Evaluating the microcirculation in early phase clinical trials: novel methodologies and interventions

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CHAPTER V

FIRST-IN-HUMAN TRIAL TO ASSESS SAFETY, TOLERABILITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF ZAGOCIGUAT (CY6463), A CNS-PENETRANT SOLUBLE GUANYLYL CYCLASE STIMULATOR

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Study highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

The NO-sGC-cGMP system is involved with memory formation and learning and has been a target for treatment of neurodegenerative disease in previous clinical trials. sGC stimulators can elevate cGMP concentrations in an NO-dependent manner. To our knowledge, no sGC stimulators have been shown to penetrate the central nervous system in humans thus far.

WHAT QUESTION DID THIS STUDY ADDRESS?

The present study investigated safety, PK and PD of zagociguat, a novel sGC stimulator.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Single doses of zagociguat up to 50 mg and multiple doses up to 15 mg QD for 14 days were well tolerated with no safety signals, with dose-proportionally increasing exposure levels. Zagociguat showed pharmacodynamic effects on blood pressure consistent with the mechanism of action and effects of other sGC stimulators. Zagociguat penetrated the CSF compartment with a mean CSF/free plasma concentration ratio of 0.43.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Zagociguat is the first sGC stimulator shown to reach the cerebrospinal fluid compartment in humans, supporting its development as a compound for treating CNS diseases in which NO-sGC-cGMP is implicated.

Abstract

Soluble guanylyl cyclase (sGC) and its product, cyclic guanosine monophosphate play a role in learning and memory formation. Zagociguat (CY6463) is a novel stimulator of sGC being developed for the treatment of neurodegenerative disease. Single zagociguat doses of 0.3, 1, 3, 10, 20, 30 and 50 mg were administered once to healthy participants in a single-ascending-dose phase; then zagociguat 2, 5, 10 and 15 mg was administered QD for 14 days in a multiple-ascending-dose phase; and finally, zagociguat 10 mg was administered once in both fed and fasted state in a food-interaction phase. Safety of zagociguat was evaluated by monitoring treatment-emergent adverse events, suicide risk, vital signs, electrocardiography, and laboratory tests. Pharmacokinetics of zagociguat were assessed through blood, urine, and cerebrospinal fluid (CSF) sampling. Pharmacodynamic effects of zagociguat were evaluated with central nervous system (CNS) tests and pharmaco-electroencephalography. Zagociguat was well tolerated across all doses evaluated. Zagociguat exposures increased in a dose-proportional manner. Median T_{max} ranged from 0.8 h to 5 h and mean $T_{½}$ from 52.8 h to 67.1 h. CNS penetration of the compound was confirmed by CSF sampling. Zagociguat induced up to 6.1 mmHg reduction in mean systolic and up to 7.5 mmHg reduction in mean diastolic blood pressure. No consistent pharmacodynamic effects on neurocognitive function were observed. Zagociguat was well tolerated, CNS-penetrant and demonstrated pharmacodynamic activity consistent with other sGC stimulators. The results of this study support further development of zagociguat.

Introduction

A range of physiological processes are controlled by nitric oxide-soluble guanylyl cyclase-cyclic guanosine monophosphate (NO-sGC-cGMP) signaling.[1–3] In this pathway, NO, produced by nitric oxide synthases (NOS), activates sGC to produce cGMP, which produces various downstream effects.[4] In the brain, the NO-sGC-cGMP pathway plays a central role in learning and memory through induction and expression of long-term potentiation, a form of synaptic plasticity that is pivotal in memory and learning,[5] and numerous *in vitro* and *in vivo* preclinical studies confirm the role of this pathway in cognitive functioning.[6–9] Dysfunction of the NO-sGC-cGMP pathway is implicated in neurodegenerative and other diseases and has been associated with a range of disruptive processes including increased inflammation, endothelial dysfunction, and dysregulation of cerebral blood flow.[10–11] In patients with Alzheimer's disease (AD), NOS activity and expression are decreased compared to healthy controls,[12] and decreased cGMP levels correlate with memory impairment.[13–14] In addition, targeting the NO-sGC-cGMP axis via inhibition of degradation of cGMP by phosphodiesterases has shown therapeutic potential in the treatment of AD by improving markers of cognition.[15–17] This evidence suggests that modulation of the NO-sGC-cGMP pathway is a promising approach for treatment of neurodegenerative diseases.[4,18–20]

One therapeutic approach to increase NO-sGC-cGMP signalling in the brain is to stimulate sGC.[21–23] Preclinical studies with the CNS-penetrant sGC stimulator zagociguat (CY6463, 8-(2-fluorobenzyl)-6-(3-(trifluoromethyl)-1H-1,2,4-triazol-5-yl)imidazo[1,2-a]pyrazine)[24] showed that this compound can act as a positive allosteric modulator to stimulate cGMP production *in vitro* in an NO-dependent and concentration-responsive manner. *In vivo* experiments showed increases in cGMP concentrations in the CSF of treated rodents, confirming CNS penetration of the compound, as well as increased gamma band power as measured with quantitative electro-encephalography (QEEG), a characteristic that is associated with increased attention and focus.[23,25] Additionally, in an *in vivo* rat model of cognitive impairment caused by administration of a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, 0.1 mg/kg and 1 mg/kg zagociguat treatment attenuated deficits in learning and memory. [23] Finally, in a model of aged rats (21 months old) with cognitive impairment in learning as assessed by the Morris water maze, 1 mg/kg and 10 mg/kg zagociguat administration decreased thigmotaxis time compared to placebo, an indication of improved spatial learning. (data on file, Cyclerion Therapeutics, Cambridge, MA, USA) Since no sGC stimulators have been shown to be CNS-penetrant in humans and no sGC stimulators have been approved for treatment of CNS disease, zagociguat may be uniquely positioned for treatment of disorders associated with deficient NO-sGC-cGMP signalling in the brain, such as AD,[26] mitochondrial encephalopathy and lactic acidosis,[27] and cognitive impairment associated with schizophrenia.

In this study, the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of zagociguat were evaluated in a first-in-human (FIH) trial in single-ascending-dose (SAD), multiple-ascending-dose (MAD), and crossover food-interaction (FI) settings.

Methods

This study was conducted at the Centre for Human Drug Research (Leiden, The Netherlands), in accordance with the principles of the Declaration of Helsinki, the International Council on Harmonisation Good Clinical Practice and ethical principles as referenced in EU Directive 2001/20/EC. The protocol was approved by the Medical Research Ethics Committee of the BEBO foundation (Assen, The Netherlands) and prospectively registered in EudraCT (number 2018-003694-99), toetsingonline.nl (CHDR1844, ABR-number 67677), and clinicaltrials.gov (NCT03856827).

