A Phase I, First-in-Human, Healthy Volunteer Study to Investigate the Safety, Tolerability, and Pharmacokinetics of CVN424, a Novel G Protein-Coupled Receptor 6 Inverse Agonist for Parkinson’s Disease

David H. Margolin, Nicola L. Brice, Antonia M. Davidson,1 Kim L. Matthews, and Mark B. L. Carlton

Cerevance, Inc., Boston, Massachusetts (D.H.M.); Cerevance, Ltd., Cambridge, United Kingdom (N.L.B., K.L.M., M.B.L.C.); and Development, LP, Austin, Texas (A.M.D., P.P.D.)

Received July 13, 2021; accepted January 20, 2022

ABSTRACT

CVN424 is a novel small molecule and first-in-class candidate therapeutic to selectively modulate GPR6, an orphan G-protein coupled receptor. Expression of GPR6 is largely confined to the subset of striatal projection neurons that give rise to the indirect (striatopallidal) pathway, important in the control of movement. CVN424 improves motor function in preclinical animal models of Parkinson’s disease. Here, we report results of a phase 1, first-in-human study investigating the safety, tolerability, and pharmacokinetics of CVN424 in healthy volunteers. The study (NCT03657030) was randomized, double-blind, and placebo controlled. CVN424 was orally administered in ascending doses to successive cohorts as inpatients in a clinical research unit. Single doses ranged from 1 mg to 225 mg, and repeated (7 day) daily doses were 25, 75, or 150 mg. CVN424 peak plasma concentrations were reached within 2 h post-dose in the fasted state and increased with increasing dose. Dosing after a standardized high-fat meal reduced and delayed the peak plasma concentration, but total plasma exposure was similar. Mean terminal half-life ranged from 30 to 41 h. CVN424 was generally well tolerated: no serious or severe adverse effects were observed, and there were no clinically significant changes in vital signs or laboratory parameters. We conclude that CVN424, a nondopaminergic compound that modulates a novel therapeutic target, was safe and well tolerated. A phase 2 study in patients with Parkinson’s disease is underway.

SIGNIFICANCE STATEMENT

This is the first-in-human clinical study of a first-in-class candidate therapeutic. CVN424 modulates a novel drug target, GPR6, which is selectively expressed in a pathway in the brain that has been implicated in the motor dysfunction of patients with Parkinson’s disease. This study paves the way for investigating this novel mechanism of action in patients with Parkinson’s disease.

Introduction

The ability to pharmacologically modulate specific cell types and circuits offers great promise for development of new therapies to improve the management of Parkinson’s disease and other neurologic disorders (Fishell and Heintz, 2013). Parkinson’s disease patients commonly experience lapses in symptom relief (e.g., motor fluctuations) despite treatment with standard-of-care dopaminergic medications, and dosage increases intended to overcome those episodes are often limited by side effects, notably drug-induced dyskinesia.

Parkinson’s disease motor symptoms result from degeneration of dopamine-producing neurons of the nigrostriatal pathway and the consequent impact on dopamine-receptive striatal neurons and their efferent circuits. There are two major types of dopamine-receptive neurons in the striatum, which differ in the type of dopamine receptor they express and that give rise to separate efferent pathways. Medium spiny neurons that express dopamine D1 receptors give rise to the striatonigral “direct” pathway, whose activity normally facilitates movement but can also drive the involuntary movements of levodopa-induced dyskinesia (Ryan et al., 2018). Medium spiny neurons that express D2 receptors give rise to the striatopallidal “indirect” pathway, whose activity inhibits movement. Normally, dopamine acting via these D2 receptors lowers the
neuronal level of 3′-5′-cyclic adenosine monophosphate (cAMP), facilitating movement by reducing neuronal activity in these striatal neurons and thus lessening the motor-inhibitory influence of the indirect pathway. Conversely, under the pathologic condition of dopamine depletion in Parkinson's disease, the indirect pathway becomes hyperactive (DeLong & Wichmann, 2015) and contributes to bradykinesia and “freezing” of gait.

CVN424 is a potent and selective inverse agonist of GPR6, an orphan G-protein coupled receptor that is selectively expressed by the D2 receptor-positive neurons of the indirect pathway (Heiman et al., 2008). Expression of GPR6 is very low or absent in D1 receptor-positive neurons of the direct pathway and in other central nervous system regions and peripheral tissues (Morales et al., 2018) (Brice et al., 2021). Since GPR6 is a Gs-coupled receptor with high constitutive activity (Uhlenbrock et al., 2002), it normally increases cAMP and thereby activates the indirect pathway, in opposition to the inhibitory effect of the Gs-coupled D2 receptors. CVN424 suppresses this constitutive activity, reducing cellular cAMP levels (Brice et al., 2021)(Sun et al., 2021) and potentially attenuating the pathologic hyperactivity of the indirect pathway seen in Parkinson's patients (Fig. 1). Thus, CVN424 is predicted to mimic the salutary effect of dopaminergic medications on the indirect pathway but without concurrent activation of the direct pathway. CVN424 should thereby facilitate voluntary movement without exacerbating levodopa-induced dyskinesia, and thus has therapeutic potential in Parkinson's disease as a monotherapy or as an adjunct to levodopa.

Preclinical testing of CVN424 included an extensive panel of pharmacology studies conducted in vitro and in vivo in rodents (Brice et al., 2021). These findings established that CVN424 was orally bioavailable and central nervous system penetrant. Importantly, CVN424 was effective at enhancing motor function in Parkinson's disease preclinical models, such as the 6-hydroxydopamine lesion model in rodents (Brice et al., 2021), supporting the GPR6 inverse agonist therapeutic hypothesis. Based on these data, a first-in-human trial was conducted to investigate the safety, tolerability, and pharmacokinetics of CVN424 in healthy subjects.

