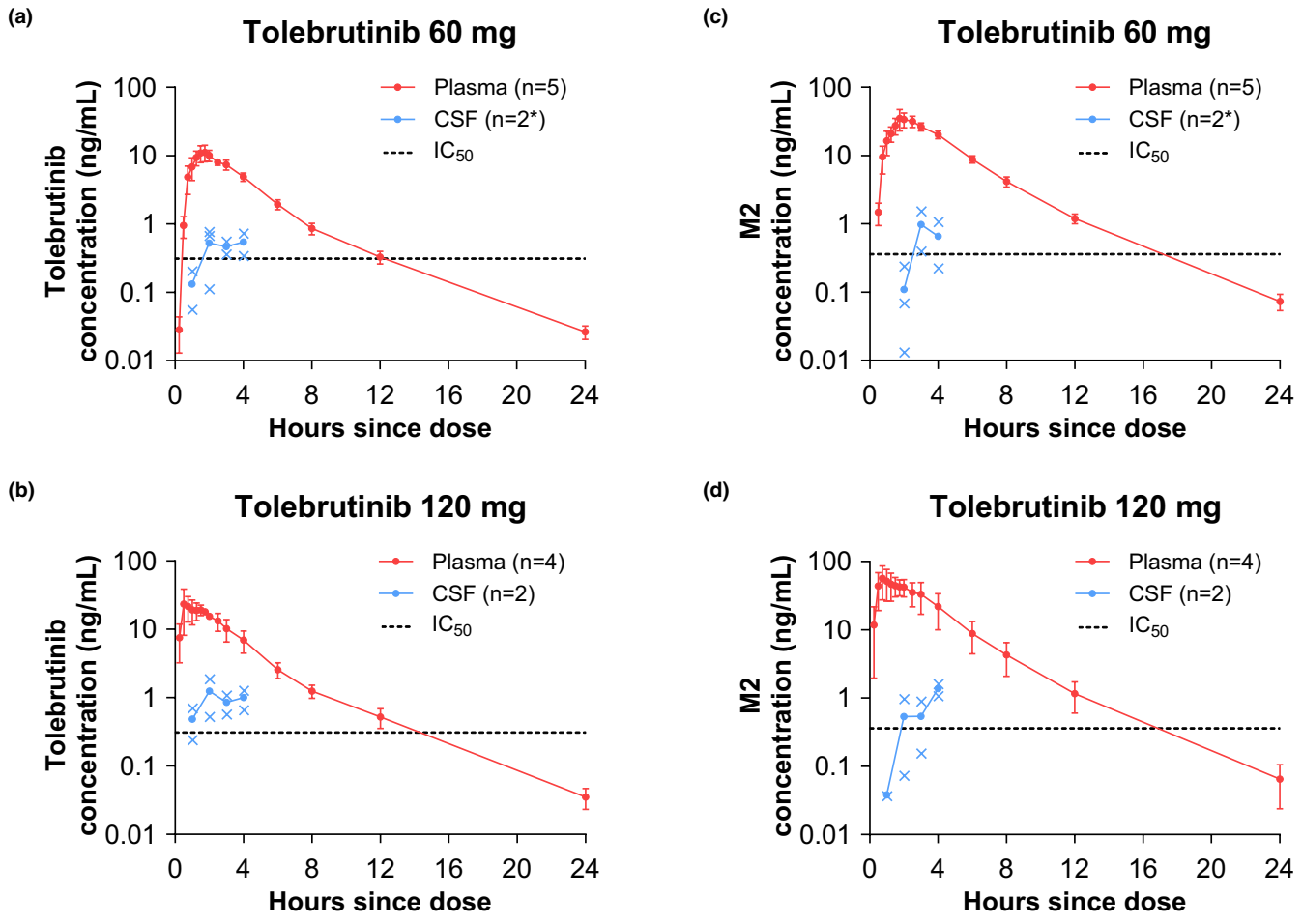

FIGURE 3 Mean CSF and plasma concentrations of tolebrutinib (a, b) and M2 (c, d) over time after single oral administration of tolebrutinib 60 mg (a, c) or 120 mg (b, d) with a moderate-fat meal. For plasma, error bars indicate standard error. At 1-hour postdose, M2 concentration in the CSF was below the lower limit of detection for both participants in the 60 mg arm and one participant in the 120 mg arm. For CSF, cross symbols indicate individual participant values. *n=2 for all timepoints except t=2 hours, which is n=3. CSF, cerebrospinal fluid; biochemical IC_{50}, half-maximal inhibitory concentration.