SUBJECTS

Healthy male and female subjects aged ≥18 and <65 years were eligible for inclusion in this FIH trial if no clinically significant abnormal findings were obtained on medical history, physical examination, 12-lead ECG, and laboratory tests (serum chemistry, haematology, coagulation, urine drug screen, and urinalysis). Pregnant and nursing women were excluded from participation, as well as subjects using any medication (exceptions were paracetamol/acetaminophen up to 4g/day, oral contraceptives, and hormone replacement therapy) and subjects with documented allergy or hypersensitivity to inactive compounds of the study drug formulation (tablets).

STUDY DESIGN

The first phase of the study was a double-blind, randomized, placebo-controlled SAD study. Seven cohorts of 8 subjects were planned to evaluate increasing dose levels of zagociguat, randomized in a 6:2 ratio to receive a single, oral dose

of zagociguat or placebo, respectively. Sentinel dosing was performed in all cohorts by randomizing the first 2 subjects to zagociguat or placebo (1 each) and dosing these subjects at least 24 h before the remainder of the cohort. The second phase was a double-blind, randomized, placebo-controlled MAD study with 4 cohorts of 10 subjects to evaluate increasing repeat-dose levels of zagociguat. Each cohort was randomized in a 4:1 ratio to receive once-daily, oral zagociguat or placebo for 14 days. The third phase was an open-label, randomized, 2-period, 2-treatment crossover FI study. Fourteen subjects were planned to receive 2 single doses of zagociguat, 1 in fasted and 1 in fed state. Subjects were randomized 1:1 to a fed-fasted or fasted-fed sequence with a 21-day washout in between. In the fed dosing period, subjects were administered study drug approximately 30 minutes after being served a high-fat meal (i.e., 800–1000 kcal total with approximately 150, 250, and 500 to 600 kcal derived from protein, carbohydrate, and fat, respectively). During the SAD and MAD phases, doses were administered after an overnight fast of at least 10 h. All doses were administered in tablet form.

The sample size for each stage of this phase 1 study was based on usual considerations regarding phase 1 studies of similar design aimed at obtaining initial information on safety, PK and PD effects.

DOSE LEVEL JUSTIFICATION

The 0.3 mg starting dose was based on 1/3 of the human equivalent dose (HED) of the lowest pharmacologically active dose observed in nonclinical QEEG assessments in rats,[23] in line with EMA recommendations. This dose was approximately 100-fold lower than the no-observed-adverse-event-level (NOAEL) HED observed in the 28-day good laboratory practice (GLP) toxicity study in the most sensitive species (rats), which was estimated to be 29 mg/subject/day, assuming a body weight of 60 kg, and was expected to result in 100-fold lower exposure. Subsequent dose levels were determined in dose escalation meetings based on safety, PK, and PD measurements from previous cohorts, using approximately log 0.5 exposure increases. These increases were slightly reduced when approaching the NOAEL, as this was the first exploration of a CNS penetrant compound with a plentiful pharmacological target in the brain. Final dose levels administered in the SAD phase of the study were 0.3 mg, 1 mg, 3 mg, 10 mg, 20 mg, 30 mg, and 50 mg. Although the 50 mg dose was predicted to exceed the NOAEL area under the curve (AUC), but not NOAEL maximum concentration (C_{max}) in the most sensitive species, since zagociguat was well tolerated in humans up to 30 mg, this dose level was added during study conduct.

The 2 mg starting dose for the MAD phase was chosen based on preliminary PK data showing an expected total exposure approximately twice the exposure of the 3 mg SAD cohort and lower than the exposure of the 10 mg SAD cohort, both of which showed no safety signals. Based on preliminary PK data from the SAD phase showing estimated steady state achieved at 11 days and $T_{½}$ supporting once daily (QD) dosing, the treatment regimen was set to 14 days QD. Subsequent dose levels were determined based on safety, PK, and PD data from the preceding SAD and MAD cohorts. Final dose levels administered were 2 mg, 5 mg, 10 mg and 15 mg in the MAD phase and 10 mg in the FI phase of the study.

STUDY ASSESSMENTS

SAFETY

Safety assessments during all phases of the study included standard FIH safety assessments, recording of adverse events (AES) and concomitant medication use, measurement of vital signs, electrocardiograms (ECG), physical examinations and safety laboratory tests. Given the potential blood pressure lowering mechanism of action of the compound, measurements of orthostatic blood pressure and continuous ECG monitoring (Holter) were also included in the safety assessments. In addition, since enhancement of NO-sGC-cGMP signalling may influence thrombocyte aggregation,[28] IVY bleeding time was monitored, and as the compound has potential CNS effects, the Columbia Suicide Severity Rating Scale was performed to assess any development of suicidality in participating subjects. Measurement of BP was assessed using the following procedure: BP was measured after 5 minutes of supine rest. Then, participants assumed sitting position for ≥1 minute and subsequently standing position for 1 to 3 minutes, and BP was measured to assess orthostasis, i.e. a systolic blood pressure (SBP) decrease of at least 20 mmHg or a diastolic blood pressure (DBP) decrease of at least 10 mmHg within three minutes of standing.[29] AES were coded according to the Medical Dictionary for Regulatory Activities version 21.1.

PHARMACOKINETIC ASSESSMENTS

PK sampling was performed prior to study drug administration and at timepoints 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 12 h, 24 h, and 48 h post study drug administration in the SAD phase. In the MAD phase, PK samples were collected daily before study drug administrations, and on treatment days 1 and 14 PK samples were collected on the same timepoints as in the SAD phase. In the FI phase, PK samples were obtained around study drug administration at the same timepoints

as in the SAD phase during both treatment periods. Additional PK samples were obtained 7 (±1) and 14 (±1) days post study drug administration in the first crossover period and 5 (±1) and 9 (±1) days post study drug administration in the second crossover period.

Urine was collected for 48 h after drug administration on day 1 in the SAD phase and for 24 h (i.e., 1 dosing interval) on days 1 and 14 in the MAD phase. A single CSF sample was obtained 3 h after drug administration on day 7 in cohort 3 (10 mg) of the MAD phase.