Materials and Methods

Study Design. The study was conducted at the Clinical Research Unit of PPD Development, LP, in Austin, TX, in compliance with the ethical principles of the Declaration of Helsinki, the Good Clinical Practice Guidelines of the International Conference on Harmonization, and local laws and regulations. This study was approved by the investigator's Institutional Review Board. All the subjects were given detailed written and oral information about the study, and written informed consent was obtained before screening for eligibility. This study was registered at ClinicalTrials.gov (https://clinicaltrials.gov/ct2/show/NCT03657030).

This was a first-in-human, randomized, double-blinded, placebo-controlled single- and multiple-dose dose escalation study in healthy subjects. Each cohort consisted of 8 subjects randomized (2:1) to receive CVN424 or placebo under overnight fasted conditions. In the single ascending dose (SAD) portion of the study, 40 healthy male or female subjects, ages 18 to 50 years, were enrolled and randomized into 1 of 5 ascending dose cohorts (designated S1 through S5) to receive 1, 5, 25, 75, or 225 mg of CVN424 or placebo. In each single-dose cohort, 2 sentinel subjects (1 subject receiving CVN424 and the other placebo) were dosed first; the remaining 6 subjects of each cohort could be dosed after blinded review of 24-hour post-dose safety and tolerability data provided that the adverse event profile of CVN424 in the first 2 subjects was considered acceptable. For the multiple ascending dose (MAD) portion of the study, 24 subjects were enrolled in 1 of 3 ascending multiple-dose cohorts (designated M1 through M3) and randomized to receive 7 daily doses of 25, 75, or 150 mg of CVN424 or placebo. Dose escalations occurred after a blinded review of all available safety, tolerability, clinical laboratory results, and available pharmacokinetic (PK) data, including at least 72-hour post-dose follow-up of the most recent cohort.

Subjects for all cohorts were admitted to the clinical research unit 1 day prior to dosing and remained in the unit through at least 48 hours after their last dose for safety and PK assessments. The total confinement period was 4 or 9 nights for single- and multiple-dose cohorts, respectively. Outpatient or telephone follow-up assessments for single- and multiple-dose cohorts, respectively, occurred on approximately days 8 and 14 or days 10, 14, and 21.

The effect of food on bioavailability of CVN424 was assessed according to United States Food and Drug Administration Guidance for Industry, 2002. After the safety of a single dose of 5 mg administered in a fasted state had been assessed, the same subjects returned to the clinic (no sooner than 14 days after their prior dose) and received the same dose as before, administered after ingesting a standardized high-fat high-calorie breakfast. Subjects finished the entire content of their breakfast within 25 minutes and received investigational product 30 minutes (±5 minutes) after beginning the meal. Sentinel dosing was not required for subjects returning to the clinic for the fed regimen.

Study Drug. Aqueous suspensions of CVN424 contained 200 mg of hydroxypropyl methylcellulose, 50 mg of Tween-80, and 10 ml of water. Placebo contained the same ingredients but omitted active drug.

Applying a 20-fold safety margin below the human-equivalent dose corresponding to the no-observed-adverse-effect level from nonclinical toxicology studies, the maximum recommended starting dose for this first-in-human study per Food and Drug Administration guidance was 0.8 mg/kg, or 48 mg for a 60-kg subject. However, based on preclinical data, that dosage was predicted to yield up to 98% receptor occupancy of GPR6 sites in the brain. To be conservative, the starting dosage for the SAD was set at 1 mg, with an expected peak receptor occupancy of 58%.

Subjects. Eligible subjects were healthy male or female adult volunteers between 18 and 50 years of age, weighing at least 45 kg with a body mass index between 18 and 30 kg/m². Subjects were excluded at screening if they had out-of-range vital signs, clinically significant abnormalities on standard clinical laboratory testing or electrocardiogram, a positive urine result for cocaine or drugs of abuse, or had evidence of any disorder or other abnormality that might have impacted the ability of the subject to participate or potentially confounded the study results. Subjects agreed to abide by the study’s contraceptive requirements. (For eligibility criteria see Supplemental Material.)

PK Assessments. In the SAD cohorts, blood samples were obtained for PK analyses on day 1 pre-dose and serially through 72 hours post-dose. In the MAD cohorts, blood samples were collected for PK analyses on day 1 pre-dose and serially through 24 hours post-dose (i.e., day 2 pre-dose), pre-dose on days 3, 4, 5, and 6, and on day 7 pre-dose and serially through 72 hours post-dose. Plasma obtained pre-dose and serially through 72 hours post-dose after their last dose for safety and PK assessments. The total concentration of CVN424 or placebo under overnight fasted conditions. In each single- and multiple-dose cohorts, respectively, occurred on approximately days 8 and 14 or days 10, 14, and 21. The effect of food on bioavailability of CVN424 was assessed according to United States Food and Drug Administration Guidance for Industry, 2002. After the safety of a single dose of 5 mg administered in a fasted state had been assessed, the same subjects returned to the clinic (no sooner than 14 days after their prior dose) and received the same dose as before, administered after ingesting a standardized high-fat high-calorie breakfast. Subjects finished the entire content of their breakfast within 25 minutes and received investigational product 30 minutes (±5 minutes) after beginning the meal. Sentinel dosing was not required for subjects returning to the clinic for the fed regimen.

Study Drug. Aqueous suspensions of CVN424 contained 200 mg of hydroxypropyl methylcellulose, 50 mg of Tween-80, and 10 ml of water. Placebo contained the same ingredients but omitted active drug.

Applying a 20-fold safety margin below the human-equivalent dose corresponding to the no-observed-adverse-effect level from nonclinical toxicology studies, the maximum recommended starting dose for this first-in-human study per Food and Drug Administration guidance was 0.8 mg/kg, or 48 mg for a 60-kg subject. However, based on preclinical data, that dosage was predicted to yield up to 98% receptor occupancy of GPR6 sites in the brain. To be conservative, the starting dosage for the SAD was set at 1 mg, with an expected peak receptor occupancy of 58%.