A positive food effect was observed for tolebrutinib and M2, consistent with preliminary findings.[12] Given that tolebrutinib is nearly completely absorbed under fasting conditions,[13] it is possible that the food effect is due to changes in first-pass metabolism via alterations in gastrointestinal and hepatic blood flow.[18] Further work will be required to understand the mechanism underlying this effect.

To date, tolebrutinib is the only BTK inhibitor to demonstrate CSF exposure at bioactive levels. After a single tolebrutinib 60 mg dose, CSF concentrations of tolebrutinib and M2 exceeded their respective biochemical IC_{50} for BTK for more than five times the half-life of BTK inactivation, indicating relevant covalent BTK inhibition in the CNS.[19] Furthermore, after the 60 mg dose, CSF concentrations of tolebrutinib increased above tolebrutinib's in vitro IC_{50} for microglia within 2 h and maintained that level up to 4 h, the longest timepoint measured. Preclinical studies have confirmed that tolebrutinib functionally modulates microglial activity in vitro and in vivo.[20,21] Therefore, tolebrutinib 60 mg may inhibit BTK activity in microglia and CNS-resident lymphocytes thought to be major drivers of disability accumulation in relapsing and non-relapsing forms of MS.[22,23] With sequential dosing, tolebrutinib may achieve higher levels of BTK occupancy in the CNS, as in the periphery,[12] consistent with its irreversible mode of binding. However, this remains speculative because the turnover rate of BTK protein in the CNS is unknown and the present study did not assess BTK occupancy because CSF cell counts were insufficient for assay.

Tolebrutinib and M2 exposures at the single dose of 300 mg and multiple daily doses of 240 mg were at least five times more than for the 60 mg dose currently being examined in phase III trials, enabling assessment of tolebrutinib safety over a wide exposure margin. No new safety signals emerged with higher doses of tolebrutinib. Except for common TEAEs related to the CSF collection procedure, TEAEs with single or multiple doses were mild in presentation and the only TEAEs reported in more than one participant were headache and back pain (2 participants each).

ECG results indicate that tolebrutinib is not associated with proarrhythmic effects. Current guidance from the International Council for Harmonisation indicates that concentration-QT interval modeling can provide supportive evidence for an investigational medicine's low proarrhythmic risk if the upper bound of the two-sided 90% CI for the QTc interval at the highest clinically relevant exposure is less than 10 ms.[24] In the present analysis, the upper bound of the 90% CIs for estimates of placebo-corrected QTcF interval changes from baseline were less than 8 ms for all C_{max} exposures for tolebrutinib and M2. Considered together with the absence of cardiac safety concerns reported in previous phase I and phase IIb trials,[12,14] the data so far have provided no indication of clinically significant cardiac repolarization effects after tolebrutinib treatment.

In conclusion, no safety or tolerability concerns were identified in healthy volunteers for single oral doses of tolebrutinib up to 300 mg and multiple daily doses up to 240 mg for 14 days. Increased exposure for tolebrutinib and its active metabolite M2 when taken with food versus fasting was confirmed, as was CSF penetrance for the 60 mg dose being evaluated in phase III trials for the treatment of MS.

AUTHOR CONTRIBUTIONS

C.J., E.K., M.-J.C., O.N., O.V., P.B., P.S., T.T., and W.S. wrote the manuscript. C.J., E.K., M.-J.C., O.N., P.B., P.S., T.T., and W.S. designed the research. W.S. performed the research. C.J., O.N., O.V., and P.S. analyzed the data.

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CONFLICT OF INTEREST STATEMENT

M.J.C., O.N., O.V., C.J., P.B., P.S., T.J.T., and E.K. are employees of Sanofi (may hold shares and/or stock options in the company). W.B.S. is the founder, president, and principal investigator of the New Orleans Center for Clinical Research and Volunteer Research Group, and the Alliance for Multispecialty Research centers in Knoxville, TN, and New Orleans, LA.

DATA AVAILABILITY STATEMENT

Qualified researchers may request access to patient-level data and related study documents, including the clinical study report, study protocol with any amendments, blank case report form, statistical analysis plan, and data set specifications. Patient-level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants. Further details on Sanofi's data sharing criteria, eligible studies, and process for requesting access can be found at: https://vivli.org/.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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