Quantification of zagociguat in plasma (in potassium ethylenediaminetetraacetic acid), urine, and CSF was conducted using a GLP-validated liquid chromatography with tandem mass spectrometry methods. Zagociguat was extracted using protein precipitation with an internal standard. (MM-501250) High-performance liquid chromatography separation was conducted at 0.7 mL/min through an ACE 50x2.1mm C18 column (Advanced Chromatography Technologies Ltd) using a gradient of 0.1% formic acid in water and in acetonitrile. The compound was detected using an API-5500 (Applied Biosystems/MDS SCIEX, Framingham, Massachusetts) in positive-ion mode, multiple reaction monitoring using parent/product transitions of 367.1/271.0 m/z for zagociguat and 367.1/271.0 m/z for the internal standard. The method for all 3 matrices was validated with a standard curve range of 1.00 to 1000 ng/mL, and linear peak area ratios were assessed with linear regression with $1/x^2$ weighting. Each analytical run included duplicate calibration curves, appropriate blanks, and triplicate quality controls at three concentrations. The inter-run accuracy and precision for plasma were -1.5 to 1.3 and 2.4 to 4.7, respectively, -3.0 to 3.0 and 2.0 to 4.0 for urine, respectively, and the accuracy for CSF was -2.3 to 4.0.

PHARMACODYNAMIC ASSESSMENTS

Vital signs were evaluated as both safety and PD endpoints.[30] In the SAD phase, BP and pulse rate were measured before study drug administration and at 30 min, 1 h, 2 h, 4 h, 6 h, 12 h, 24 h, and 48 h after administration, and orthostatic BP was assessed at the same timepoints as well as 24 h and 48 h after study drug administration. In the MAD phase, BP and pulse rate were measured before study drug administration on all treatment days and, on days 1, 2, and 14, were additionally collected 1 h, 2 h, 4 h, 6 h, and 12 h after study drug administration. Orthostatic BP was measured at 24 h and 48 h after the last study drug administration.

The CNS PD tests consisted of pharmaco-electroencephalography (PEEG) and a CNS test battery (NeuroCart®). PEEG was conducted using a 40-channel recording system under vigilance-controlled conditions for 10 minutes per assessed timepoint employing alternating 64-second periods of eyes open and eyes closed. The CNS test battery included measurements of saccadic eye movement and smooth pursuit eye movements, body sway, adaptive tracking, Visual Verbal Learning test (VVLT), Milner Learning Maze Test (MMT), simple reaction time test, and choice reaction time test. These tests were chosen because they are sensitive to low doses of CNS-active agents.[31] Eye movements, body sway, and reaction time tests have been used extensively to assess the CNS effects of sleep deprivation,[32] alcohol,[33–34] and benzodiazepines.[35–37] Adaptive tracking testing according to Borland and Nicholson[38] was conducted to assess zagociguat effects on visuo-motor coordination and vigilance, while MMT was used to assess spatial working memory,[39] and VVLT to assess acquisition and consolidation of information, active retrieval from long-term memory, and memory storage using respectively immediate recall, delayed recall, and delayed recognition tasks.[40–42] To avoid learning effects during the study, subjects were familiarized with the tests during a training session within 21 days prior to admission to the clinic. In the SAD phase, the CNS assessments including PEEG but excluding VVLT were conducted twice before study drug administration and at 2 h, 4 h, 6 h, 10 h, and 24 h after administration. In the MAD phase, CNS tests including PEEG but excluding VVLT were conducted before study drug administration on day 1 and 14, and at 1 h, 5 h, and 10 h after administration on these days. On day 1, 13 and 15, CNS tests were performed at approximately T_{max} (90 min). VVLT was conducted at 2.5 h (immediate recall) and 3.5 h (delayed recall) after study drug administration in the SAD phase, and at 2 h (immediate recall) and 2.5 h (delayed recall) after study drug administration on day 1 and 14 in the MAD phase.

STATISTICAL ANALYSIS

All statistical analyses were conducted according to a statistical analysis plan written before unblinding of treatment allocation. All safety and statistical programming was conducted using SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA), with non-compartmental analysis done using Phoenix WinNonlin version 8.1 (Certara USA, Inc., Princeton, NJ, USA).

ANALYSIS OF SAFETY DATA

All subjects who received ≥1 dose of study drug were included in the safety analyses. Treatment-emergent AES (TEAES) were summarized, and percentages

calculated by treatment, system organ class, preferred term, severity, and study drug-relatedness. ECG, safety laboratory results, and vital signs were summarized similarly. The number and percentage of out-of-range values were calculated by treatment and time point.

ANALYSIS OF PK DATA

All subjects who received ≥1 dose of zagociguat and had ≥1 post dose PK assessment were included in the PK analyses. In the SAD, AUC until last PK sample (AUC_{last}), C_{max}, and time to maximum concentration (T_{max}) were calculated for dose levels 0.3 mg, 1.0 mg, and 3.0 mg. For the subsequent dose levels these parameters plus AUC extrapolated to infinity (AUC_{inf}), total clearance (CL/F), and terminal elimination half-time ($T_{½}$) were calculated. The excreted amount of zagociguat, both absolute (AE_{last}) and as a percentage of plasma concentrations (AE_{last}%), as well as renal clearance (CLR) were calculated from urine data. In the MAD phase, the C_{max}, T_{max}, and $T_{½}$ were calculated, as well as the AUC during one dosing interval (AUC_T), trough concentration (C_{trough}), peak-to-trough ratio (PTR), and accumulation ratios based on AUCS (R_{auc}), maximum concentrations (R_{max}), and trough concentrations (R_{trough}). The same urine PK parameters calculated in the SAD were calculated per dosing interval in the MAD. For CSF PK, ratio between plasma and CSF concentration was calculated (R_{csf}) and corrected for protein binding ($R_{csf–free}$). In *in vitro* experiments, the mean percentage of protein-bound zagociguat (1 and 10 µM) was 97.27%, 98.47%, 96.71%, 98.69%, and 98.89% in mouse, rat, dog, monkey, and human plasma, respectively, determined using equilibrium dialysis method. (data on file, Cyclerion Therapeutics, Cambridge, MA, USA) Hence, to calculate the ratio between concentration in the CSF and the unbound concentration in plasma, it was assumed that the free zagociguat concentration in human plasma was 1.11% of total zagociguat concentration in plasma. All PK parameters were expressed as geometric means with 95% confidence intervals (CI) and medians with minimum and maximum values.

Dose proportionality was calculated using log transformed AUC_{last}, AUC_{inf}, and C_{max} for the SAD phase and using AUC_T and C_{max} for day 1 and day 14 for the MAD phase. Above PK parameters were fitted to a regression model and resulting slope estimates compared to a pre-specified critical interval of 0.5 to 2.0.[43] Steady state analysis was performed by calculating Helmert contrasts between subsequent trough concentrations until the contrast was not statistically significant.[44] Food effects were assessed by comparing C_{max}, AUC_T, and AUC_{inf} in the fed and fasted state using an analysis of variance model with food condition, sequence and period as fixed effects and subject within sequence as a random effect.