Subjects. Eligible subjects were healthy male or female adult volunteers between 18 and 50 years of age, weighing at least 45 kg with a body mass index between 18 and 30 kg/m². Subjects were excluded at screening if they had out-of-range vital signs, clinically significant abnormalities on standard clinical laboratory testing or electrocardiogram, a positive urine result for cocaine or drugs of abuse, or had evidence of any disorder or other abnormality that might have impacted the ability of the subject to participate or potentially confounded the study results. Subjects agreed to abide by the study’s contraceptive requirements. (For eligibility criteria see Supplemental Material.)

PK Assessments. In the SAD cohorts, blood samples were obtained for PK analyses on day 1 pre-dose and serially through 72 hours post-dose. In the MAD cohorts, blood samples were collected for PK analyses on day 1 pre-dose and serially through 24 hours post-dose (i.e., day 2 pre-dose), pre-dose on days 3, 4, 5, and 6, and on day 7 pre-dose and serially through 72 hours post-dose. Plasma obtained after centrifugation was stored frozen at -70°C until analysis.

Plasma concentrations of CVN424 were quantitated using a validated assay based on liquid chromatography coupled with tandem mass spectrometry (Frontage Laboratories, Inc.; Exton, PA). The assay has a dynamic range of 0.100–100 ng/mL; to extend the range, high-concentration samples were retested after dilution.

The following PK parameters were calculated for CVN424 from plasma concentration and actual time data for each subject by non-compartmental analysis using Phoenix WinNonlin (Certara LP, Princeton, NJ) Version 8.0: area under the concentration-time curve from time 0 to 24 hours (AUC(0-24)), from time 0 to time of the last quantifiable concentration (AUC(t)), from time 0 to infinity (AUC(∞)) and during a dosing interval (AUC(0→τ) for Day 7 in multiple-dose cohorts), time...
to the observed maximum plasma concentration (tmax), the maximum observed plasma concentration after a single dose (Cmax) and at steady state (Cmax,ss), the minimum plasma concentration at steady state (Cmin,ss), apparent clearance CL/F, apparent volume (Vz/F) and terminal half-life (t1/2z).

Accumulation ratios based on AUC (Rac(AUC)) and Cmax (Rac(Cmax) and dose-normalized AUCs, Cmax, and Cmax,ss were also calculated.

The PK parameters were summarized by treatment using summary statistics. Approximate attainment of steady state was visually assessed by plotting mean trough concentrations.

To evaluate dose proportionality, a power model was fitted to describe the relationship between Y (Cmax, AUC24, AUCt, and AUCtau for single-dose cohorts and Cmax,ss, AUC24, AUCt, and AUCtau for multiple-dose cohorts) and X (dose) using the least-squares linear regression model \[ \ln(Y) = \ln(a) + b \times \ln(X) \]. Dose proportionality was concluded if the 90% confidence interval (CI) of the slope b lies entirely within \[ [1 + \ln(0.8) / \ln(r), 1 + \ln(1.25) / \ln(r)] \], where r is a ratio that describes the dose range and was defined as the ratio of highest dose/lowest dose (Smith et al., 2000).

To evaluate the effect of food on PK of CVN424 in suspension formulation, a linear mixed-effect model (SAS PROC MIXED) with treatment as a fixed effect and measurements within subject as repeated measures was fitted to the natural log-transformed PK parameters Cmax, AUC24, AUCt, AUCtau, and for use in estimation of effects and construction of CIs for SAD cohort S2 Fed compared with SAD cohort S2 Fasted. Point estimates and 90% CIs for differences on the log scale were exponentiated to obtain estimates for the ratios of geometric means and respective 90% CIs on the original scale.

Safety Assessment. The study’s primary objective was to characterize in healthy subjects the safety and tolerability profile of escalating dose levels of a CVN424 suspension when administered as a single oral dose or daily oral doses for 7 days. Safety parameters included adverse events, vital sign measurements, physical examinations, clinical laboratory results, electrocardiographs (ECG), and assessment of suicidal ideation and behavior. Blood and urine samples were analyzed using hematology, coagulation, serum chemistry, urinalysis, and drug screen test panels. Additionally, serum prolactin and thyrotropin levels were monitored.

For single-dose cohorts, vital signs (oral temperature, respiration, pulse, and blood pressure) were obtained at screening and at inpatient check-in, Day 1 pre-dose, at 1, 2, 4, 6, 8, and 12 hours post-dose, then every 12 hours through 72 hours post-dose, at Outpatient Visit (Day 8) or Early Termination (if applicable), and as appropriate at follow-up (Day 14 ±2 days). Triplicate orthostatic vital signs (blood pressure and heart rate) were recorded at baseline (inpatient check-in) 15 minutes apart. For multiple-dose cohorts, vital signs (oral temperature, respiration, pulse, and blood pressure) were obtained at screening and at inpatient check-in, on days Day 1 pre-dose and at 1,
2, 4, 6, 8, and 12 hours post-dose, on days 2 through 6 pre-dose and 12 hours post-dose, and in the morning on days 8 and 9 (24 and 36 hours post-dose, respectively). TriPLICATE orthostatic vital signs (blood pressure and heart rate) were recorded at baseline (inpatient check-in) 15 minutes apart.

**Statistical Analyses.** The safety set included all subjects who were enrolled and received study drug. The safety set was used for demographic, baseline characteristics, and safety summaries. The PK set included all subjects who received study drug and had at least 1 measurable plasma concentration.

Medical history and adverse events were coded using the Medical Dictionary for Regulatory Activities (Medical Dictionary for Regulatory Activities; Version 21.0); adverse events were coded by system organ class and preferred term. At each level of subject summarization, a subject was counted once if the subject reported one or more events. For the pooled treatment groups, subjects in the fed/fasted cohort (CVN424 5 mg or matching placebo) were counted once if the subject reported one or more events in either the fasted or fed treatment period. Concomitant medications were coded using World Health Organization Drug Dictionary, Version 9.3 (SAS Institute, Cary, NC). For categorical variables, number of subjects and percentages (rounded to 1 decimal place) were reported. Continuous variables were summarized using descriptive statistics including number of subjects, mean, median, SD, minimum, and maximum, unless otherwise specified. Geometric mean and coefficient of variation (CV) were presented for PK parameters. If there were repeated assessments at a time point, the first non-missing assessment was included in the summary tables.