ANALYSIS OF PD DATA

All repeatedly measured PD parameters were summarized (n, mean, SD, SEM, median, minimum, and maximum values) by treatment and time, while single measured PD variables were only summarized by treatment. Treatment effects on PD variables, pulse rate, and BP were assessed using an analysis of covariance model with fixed factors treatment, time, and treatment by time, random factor subject and the average of the value before drug administration were used as covariates. From these models estimated differences, the least square mean (LSM) estimates, and the p-value were calculated. Differences between zagociguat and placebo for each dose level were calculated both pooled and for each dose level, and for both day 1 and day 14 in the MAD phase.

Results

SUBJECT DISPOSITION

In the SAD phase, 56/56 planned subjects received study drug and completed the study, of which 42 received zagociguat and 14 placebo. In the MAD phase, 40 subjects received at least 1 dose of study drug, of which 32 received zagociguat and 8 placebo. 38 subjects completed the MAD phase; 1 subject dropped out due to non-study related reasons after 1 dose of zagociguat (10 mg), and 1 subject was lost to follow-up after completion of 14-day zagociguat (2 mg) treatment. Study drug (placebo) was discontinued in 1 subject on day 10 due to a liver enzyme elevation. This subject completed study assessments including follow-up as planned. In the FI phase, 13/14 subjects who received study drug completed both treatment periods while 1 subject dropped out after the 1st treatment period due to non-study related reasons.

BASELINE STATISTICS

No remarkable differences in age, height, weight, or BMI were noted between study cohorts in any phases of the study (Table S1). Sex distribution was also similar across the SAD and FI phases but in the MAD phase, the percentage of male subjects in the placebo group was 100% compared to 37.50-75% across the zagociguat groups.

SAFETY DATA

No deaths or other serious AES were observed. Paracetamol was occasionally used as concomitant medication for headache or malaise. Across all study phases, observed TEAES were mild and transient. An overview of TEAES across study phases is provided in Table 1.

TABLE 1 Overview of TEAES across study phases.

SAD Dose level	TEAES, n	Subjects with ≥1 TEAE, n (%)	Subjects with ≥1 study drug related TEAE, n	Subjects with ≥1 SAE, n	Subjects discontinued from study drug due to ≥1 TEAE, n
0.3 mg (n=6)	5	2 (33.3%)	2	0	0
1 mg (n=6)	5	2 (33.3%)	1	0	0
3 mg (n=6)	7	3 (50.0%)	2	0	0
10 mg (n=6)	7	2 (33.3%)	2	0	0
20 mg (n=6)	5	3 (50.0%)	2	0	0
30 mg (n=6)	13	5 (83.3%)	5	0	0
50 mg (n=6)	15	5 (83.3%)	3	0	0
Pooled Zagociguat group (n=42)	57	22 (52.4%)	17	0	0
Placebo (n=14)	11	9 (64.3%)	6	0	0
MAD **Dose Level**					
2 mg (n=8)	17	6 (75%)	6	0	0
5 mg (n=8)	27	8 (100%)	7	0	0
10 mg (n=8)	19	8 (100%)	5	0	0
15 mg (n=8)	18	7 (87.5%	5	0	0
Pooled Zagociguat group (n=32)	81	29 (90.1%)	23	0	0
Placebo (n=8)	13	7 (87.5)	6	0	1
FI **Sequence**					
10mg Fed-Fasted (n=7)	7	5 (35.7%)	1	0	0
10mg Fasted-Fed (n=7)	7	4 (30.8%)	2	0	0

FI=food interaction; MAD=multiple ascending dose; SAD=single ascending dose; SAE=serious adverse event; TEAE=treatment emergent adverse event.

SAD PHASE

The overall incidence of TEAES was lower in the pooled zagociguat group (22/42 subjects, 52.4.%) than in the placebo group (9/14 subjects, 64.3%). A greater number of subjects experienced ≥1 TEAE in the groups with the higher zagociguat dosages of 30 mg (5/6 subjects, 83.3%) and 50 mg (5/6 subjects, 83.3%) when compared with the 0.3, 1, 3, 10 and 20 mg dose groups (range of 2/6 (33.3%) to 3/6 subjects (50%)). For all zagociguat treated subjects, 8/42 subjects (19.0%) experienced at least 1 dizziness TEAE, compared to 0 subjects in the placebo group. One orthostatic hypotension TEAE was reported in a subject who received 3 mg zagociguat. Gastro-intestinal (GI)-related TEAES were reported more frequently in zagociguat groups (1/6 subjects, 16.7%, all cohorts except 50 mg, 4/6 subjects, 66.7%) when compared with placebo (1/14 subjects, 7.1%) (Table S2). No clinically relevant abnormalities were seen in safety laboratory tests (including IVY bleeding time), vital signs measurements, and ECG analysis (including safety Holter).

MAD PHASE

Comparable overall percentages of subjects with TEAES were seen across treatment groups and compared with placebo (Table S3). Two subjects in the 2 mg, 5 mg, and 15 mg zagociguat groups and 5 subjects in the 10 mg group experienced GI TEAES, compared to 0 subjects in the placebo group. Postural dizziness was observed in 1 subject who received 5 mg zagociguat. One subject in the 2 mg, 5 mg, and 10 mg zagociguat group each reported a non-postural dizziness TEAE, compared to 0 subjects in the placebo group. No orthostatic hypotension events were reported.

Two subjects experienced mild increases in liver enzymes. For one subject, this manifested after 10 placebo administrations (peak AST 53 U/L [normal range<35 U/L], ALT 97 U/L [normal range<45 U/L], GGT 25 U/L [normal range < 55 U/L]). The subject was subsequently withdrawn from dosing. At follow-up, liver enzyme elevation was resolving (AST 34 U/L, ALT 49 U/L, GGT 19 U/L). The second subject developed liver enzyme elevation on day 6 of 2 mg zagociguat QD. Liver enzyme elevation peaked after the last dose of zagociguat (day 16, AST 49 U/L, ALT 92 U/L, GGT 22 U/L). Upon follow-up on day 30, liver enzymes were decreasing (AST 43 U/L, ALT 49 U/L, GGT 23 U/L). Review of all other safety laboratory parameters (including IVY bleeding time), vital signs, and ECG analysis (safety Holter) revealed no other clinically significant changes.

FI PHASE

A comparable number of subjects experienced ≥1 TEAE under fasted (n=5/14, 35.7%) and fed conditions (n=4/13, 30.8%) (Table S4). Three subjects had dizziness TEAES: 2 different subjects experienced respectively postural and non-postural dizziness during the fasted condition, while another subject experienced both a non-postural and separate postural dizziness event during the fed condition. No orthostatic hypotension events were recorded.

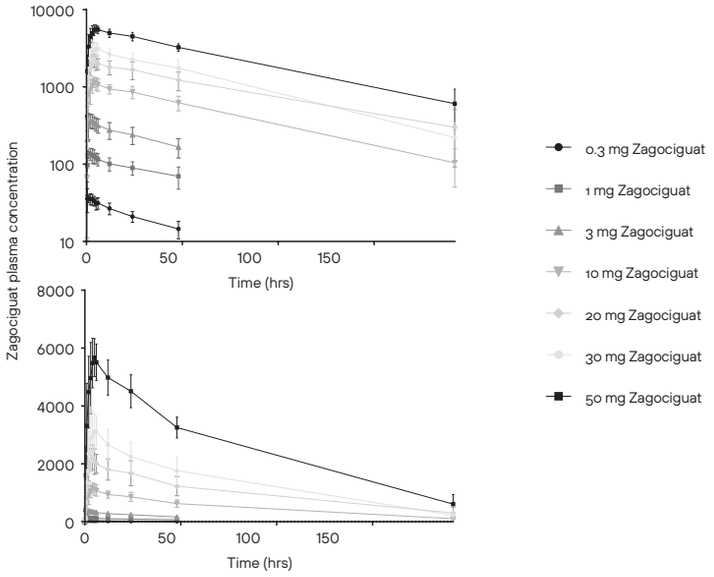
PHARMACOKINETIC ANALYSES

Data from 42 subjects in the SAD phase, 32 subjects in the MAD phase, and 14 subjects in FI phase were included in PK analyses.

SAD PHASE

Mean zagociguat concentrations in plasma up to 192 h post administration in the SAD phase are shown in Figure 1. A summary of PK parameters across cohorts is given in Table 2. Mean terminal $T_{½}$ ranged between 57 and 68 hr after single administrations of 10 to 50 mg. Zagociguat was absorbed more rapidly at doses of 0.3 to 3 mg (median T_{max} 0.5 - 2.1 hr) than at doses of 10 to 50 mg (3.5 to 5 hr), and a dose-linear increase in C_{max} and AUC after single doses of 0.3 to 50 mg zagociguat was observed. Zagociguat was detectable in urine from the 10 mg dose level, with a low percentage of dose administered excreted in urine (median 0.01% - 0.02%). Dose linearity analysis using AUC_T and C_{max} indicated dose proportionality (Table S5).

FIGURE 1 Mean (SD) zagociguat concentration in plasma (ng/mL) (top: semi-log scale, bottom: linear scale) on Day 1 during the SAD phase.



First dose is at protocol time=0. Concentrations below limit of quantification (1.00 ng/mL) were set to 0. SAD=single ascending dose; SD=standard deviation.

TABLE 2 Summary of Plasma and CSF PK Parameters of Zagociguat-SAD Phase.

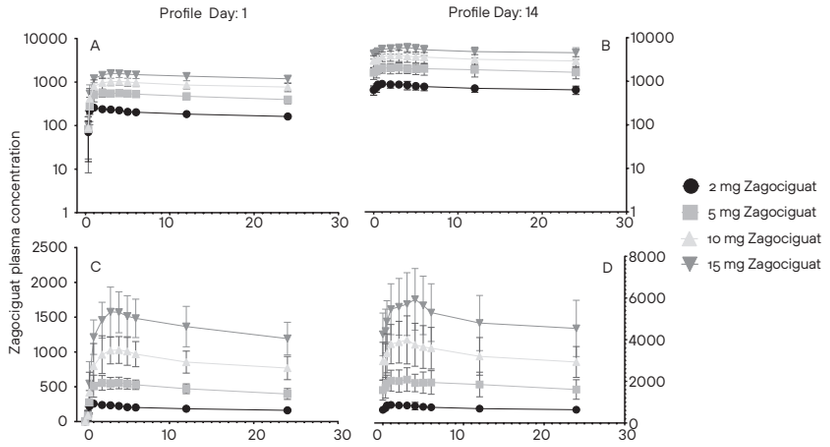
Treatment	Parameter	N	GEOMEAN	95% CI (lower, upper)	Median	MIN	MAX
0.3 mg	C_{max} (ng/mL)	6	39.95	33.55, 47.58	40.45	31.70	50.70
	T_{max} (h)	6			0.8	0.5	4.0
	AUC_{last} (h*ng/mL)	6	1062	885, 1275	1087	844	1291
1 mg	C_{max} (ng/mL)	8	142.46	123.75, 164.00	150.00	114.00	161.00
	T_{max} (h)	8			1.0	0.5	3.0
	AUC_{last} (h*ng/mL)	8	4337	3497, 5377	4456	3264	5456
3 mg	C_{max} (ng/mL)	8	372.93	292.01, 476.27	372.00	285.00	514.00
	T_{max} (h)	8			2.1	0.5	4.0
	AUC_{last} (h*ng/mL)	8	11381	9031, 14342	11220	8785	16427
10 mg	C_{max} (ng/mL)	6	1190.17	999.97, 1416.55	1290.00	889.00	1340.00
	T_{max} (h)	6			3.5	3.0	6.0
	$T_{½}$ (h)	3	63.4	29.1, 138.3	64.8	45.8	85.8
	AUC_{inf} (h*ng/mL)	3	92293	55049, 154736	95039	73984	111808
	AUC_{last} (h*ng/mL)	6	57602	37016, 89639	57922	32439	103436
20 mg	C_{max} (ng/mL)	6	2191.70	1783.28, 2693.65	2265.00	1690.00	2730.00
	T_{max} (h)	6			4.0	3.0	4.0
	$T_{½}$ (h)	6	67.1	54.44, 82.8	68.0	50.4	88.9
	AUC_{inf} (h*ng/mL)	6	194329	132134, 285800	180286	112219	313840
	AUC_{last} (h*ng/mL)	6	166913	122796, 226878	158060	109973	237435
30 mg	C_{max} (ng/mL)	6	3365.05	2703.22, 4188.91	3650.00	2270.00	3950.00
	T_{max} (h)	6			4.5	3.0	6.0
	$T_{½}$ (h)	6	65.7	46.7, 92.4	62.2	42.0	101.8
	AUC_{inf} (h*ng/mL)	6	271998	181509, 407599	288299	171361	492575
	AUC_{last} (h*ng/mL)	6	251099	171612, 367296	273032	156449	425747
50 mg	C_{max} (ng/mL)	6	5727.44	5033.81, 6516.64	5815.00	4820.00	6680.00
	T_{max} (h)	6			5.0	4.0	6.0
	$T_{½}$ (h)	6	57.0	46.4, 70.0	57.3	45.5	74.5
	AUC_{inf} (h*ng/mL)	6	478098	412224, 554497	471151	413139	604738
	AUC_{last} (h*ng/mL)	6	424802	374171, 482284	427283	344645	498002

AUC_{inf}=area under concentration-time the curve from time zero to infinity; AUC_{last}=area under the concentration-time curve from time zero to last measurable concentration; CI=confidence interval; C_{max}=maximum concentration, CSF=cerebrospinal fluid; GEOMEAN=geometric mean; N=Number of subjects; MAX=maximum; MIN=minimum; PK=pharmacokinetic; SAD=single ascending dose; SD=standard deviation; $T_{½}$= Apparent terminal phase half-life; TMAX=time of maximum concentration.