Baseline values were defined as the last non-missing assessment (including unscheduled assessments) before the first dose of study drug. Where values were obtained in triplicate, average of the triplicate assessments instead of each individual triplicate assessment was used as baseline. For MAD cohorts, a mixed-effect model with the treatment, visit, and interaction between treatment and visit as fixed effects, baseline weight as the covariate, and subjects nested within visit as the repeated measures was fitted to the post-baseline weight for the use in estimation of the effects of CVN424 on body weight. Kenward-Rogers degrees of freedom were specified in the model. The estimated least squares mean and standard error were presented for each treatment at each post-baseline visit. The estimated mean difference between each CVN424 dose cohort and the corresponding pooled placebo group along with the standard error, 90% CI, and the 2-sided P value were presented at each post-baseline visit.

**Results**

**Subjects.** A total of 64 subjects were enrolled, 48 received CVN424 (30 in the SAD cohorts and 18 in the MAD cohorts), and 16 received placebo (10 in the SAD cohorts and 6 in the MAD cohorts). One MAD subject who received a 75-mg dose of CVN424 discontinued early because of an adverse event of

### TABLE 1
Demographic characteristics of the study subjects

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>CVN424</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo(^a)</td>
<td>1 mg</td>
<td>5 mg</td>
<td>25 mg</td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td>(N = 10)</td>
<td>(N = 6)</td>
<td>(N = 6)</td>
<td>(N = 6)</td>
<td>(N = 6)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.0 (10.1)</td>
<td>31.3 (7.8)</td>
<td>36.5 (9.8)</td>
<td>35.3 (8.2)</td>
<td>34.2 (7.1)</td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>3 (30.0)</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
<td>2 (33.3)</td>
<td>0</td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>7 (70.0)</td>
<td>4 (66.7)</td>
<td>4 (66.7)</td>
<td>4 (66.7)</td>
<td>6 (100.0)</td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td>White</td>
<td>9 (90.0)</td>
<td>4 (66.7)</td>
<td>5 (83.3)</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td></td>
<td>Black or African American</td>
<td>1 (10.0)</td>
<td>2 (33.3)</td>
<td>0</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td></td>
<td>American Indian or Alaska Native</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td>Hispanic or Latino</td>
<td>6 (60.0)</td>
<td>1 (16.7)</td>
<td>4 (66.7)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td></td>
<td>Not Hispanic or Latino</td>
<td>4 (40.0)</td>
<td>5 (83.3)</td>
<td>2 (33.3)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.0 (10.4)</td>
<td>176.0 (12.3)</td>
<td>167.3 (9.0)</td>
<td>169.5 (9.9)</td>
<td>175.4 (7.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.7 (11.9)</td>
<td>78.1 (12.2)</td>
<td>72.6 (8.1)</td>
<td>72.0 (8.2)</td>
<td>75.6 (3.9)</td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26.1 (2.6)</td>
<td>25.2 (2.5)</td>
<td>25.9 (1.9)</td>
<td>25.1 (2.8)</td>
<td>24.7 (2.1)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>CVN424</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo(^a)</td>
<td>25 mg</td>
<td>75 mg</td>
<td>150 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(N = 6)</td>
<td>(N = 6)</td>
<td>(N = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.5 (7.71)</td>
<td>36.8 (8.93)</td>
<td>31.7 (9.81)</td>
<td>38.0 (7.87)</td>
<td></td>
</tr>
<tr>
<td>Female, No. (%)</td>
<td>1 (16.7)</td>
<td>2 (33.3)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Male, No. (%)</td>
<td>5 (83.3)</td>
<td>4 (66.7)</td>
<td>6 (100.0)</td>
<td>6 (100.0)</td>
<td></td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td></td>
<td>2 (33.3)</td>
<td>5 (83.3)</td>
<td>3 (50.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Black or African American</td>
<td>4 (66.7)</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>American Indian or Alaska Native</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td>Hispanic or Latino</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
<td>2 (33.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not Hispanic or Latino</td>
<td>4 (66.7)</td>
<td>5 (83.3)</td>
<td>4 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.6 (9.7)</td>
<td>173.0 (8.7)</td>
<td>174.7 (5.5)</td>
<td>175.8 (5.0)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.1 (9.4)</td>
<td>84.0 (9.7)</td>
<td>83.0 (7.3)</td>
<td>83.9 (9.2)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>26.9 (2.1)</td>
<td>25.0 (1.1)</td>
<td>27.2 (1.9)</td>
<td>27.1 (1.7)</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{N, number.}\)

\(^a\)Subjects who received placebo were pooled across single-dose cohorts and similarly for multiple-dose cohorts.
dysphagia; all other subjects completed the study as per protocol. The demographic characteristics of the enrolled subjects are summarized in Table 1.

**Pharmacokinetics.** The mean plasma concentration-time profiles of CVN424 in the SAD and MAD cohorts are presented in Fig. 2.
Following administration of a single dose of CVN424 oral suspension in a fasted state, mean plasma CVN424 concentrations (Table 2) increased in a similar fashion for all dose levels, reaching a peak within 2 hours post-dose. Mean plasma CVN424 concentrations declined with a multiphasic elimination and showed a similar trend across all dose levels. Mean plasma CVN424 concentrations were detectable throughout 72 hours of post-dose sampling in all dosed subjects. Following administration of a single dose (5 mg) of CVN424 oral suspension under fed state, mean plasma CVN424 concentrations were slower to reach the peak level post-dose than in the fasted state, and the peak plasma concentration was lower.