MAD PHASE

Mean zagociguat concentrations in plasma on day 1 and 14 of are shown in Figure 2. A summary of PK parameters across cohorts is given in Table 3. Mean PTR ranged from 1.35 (15 mg cohort, day 14) to 1.62 (2 mg cohort, day 1), indicating $T_{½}$ longer than the dosing interval (>24 h), and median observed T_{max} ranged from 1 to 3.5 h. CSF samples showed a mean CSF/free plasma concentration ratio of 0.43. Zagociguat was detectable in urine from the 2 mg dose level onwards, and as in the SAD phase a very low percentage of administered zagociguat was excreted in urine (median 0.00% - 0.04%). Dose linearity analysis using AUC_T and C_{max} indicated dose proportionality both on day 1 and day 14 (Table S6). Steady state analysis showed that for all doses administered, steady state was attained on day 11.

FIGURE 2 Mean (SD) zagociguat concentration in plasma (ng/mL) (A and B: semi-log scale, C and D: linear scale) on Day 1 (A and C) and Day 14 (B and D) during the MAD phase.



First dose is at protocol time=0. Concentrations below limit of quantification (1.00 ng/mL) were set to 0. MAD=multiple ascending dose; SD=standard deviation.

TABLE 3 Summary of Plasma and CSF PK Parameters of Zagociguat-MAD Phase (Days 1 and 14).

Treatment	Parameter	Day	N	GEOMEAN	95% CI (lower, upper)	Median	MIN	MAX
2 mg	C_{max} (ng/mL)	1	8	260.40	243.42, 278.56	268	224	282
		14	8	879.83	771.21, 1003.75	888	704	1170
	T_{max} (h)	1	8			1.0	0.5	2.0
		14	8			1.0	0.5	4.0
	$T_{½-eff}$ (h)		8	54.60	43.60, 68.37	54.46	42.63	66.67
	AUC_T (h*ng/mL)	1	8	4500	4209, 4812	4524	4067	5201
		14	8	16895	14508, 19674	16960	12894	23549
	CTROUGH (ng/mL)	1	8	161.13	149.10, 174.13	157.0	147	192
		11	8	587.49	484.50, 712.38	579.0	407	911
		12	8	593.32	491.16, 716.74	584.0	427	911
		13	8	616.39	510.84, 743.74	609.5	439	938
		14	8	624.08	524.04, 743.20	631.5	454	914
	PTR	1	8	1.62	1.54, 1.70	1.64	1.47	1.77
		14	8	1.41	1.34, 1.48	1.40	1.28	1.55
	R_{auc}		8	3.75	3.37, 4.18	3.80	3.09	4.53
	R_{max}		8	3.38	3.09, 3.70	3.32	2.9	4.15
	RTHROUGH		8	3.87	3.40, 4.41	4.06	3.09	4.76
5 mg	C_{max} (ng/mL)	1	8	601.97	535.60, 676.56	597	464	707
		14	8	2079.08	1661.53, 2601.56	2230	1230	2950
	T_{max} (h)	1	8			2.0	1.0	5.0
		14	8			3.0	0.6	3.0
	$T_{½-eff}$ (h)		8	54.60	43.60, 68.37	56.63	32.27	83.04
	AUC_T (h*ng/mL)	1	8	11004	9597, 12517	11488	8329	12874
		14	8	42085	32189, 55024	43934	22299	64599
	CTROUGH (ng/mL)	1	8	389.14	325.85, 464.71	410.5	267	482
		11	8	1478.18	1110.15, 1968.22	1545.0	781	2380
		12	8	1495.10	1143.49, 1954.82	1575.0	785	2260
		13	8	1507.11	1125.74, 2017.68	1580.0	740	2290
		14	8	1539.88	1167.04, 2031.84	1635.0	778	2350
	PTR	1	8	1.55	1.39, 1.72	1.50	1.37	2.05
		14	8	1.35	1.27, 1.43	1.34	1.26	1.58
	R_{auc}		8	3.83	3.17, 4.62	3.93	2.48	5.51
	R_{max}		8	3.45	2.88, 4.15	3.56	2.24	4.89
	RTHROUGH		8	3.98	3.41, 4.59	3.79	2.91	5.33
10 mg	C_{max} (ng/mL)	1	8	1071.70	903.40, 1271.37	1140	728	1370
		14	7	3911.16	2996.09, 5105.72	4050	2570	5730
	T_{max} (h)	1	8			3.0	2.0	6.0
		14	7			2.2	1.0	3.0
	$T_{½-eff}$ (h)		7	54.29	42.76, 68.92	54.53	37.32	82.55

(Continuation Table 3)