Following single dose oral administration of 1 mg to 225 mg of CVN424 under fasted conditions, mean peak (Cmax) and total (AUC24, AUCt, and AUC∞) plasma exposure increased with increase in CVN424 dose, with inter-individual variability (CV%) ranging from 9.3 to 41.6%. Median Tmax ranged from 1.5 to 2 hours across the fasted SAD cohorts. Mean t1/2 and V/F ranged from 29.6 hours to 41.4 hours and 306 L to 560 L, respectively, with no apparent trend across SAR cohorts. Mean CL/F ranged from 6.6 L/h to 9.6 L/h with CV ranging from 20.5 to 40.9%.

Following single-dose oral administration of 5 mg of CVN424 in the fed state, the presence of food lowered mean peak plasma CVN424 concentration (Cmax: 29.03 ng/ml in the fasted state, 44.03 ng/ml in the fed state). Median Tmax was delayed in presence of food. The 90% CI of the ratio of geometric LS means for Cmax for the comparison under fed state, mean plasma CVN424 concentrations were detectable up to 72 hours post-dose following the last dose of CVN424 on Day 7.

Following a once-daily administration of CVN424 oral suspension for 7 days (Table 3), Cmax,ss, Cmin,ss, and AUCtau increased with increase in dose in the 25 mg to 150 mg dose range. Mean plasma Cmax,ss values were 315 ng/ml, 776 ng/ml, and 1097 ng/ml, and Cmin,ss values were 74 ng/ml, 241 ng/ml, and 497 ng/ml for 25 mg, 75 mg, and 150 mg MAD cohorts, respectively. Mean plasma AUCtau values on Day 7 were 2954 hours*ng/ml, 9425 hours*ng/ml, and 16,200 hours*ng/ml for 25 mg, 75 mg, and 150 mg MAD cohorts, respectively. Median Tmax ranged from 1.75 to 2.5 hours, and mean t1/2 ranged from 30.6 to 34.1 hours on Day 7 across MAD cohorts.

Point estimates of Cmax and AUCs (AUC24, AUCt) for both Day 1 and Day 7 using a power model suggest a less than dose-proportional increase in the dose range of 1 mg to 225 mg following a single dose.

For repeated daily dose cohorts on both Day 1 and Day 7, the time course for mean plasma CVN424 concentrations following drug administration under fasted state appeared similar to that for the fasted single dose cohorts. Mean plasma CVN424 concentrations were detectable up to 72 hours post-dose following the last dose of CVN424 on Day 7.

Dose-normalized exposures showed a decline as the dose increased, indicating a non-linear increase in all exposure parameters. However, these were within 2-fold for Cmax over the dose range 1 to 25 mg and for AUC24 over the dose range 1 to 75 mg. Statistical analyses of Cmax and AUCs (AUC24, AUCt, and AUC∞) using a power model showed less than dose-proportional increase in the dose range of 1 mg to 225 mg following a single dose.

Safety and Tolerability. A summary of treatment emergent adverse events (TEAEs) is presented in Table 4. Overall, 6 of 40 subjects (15.0%) in the single-dose cohorts reported TEAEs during the study. Study drug-related TEAEs were reported by 2 of 40 subjects (5.0%) overall in the single-dose cohorts: 1 of 6 subjects each (16.7%) after receiving CVN424 75 mg (feeling hot) and 225 mg (headache).
No study drug-related TEAEs were reported by subjects after receiving CVN424 1 mg, 5 mg (fasted or fed conditions), or 25 mg or placebo.

Overall, 6 of 24 subjects (25.0%) in the multiple-dose cohorts reported TEAEs during the study. Study drug-related TEAEs were reported by 2 of 24 subjects (8.3%) overall in the multiple-dose cohorts: 1 of 6 subjects each (16.7%) while receiving CVN424 75 mg (dysphagia) and 150 mg (chills). No study drug-related TEAEs were reported by subjects while receiving CVN424 25 mg or placebo.

All TEAEs were mild in severity apart from one moderate TEAE of dysphagia in the 75 mg cohort, which was unrelieved by antacids or simethicone, and led to study drug discontinuation after Day 3 and early discontinuation from the study. The dysphagia resolved fully by study Day 7. No other subject

### TABLE 3
Mean (CV) plasma pharmacokinetic parameters of CVN424: multiple-dose cohorts

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Treatment</th>
<th>25 mg (N = 6)</th>
<th>75 mg (N = 6)</th>
<th>150 mg (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg</td>
<td>5 mg Fasted</td>
<td>5 mg Fed</td>
<td>25 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75 mg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>225 mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>188.5 (29.2)</td>
<td>–</td>
<td>407.2 (30.7)</td>
<td>561.5 (23.2)</td>
</tr>
<tr>
<td></td>
<td>314.7 (47.8)</td>
<td>73.83 (26.7)</td>
<td>776.0 (37.3)</td>
<td>1097 (29.6)</td>
</tr>
<tr>
<td></td>
<td>241.0 (41.6)</td>
<td>–</td>
<td>240.0 (25.0)</td>
<td>496.8 (35.9)</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>1.750</td>
<td>1.750</td>
<td>2.000</td>
<td>2.008</td>
</tr>
<tr>
<td></td>
<td>(1.50, 3.00)</td>
<td>(1.00, 3.00)</td>
<td>(1.50, 2.08)</td>
<td>(1.50, 3.00)</td>
</tr>
<tr>
<td>AUC24 (h*ng/mL)</td>
<td>1377 (6.7)</td>
<td>–</td>
<td>4567 (27.4)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>1097 (29.6)</td>
<td>9425 (42.5)</td>
<td>16200 (31.4)</td>
</tr>
<tr>
<td>AUC(tau)</td>
<td>2157 (15.5)</td>
<td>2954 (25.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>1097 (29.6)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>t1/2z (h)</td>
<td>0.0456 (17.4)</td>
<td>0.0211 (21.1)</td>
<td>0.175 (35.0)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.0211 (21.1)</td>
<td>NE</td>
<td>0.175 (35.0)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.0211 (29.0)</td>
<td>NE</td>
<td>0.175 (35.0)</td>
<td>–</td>
</tr>
<tr>
<td>Rac(Cmax)</td>
<td>0.0456 (17.4)</td>
<td>0.0211 (21.1)</td>
<td>0.175 (35.0)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>0.0211 (21.1)</td>
<td>NE</td>
<td>0.175 (35.0)</td>
<td>–</td>
</tr>
<tr>
<td>Rac(AUC)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NE, not estimable; –, not calculated.

For tmax, the median (minimum, maximum) values are presented.

n = 5

### TABLE 4
Summary of treatment emergent adverse events

A TEAE was defined as any AE with onset occurring within 30 days after study drug administration. N represents the number of subjects within each treatment and overall; n represents the number of subjects at each level of summarization.

#### SAD Subjects

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo (N = 10)</th>
<th>1 mg (N = 6)</th>
<th>5 mg Fasted (N = 6)</th>
<th>5 mg Fed (N = 6)</th>
<th>25 mg (N = 6)</th>
<th>75 mg (N = 6)</th>
<th>225 mg (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any TEAE</td>
<td>2 (20.0)</td>
<td>1 (16.7)</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Feeling hot</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tooth abscess</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dizziness</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Medical device site dermatitis</td>
<td>1 (10.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tinea cruris</td>
<td>1 (10.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

#### MAD Subjects

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Placebo (N = 6)</th>
<th>25 mg (N = 6)</th>
<th>75 mg (N = 6)</th>
<th>150 mg (N = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any TEAE</td>
<td>0</td>
<td>3 (50.0)</td>
<td>1 (16.7)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Any TEAE Leading to Early Discontinuation</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
</tr>
<tr>
<td>Medical device site dermatitis</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Chills</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Back pain</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Neck pain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Dysphagia</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>1 (16.7)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

AE, adverse event.
reported dysphagia. There were no serious adverse effects or deaths reported, and no other subject discontinued early from the study. All TEAEs resolved by the end of the study.

No subject had a clinically significant abnormal vital sign. Body weight changes from baseline were similar across placebo and CVN424 cohorts. Modest elevations in body temperature and pulse rate were observed following administration of CVN424 (Fig. 3). These changes were detectable at 1 hour post-dose (the earliest scheduled post-dose assessment), increased further by 6 hours post-dose, and spontaneously returned to baseline by 24 hours post-dose. During the first 24 hours post-dose, the individual maximum observed temperature change from baseline was 1.6°C for CVN424 versus 0.8°C for placebo, and the maximum heart rate change from baseline was 57 beats per minute for CVN424 versus 33 beats per minute for placebo. These observations were restricted to the first dose of CVN424; elevations in temperature or other vital sign trends were not observed after administration of subsequent doses to the multiple-dose cohorts. Orthostatic vital sign changes were similar for CVN424 and placebo groups.

**Discussion**

The current standard of care for Parkinson’s disease is symptomatic treatment by dopamine replacement, dopamine agonists, or analogous mechanisms. After several years of treatment, many patients experience motor fluctuations that limit the effectiveness of those drugs and may also develop dyskinesias that are exacerbated by dopaminergic agents (e.g., levodopa-induced dyskinesias). Novel, non-dopaminergic therapies like CVN424 have potential to improve treatment of such patients.

This first-in-human study investigated the safety, tolerability, and PK of single and multiple escalating doses of CVN424 in healthy volunteers. CVN424 mean peak ($C_{\text{max}}$) and total ($\text{AUC}_{24}$, $\text{AUC}_1$, and $\text{AUC}_\infty$) plasma exposure increased with increase in CVN424 dose across the evaluated dose range (1–225 mg once daily, or 25–150 mg once daily for 7 days), although the increase appeared to be less than dose proportional. The food effect comparison showed a 33% reduction in $C_{\text{max}}$ and a delay in $t_{\text{max}}$ with food. A small increase in the overall exposure was noted when given with food (comparison of $\text{AUC}_\infty$, 19%), although $\text{AUC}_{24}$ and $\text{AUC}_1$ values met equivalence criteria. Moderate plasma accumulation of CVN424 was observed following 7-day once daily dosing of CVN424 oral suspension, as expected given the drug’s half-life, with apparent steady-state concentrations of CVN424 being achieved following 4 or 5 days of dosing. The administration of CVN424 was safe and well tolerated and resulted in no serious adverse events. The only early discontinuation was for dysphagia (75 mg group), an adverse event not reported by any other subject.

Transient increases in temperature and heart rate, observed as first-dose effects, were not clinically significant in the study subjects. It is uncertain whether similar vital sign changes

---

**Fig. 3.** (A) Mean (+/−S.D.) change from Baseline in Temperature. (B) Mean (+/−S.D.) change from baseline in pulse rate.
will be observed in Parkinson’s disease patients, who may be
everly with various cardiac and autonomic co-morbidities and
using concomitant medications. Curiously, the rise in heart
rate averaged across all CVN424 cohorts was about 8 beats
per minute per degree Celsius rise in temperature (data not
shown), which is close to the 8.5 beats per minute physiologic
rise in heart rate linked to each degree Celsius rise in febrile
patients in previous studies (Karjalainen and Viitasalo, 1986).
If that is not coincidental, perhaps CVN424 induces a temperature
rise that indirectly leads to the increase in heart rate. An
in vitro selectivity screen of CVN424 against 110 receptors,
channels, and enzymes showed inhibition greater than 50% at
10 μM for adrenergic α1 receptors (maximum inhibition seen
was 70% for α1D) and the sigma receptor (Brice et al., 2021),
although it was not determined whether this inhibition in bind-
ing translates to a functional effect. The maximum total plasma
concentration achieved in this trial was 1097 ng/ml (2.3 μM) at
the top dose of the SAD cohort. Although adrenergic receptors
are known to affect heart rate, it is unlikely that the free drug
concentration was sufficient to modulate these receptors. There-
fore, the mechanism by which CVN424 or modulation of GPR6
might cause the observed changes in vital signs is uncertain.