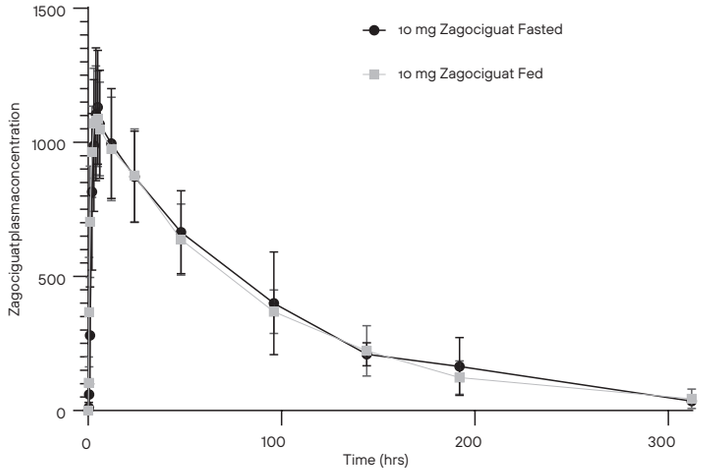
Treatment	Parameter	Day	N	GEOMEAN	95% CI (lower, upper)	Median	MIN	MAX
	AUC_T (h*ng/mL)	1	8	20065	16847, 23898	21523	12966	25246
		14	7	76786	58939, 100037	82073	49313	107639
	CTROUGH (ng/mL)	1	8	751.32	614.64, 918.40	750.5	455	989
		11	7	2763.22	2034.92, 3752.18	3090.0	1650	4170
		12	7	2912.46	2187.20, 3878.23	3240.0	1880	4450
		13	7	2837.85	2095.18, 3843.78	3190.0	1710	4220
		14	7	2847.64	2239.24, 3621.34	2820.0	1980	3910
	PTR	1	8	1.35	1.27, 1.43	1.36	1.27	1.67
		14	7	1.37	1.31, 1.44	1.37	1.30	1.47
	R_{csf}	7	7	0.005	0.003, 0.006	0.005	0.003	0.007
	$R_{csf\ free}$	7	7	0.415	0.307, 0.560	0.437	0.251	0.664
	R_{auc}		7	3.80	3.10, 4.66	3.8	2.78	5.48
	R_{max}		7	3.60	2.86, 4.53	3.68	2.62	5.25
	RTHROUGH		7	3.79	3.17, 4.54	3.56	3.03	5.37
15 mg	C_{max} (ng/mL)	1	8	1616.87	1371.50, 1906.14	1530	1190	2270
		14	8	5850.17	4746.81, 7210.00	5805	4420	8420
	T_{max} (h)	1	8			3.0	1.0	5.0
		14	8			3.5	1.0	4.0
	$T_{½-eff}$ (h)		8	52.80	44.05, 63.28	51.81	40.69	75.26
	AUC_T (h*ng/mL)	1	8	31299	26949, 36350	30092	24204	42871
		14	8	116128	93512, 144213	111532	83912	175583
	CTROUGH (ng/mL)	1	8	1173.20	997.97, 1379.19	1165	884	1580
		11	8	4149.25	3342.27, 5151.05	4140	3000	5880
		12	8	4246.79	3480.29, 5182.12	4140	3190	6500
		13	8	4157.21	3434.86, 5031.59	4160	3090	6290
		14	8	4377.90	3446.62, 5560.81	4275	2930	6980
	PTR	1	8	1.38	1.30, 1.46	1.37	1.22	1.50
		14	8	1.35	1.20, 1.49	1.33	1.12	1.61
	R_{auc}		8	3.71	3.18, 4.33	3.64	2.98	5.04
	R_{max}		8	3.61	3.16, 4.15	3.54	2.95	4.46
	RTHROUGH		8	3.73	3.17, 4.39	3.81	2.97	4.85

AUC_T=area under the concentration - time curve between consecutive dosing; CI=confidence interval; C_{max}=maximum concentration, CSF=cerebrospinal fluid; C_{trough}=through concentration; $GEOM_{ean}$=geometric mean; MAD=multiple ascending dose; PK=pharmacokinetic; PTR=peak-to-trough ratio; R_{auc}=accumulation ratio calculated from AUC_T at steady state and after a single dose; RCSF=ratio of CSF concentration divided by plasma concentration; $R_{csf\ free}$=ratio of CSF concentration divided by free plasma concentration; R_{max}=accumulation ratio calculated from C_{max} at steady state and after a single dose; R_{trough}=accumulation ratio calculated from C_{trough} at steady state and after a single dose; SD=standard deviation; $T_{½-eff}$=effective half-life based on accumulation; T_{max}=time of maximum concentration.

FI PHASE

No food effects were observed in C_{max} (LSM ratio fed vs fasted 0.99, 95%CI: 0.94, 1.03) or AUC_{inf} (LSM ratio fed vs fasted 0.99, 95%CI: 0.95, 1.04) (Figure 3). For all subjects, zagociguat concentration in plasma was below limit of quantification in pre-dose samples in the second treatment period, indicating a sufficiently long washout period.

FIGURE 3 Mean (SD) zagociguat concentration in plasma (ng/mL) (normal scale) on Day 1 of fed and fasted treatment periods during the FI phase.



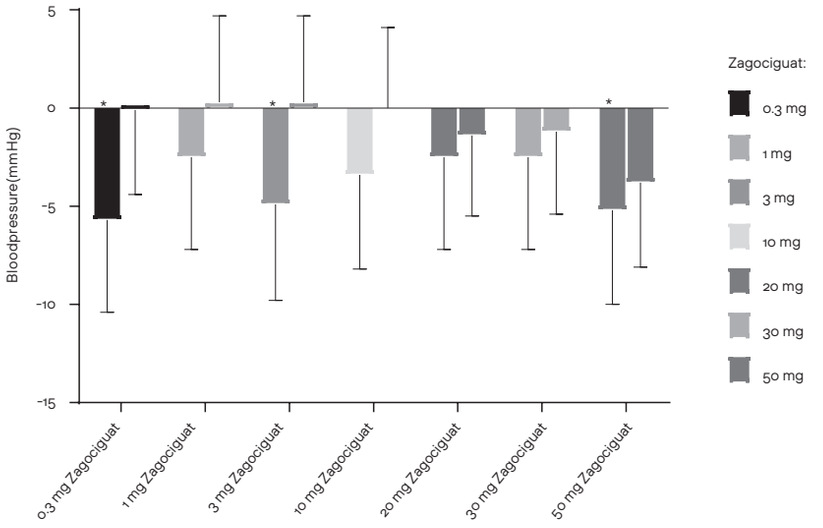
First dose is at protocol time=0. Concentrations below limit of quantification (1.00 ng/mL) were set to 0. FI=food interaction; SD=standard deviation.

PHARMACODYNAMIC ANALYSES

BLOOD PRESSURE

In the SAD phase, decreases in mean SBP over 48 h were observed after 0.3 mg zagociguat (LSMs difference from placebo: -5.7, 95% CI: -10.4, -1.0, p=0.019); 3 mg zagociguat (LSMs difference: -4.9, 95% CI: -9.8, -0.1, p=0.047), and 50 mg zagociguat (LSMs difference: -5.2, 95% CI: -10.0, -0.5, p=0.032). No decreases in SBP in other dose groups were observed, and no decreases in DBP were observed. LSMs of SBP and DBP across treatment groups over the course of the study are presented in Figure 4.

FIGURE 4 SAD phase: SBP (left bar) and DBP (right bar), mean difference in LSMs (SD) over entire treatment period, zagociguat vs placebo.