The data presented here detail the PK profile of a suspen-
sion formulation, while use of solid form in future clinical stud-
ies may require bridging PK analysis to assess bioequivalence.

While GPR6 expression is most prominent in striatal indi-
rect pathway neurons, GPR6 expression has been observed in
a subset of hypothalamic neurons in mice and humans (Henry
et al., 2015; Cerevance unpublished data), which suggests a
possible mechanism to account for our observations given the
hypothalamus’s role in temperature and energy regulation and
autonomic balance.

CVN424 is a non-dopaminergic compound that modulates a
novel Parkinson’s disease therapeutic target. Previously pub-
lished pre-clinical data has shown that CVN424 is efficacious
in models relevant to Parkinson’s disease, such as the rat 6-
OHDA lesion model, and achieves good levels of brain ex-
posure with a total plasma concentration that achieves 50%
receptor occupancy (RO50) of 7.4 ng/ml (Brice et al., 2021). In
humans, CVN424 appears to be safe and well tolerated in
healthy adults over a range that includes plasma exposures
predicted from pre-clinical studies to achieve near saturation of
GPR6 sites in the brain. These results supported the design of
a phase II study of CVN424 adjunctive to levodopa in Par-
kinson’s disease patients with motor fluctuations, currently
ongoing (NCT04191577).

Acknowledgments

The authors thank the volunteers who participated in the trial,
as well as staff from PPD Development, including Carmichael
Angeles, Yuansi Liu, Nancy Zheng, Bradley Wetzell, and Kristi
Wheeler, who assisted in the conduct of the study and/or data
analysis, James Woodworth, who assisted with data analysis,
Mira Hong and colleagues at Frontage Laboratories, who de-
veloped and performed the PK analyses, and Ricardo Soto and Emily
Marschok from Halloran Consulting Group, who assisted with
operational oversight of study conduct. Natalie Hosea (Takeda
Pharmaceutical Company) assisted with dose selection. At Cere-
varce, Andrew Ayscough assisted with study drug manufacture
and importation, Rob Middlebrook managed budgets and contract-
ing, Lee Dawson provided comments on the manuscript, and Brad
Margus provided strategic guidance. Nathaniel Heintz (Rockefeller U.,
Howard Hughes Medical Institute) provided critical insight
into selection of GPR6 as a therapeutic target for Parkinson’s
disease.

Authorship Contributions

Participated in research design: Margolin, Brice, Carlton.
Conducted experiments: Davidson.
Performed data analysis: Davidson, Margolin.
Wrote or contributed to the writing of the manuscript: Margolin,
Brice, Matthews, Carlton, Davidson.

References

Brice NL, Schiffer HH, Menenschein H, Mulligan VJ, Page K, Powell J, Xu X, Cheung
GPR6 Inverse Agonist Effective in Models of Parkinson Disease. J Pharmacol Exp
Ther 377:407–416 10.1124/jpet.120.2090438.
DeLeng MR and Wichmann T (2015) Basal Ganglia Circuits as Targets for Neuromodu-
2397.
Heiman M, Schaeffer A, Gong S, Peterson JD, Day M, Ramsey KE, Suarez-Farinas M,
approach for the molecular characterization of CNS cell types. Cell 135:738–748
transcriptomics of hypothalamic energy-sensing neuron responses to weight-loss.
Cell Life 4: 10.7554/elife.09880.
Morales P, Isawi I, and Reggio PH (2018) Towards a better understanding of the can-
namaboid-related orphan receptors GPR3, GPR6, and GPR12. Drug Metab Rev
j.celrep.2018.05.059.
Smith BP, Vandenheede FR, DeSante KA, Farid NA, Welch PA, Callaghan JT, and
Forgue ST (2000) Confidence interval criteria for assessment of dose proportion-
Sun H, Menenschein H, Schiffer BH, Reichard HA, Kikuchi S, Hoskins M, Macklin
TK, Hitchock S, Adams M, Green J, et al. (2021) First-Time Disclos-
ure of CVN424, a Potent and Selective GPR6 Inverse Agonist for the Treat-
mant of Parkinson’s Disease: Discovery, Pharmacological Validation, and
acsmedchem.0c02081.
Uhlenbrock K, Gassenhuber H, and Kostenis E (2002) Sphingosine 1-phosphate is a
ligand of the human gpr3, gpr6 and gpr12 family of constitutively
active G protein-coupled receptors. Cell Signal 14:941–953 10.1016/S0898-
6568(02)00641-4.

Address correspondence to: Mark Carlton, Cerevance, Ltd., 418 Cam-
bridge Science Park, CB4 0PZ, Cambridge UK. E-mail: mark.carlton@
cerevance.com
A Phase I, First-In-Human, Healthy Volunteer Study to Investigate the Safety, Tolerability, and Pharmacokinetics of CVN424, a Novel GPR6 Inverse Agonist for Parkinson’s Disease *

Authors: David H Margolin, Nicola L Brice, Antonia M Davidson, Kim L Matthews and Mark B L Carlton.

*The Journal of Pharmacology and Experimental Therapeutics
JPET-AR-2021-000842

Online Supplement: Complete Eligibility Criteria

Screening for eligible subjects must be completed within 28 days prior to randomization or first dose.

**Inclusion Criteria**

Subject eligibility is determined according to the following criteria prior to entry into the study:

1. In the opinion of the Investigator, the subject is capable of understanding and complying with protocol requirements.
2. The subject signs and dates a written informed consent form (ICF) and any required privacy authorization prior to the initiation of any study procedures.
3. Subject is a healthy male or female adult who is 18 to 50 years of age inclusive at the time of ICF and study drug dosing.
4. Subject weighs at least 45 kg (99 lbs) and has a BMI between 18.0 and 30.0 kg/m², inclusive at Screening.
5. A male subject who is nonsterilized and sexually active with a female partner of childbearing potential agrees to use adequate contraception from signing of the ICF throughout the duration of the study and for 12 weeks after last dose.
6. A female subject with no childbearing potential, defined as the subject has been surgically sterilized (hysterectomy, bilateral oophorectomy or tubal ligation) or who are postmenopausal (defined as continuous amenorrhea of at least 2 years and FSH>40 IU/L).

**Exclusion Criteria**

Any subject who meets any of the following criteria will not qualify for entry into the study:

1. Subject has received any investigational compound within 30 days prior to the first dose of study medication, or within 5 half lives, whichever is greater.
2. Subject is a study site employee or an immediate family member of a study site employee.
3. Subject has evidence of CS neurologic, cardiovascular, pulmonary, hepatic, hematopoietic disease, renal, metabolic, gastrointestinal, urologic, immunologic, endocrine disease, serious allergy, allergic skin rash, psychiatric disorder, or other abnormality that may impact the ability of the subject to participate or potentially confound the study results.
4. There is any finding in the subject’s medical history, physical examination, or safety laboratory tests giving reasonable suspicion of a disease that would contraindicate taking
CVN424, or a similar drug in the same class, or that might interfere with the conduct of the study

5. Subject has a known hypersensitivity to any component of the formulation of CVN424.

6. Subject has a positive urine result for drugs of abuse at Screening or Inpatient Check-in (Day -1).

7. Subject has a history of drug abuse (defined as any illicit drug use) or a history of alcohol abuse within 1 year prior to the Screening Visit or is unwilling to agree to abstain from alcohol and drugs throughout the study.

8. Subject has taken any excluded medication, supplements, or food products listed in the Excluded Medications and Dietary Products table as listed in the Supplementary Table “Excluded Medications and Dietary Products”.

9. If subject is female of childbearing potential (e.g., premenopausal, not sterilized).

10. If subject is male and intends to donate sperm during the course of this study or for 12 weeks thereafter.

11. Subject has previously had a seizure or convulsion (lifetime), including absence seizure and febrile convulsion.

12. Subject has current or recent (within 6 months) gastrointestinal disease that would be expected to influence the absorption of drugs (i.e., a history of malabsorption, any surgical intervention known to impact absorption [e.g., bariatric surgery or bowel resection], esophageal reflux, peptic ulcer disease, erosive esophagitis, or frequent [i.e., more than once per week] occurrence of heartburn).

13. Subject has a history of cancer or other malignancy, with the exception of basal cell carcinoma that has been in remission for at least 5 years prior to Day 1.

14. Subject has a positive test result for hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody or a human immunodeficiency virus infection at Screening.

15. Subject has used nicotine-containing products (including but not limited to cigarettes, electronic cigarettes, pipes, cigars, chewing tobacco, nicotine patch or nicotine gum) within 28 days prior to Inpatient Check-in (Day -1) or a positive urine cotinine test at Screening or Inpatient Check-in (Day -1).

16. Subject has poor peripheral venous access.

17. Subject has donated or lost 450 mL or more of his or her blood volume (including plasmapheresis) or had a transfusion of any blood product, within 45 days prior to Day 1.

18. Subject has a Screening or Inpatient Check-in (Day -1) abnormal (CS) ECG. Entry of any subject with an abnormal (NCS) ECG must be approved and documented by signature by the Investigator or medically qualified sub-investigator.

19. Subject has a supine blood pressure outside the ranges of 90 to 140 mm Hg for systolic and 60 to 90 mm Hg (males) and 50 to 90 mm Hg (females) for diastolic, confirmed with repeat per PI discretion, at the Screening Visit or Inpatient Check-in (Day -1).

20. Subject has a resting heart rate outside the range 40 to 90 bpm, confirmed with repeat per PI discretion, at the Screening Visit or Inpatient Check-in (Day -1).
21. Subject has a QT interval with Fridericia’s correction method (QTcF) >430 ms (males) or >450 ms (females) or PR outside the range of 120 to 220 ms, confirmed with one repeat testing, at the Screening Visit or Inpatient Check-in (Day -1) Visit.

22. Subject has abnormal Screening or Inpatient Check-in (Day -1) laboratory values that suggest a CS underlying disease or subject with the following lab abnormalities: ALT and/or AST >1.5 the ULN, confirmed with one repeat testing.

23. Subject has a risk of suicide according to the investigator’s clinical judgment (e.g., per Columbia-Suicide Severity Rating Scale) or has made a suicide attempt in the previous 2 years.

Excluded Medications and Dietary Products
Use of the agents in the Supplementary Table “Prohibited Medications and Dietary Products” is prohibited from the time points specified until completion of all study activities.
**Supplementary Table 1  Prohibited Medications and Dietary Products**

<table>
<thead>
<tr>
<th>28 days prior to Inpatient Check-in (Day -1)</th>
<th>7 days prior to Inpatient Check-in (Day -1)</th>
<th>72 hours prior to Inpatient Check-in (Day -1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prescription medications (including oral contraceptives)</td>
<td>OTC medications, including antacids, proton-pump inhibitors, and H2 receptor antagonists (a)</td>
<td>Products containing caffeine or xanthine (e.g., tea or coffee)</td>
</tr>
<tr>
<td>Nicotine-containing products</td>
<td>Vitamin supplements</td>
<td>poppy seeds</td>
</tr>
<tr>
<td>Nutraceuticals (e.g., St. John’s wort, ginseng, kava kava, ginkgo biloba, Chinese herbs, and melatonin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunization/Vaccines (b)</td>
<td>Alcohol-containing products</td>
<td></td>
</tr>
<tr>
<td>Known strong inhibitors/inducers of CYPs 3A4/5 (c)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CYP= cytochrome P-450, OTC=over the counter.  
(a) Occasional use of acetaminophen (~1 g/day) or other medication as approved by sponsor’s Medical Monitor on a case-by-case basis is allowed except on Day 1.  
(b) Inclusive of but not limited to H1N1 and flu vaccinations.  
(c) e.g., chloramphenicol, clarithromycin, ketoconazole.