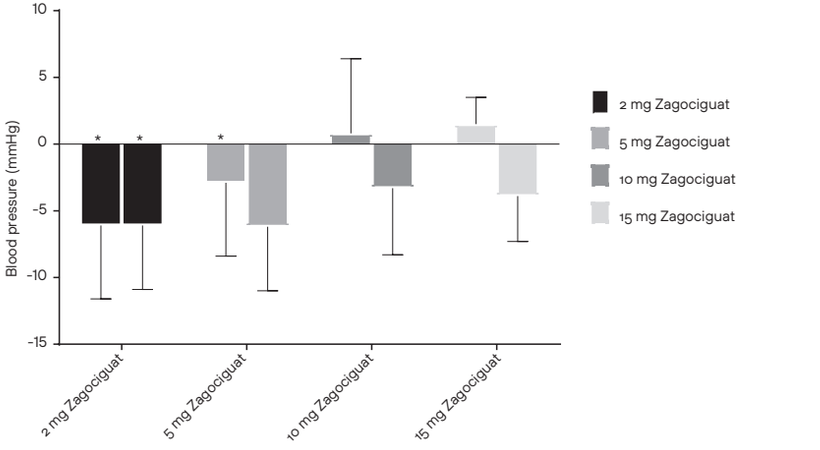
** denotes difference in LSMs of zagociguat vs placebo with p<0.05. DBP=diastolic blood pressure; LSM=least squares mean; SAD=single ascending dose; SBP=systolic blood pressure; SD=standard deviation.*

In the MAD phase (Figure 5), over 14 days of 2 mg zagociguat once-daily dosing, compared with placebo, a mean decrease in SBP and DBP was observed (LSMs difference: -6.1 95% CI: -11.6, -0.6, p=0.032 and LSMs difference: -6.1, 95% CI: -10.9, -1.3, p=0.015; respectively). DBP over 14 days was also lower compared to placebo after 5 mg zagociguat administration (LSMs difference: -6.2, 95% CI: -11.0, -1.4, p=0.014), with a maximum difference from placebo of -7.5 mmHg (95% CI: -13.0, -2.0, p=0.009) on Day 14. No decreases in SBP or DBP were observed in the 10 and 15 mg zagociguat groups.

CNS FUNCTION AND pEEG ASSESSMENTS

An overview of analysis results of CNS function and PEEG assessments is provided in Tables S7 and S8. No dose-dependent or consistent statistically significant differences between placebo and zagociguat groups were observed in PEEG or CNS tests in either the SAD or MAD phase.

FIGURE 5 MAD phase: SBP (left bar) and DBP (right bar), overall difference in LSMs (SD), zagociguat vs placebo.



** denotes difference in LSMs of zagociguat vs placebo with p<0.05. DBP=diastolic blood pressure; LSM=least squares mean; MAD=multiple ascending dose; SBP=systolic blood pressure; SD=standard deviation.*

Discussion

Zagociguat was well tolerated across all doses evaluated in this study with no safety concerns. A higher proportion of zagociguat-treated subjects reported mild dizziness and GI-related TEAEs, which is consistent with AEs reported for other sGC stimulators.[45–46] Both dizziness and GI-related events can be linked to the mechanism of action of zagociguat, since NO-sGC-cGMP signalling is involved in smooth muscle relaxation in both blood vessels and the GI tract, including the lower esophageal sphincter.[47] No clinically relevant abnormalities in ECGS, vital signs or safety laboratory assessments were observed. Of note, although enhancement of NO-sGC-cGMP signalling has been shown to inhibit platelet function *in vitro*,[28] and serious bleeding events were reported more frequently with riociguat treatment in the phase 3, placebo-controlled clinical trials in pulmonary hypertension,[48] no increases in bleeding time nor any bleeding events were associated with zagociguat administration in this study. One subject had elevated liver enzymes after 6 administrations of 2 mg zagociguat, but

no other indications of potential drug-induced liver injury were observed at higher dose levels, indicating a low likelihood of drug relatedness of this AE.

Zagociguat exposures increased in a dose-proportional manner following both single and multiple escalating doses. Effective $T_{1/2}$ and terminal elimination $T_{1/2}$ are supportive of QD dosing and suggest steady state will be achieved after 11 days of zagociguat treatment, with moderate drug accumulation over a treatment period of 14 days. Zagociguat can be given with or without food since no food effects were observed in this study. Additionally, CNS penetration of the compound was confirmed by CSF sampling during the MAD phase, with a mean CSF/free plasma concentration ratio of 0.43, comparable to CSF/free plasma ratios in preclinical experiments with dogs (0.45 at 1 h, 0.32 at 2 h, 0.3 at 4 h and 0.28 at 8 h after oral administration of 1 mg/kg zagociguat) and monkeys (geometric mean CSF/free plasma ratio of 0.57 and 0.48 after intravenous and oral administration, respectively). Although sGC stimulators have been shown to penetrate the blood-brain barrier in preclinical studies,[49] zagociguat is the first confirmed CNS-penetrant sGC stimulator to be in clinical development for the treatment of CNS diseases. Based on the totality of preclinical pharmacology across multiple models (e.g., EEG, NMDA), a CSF steady state concentration of >30 nM was established as the target threshold for pharmacological activity of zagociguat. Simulations based on plasma exposures achieved in the 10 mg and 15 mg MAD cohorts and CSF exposure achieved in the 10 mg MAD cohort predicted CSF exposures above the 30 nM threshold from day 3 of 15 mg zagociguat QD, indicating that the investigated dose range reached theoretical efficacious exposures in the CNS. Effects of zagociguat on BP were observed, which is a documented effect of sGC stimulation through vasodilation induced by smooth muscle relaxation in response to cGMP.[50] Though this observation was not dose dependent, it suggests that zagociguat engages sGC. Although no consistent or dose dependent PD CNS effects were observed across dose levels using PEEG or the NeuroCart® test battery, we hypothesize that in healthy subjects with normal neurocognitive functioning, sGC stimulation may not measurably improve cognitive function.[31] Importantly, no negative effects of zagociguat on tests for cognitive function were detected.

LIMITATIONS

The population tested in this study likely had normal neurocognitive function, which may have caused a ceiling effect and prevented the detection of neurocognitive improvement. Additionally, to induce cognitive improvement, as opposed to reversing impairment as shown in preclinical studies, treatment duration longer than the evaluated 14 days may be needed. A single CSF sample was obtained at a timepoint when steady state was not yet achieved in plasma, limiting the evaluation of PK of zagociguat in CSF. Lastly, cohorts were not balanced with respect to sex, which may have limited the extent to which conclusions can be drawn regarding sex differences in PK or PD effects of zagociguat.

CONCLUSION

Based on this study, zagociguat is a safe and CNS-penetrant sGC stimulator with potential for pharmacological action in neurodegenerative disease. These data support further development of zagociguat.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.


Figure S1


Figure S2


Figure S3


Figure S4


Figure S5


Figure S6


Figure S7


Figure S8

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