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 *

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Nonstandard abbreviations:

AUC	Area under the (plasma concentration – time) curve
AUC _t	Area under the plasma concentration – time curve from time 0 to time t
AUC _{tau}	Area under the plasma concentration-time curve during a dosing interval τ
CL/F	Apparent total clearance of the drug from plasma (volume/time)
C _{max}	Maximum observed plasma concentration
C _{min}	Minimum observed plasma concentration
FDA	United States Food and Drug Administration
MAD	Multiple ascending dose
MedDRA	Medical Dictionary for Regulatory Activities
NOAEL	No-observed-adverse-effect level
PK	Pharmacokinetic
R _{AC}	Accumulation ratio

SAD	Single ascending dose
ss	Steady state
TEAE	Treatment-emergent adverse effect
Tmax	Time of observed maximum plasma concentration
V_z/F	Apparent volume of distribution during terminal phase

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ABSTRACT (227 words):

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 mg, 75 mg, or 150 mg. CVN424 peak plasma concentrations were reached within 2h 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 30h to 41h. 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 neurological 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 pathological 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 $G_{\alpha s}$ -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 $G_{\alpha i}$ -coupled D2 receptors. CVN424 suppresses this constitutive activity, reducing cellular cAMP levels (Brice et al., 2021)(Sun et al., 2021) and potentially attenuating the pathological hyperactivity of the indirect pathway seen in Parkinson's patients (Figure 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 (3:1) 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 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 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 (FDA) Guidance for Industry, 2002. After the safety of single-dose 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 hydroxypropyl methylcellulose 200 mg, Tween-80 50 mg, and 10 mL water. Placebo contained the same ingredients but omitted active drug.

Applying a 20-fold safety margin below the human-equivalent dose corresponding to the NOAEL from nonclinical toxicology studies, the maximum recommended starting dose for this first-in-human study per FDA 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 cotinine 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 Supplementary Information)

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 noncompartmental analysis using Phoenix® WinNonlin® (Certara L.P., Princeton, NJ) Version 8.0: Area under the concentration-time curve from time 0 to 24 hours (AUC_{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_{τ} for Day 7 in multiple-dose cohorts), time to the observed maximum plasma concentration (t_{max}), the maximum observed plasma concentration after a single dose (C_{max}) and at steady state ($C_{max,ss}$), the minimum plasma concentration at steady state ($C_{min,ss}$), apparent clearance CL/F , apparent volume V_z/F and terminal half-life $t_{1/2z}$.

Accumulation ratios based on AUC ($R_{ac(AUC)}$) and C_{max} ($R_{ac(C_{max})}$) and dose-normalized AUCs, C_{max} , and $C_{max,ss}$ 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 (C_{max} , AUC_{24} , AUC_t , and AUC_{∞} for single-dose cohorts and $C_{max,ss}$, AUC_{24} , AUC_t , and AUC_{τ} for multiple-dose cohorts) and X (dose) using the least-squares linear regression model [$\ln(Y) = \ln(\alpha) + \beta \times \ln(X)$]. Dose proportionality was concluded if the 90% CI of the slope β 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 C_{max} , AUC_{24} , AUC_t , AUC_{∞} , 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 assessments

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 1 Day 7 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 (MedDRA; 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 March 2018. The Statistical Analysis Plan was pre-specified. Statistical analyses were performed using SAS[®] 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, S.D., 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% confidence interval, 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 75 mg dose of CVN424 discontinued early because of an adverse event of 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 **Error! Reference source not found..**

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 (C_{max}) and total (AUC_{24} , AUC_t , and AUC_{∞}) plasma exposure increased with increase in CVN424 dose, with inter-individual variability (CV%) ranging from 9.3% to 41.6%. Median T_{max} ranged from 1.5 to 2 hours across the fasted SAD cohorts. Mean $t_{1/2}$ and V_z/F ranged from 29.6 hours to 41.4 hours and 306 L to 560 L, respectively, with no apparent trend across SAD 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 (C_{max} ; 29.03 ng/mL in the fed state, 44.03 ng/mL in the fasted state). Median T_{max} was delayed in presence of food (median T_{max} ; 3 h in the fed state, 1.5 h in the fasted state). Mean AUCs (AUC_{24} , AUC_t , and AUC_{∞}) were higher in the fed cohort (345, 635, and 860 h*ng/mL, respectively) compared to the fasted cohort (325, 554, and 706 h*ng/mL, respectively). Mean $t_{1/2}$ values were similar (35.9 h for fasted cohort and 36.7 h for fed cohort) as were mean CL/F values (7.3 L/h under fasted conditions and 6.2

L/h under fed conditions). Statistical analysis of $C_{\max}$ and AUCs (AUC_{24} , AUC_t , and AUC_{∞}) using a power model, showed the 90% CI of the ratio of geometric LS means for $C_{\max}$ for the comparison of fed/fasted S2 cohort was not contained within 80% to 125%. Geometric mean $C_{\max}$ following a single dose administration of 5 mg CVN424 decreased by approximately 33% in presence of food. The 90% CI of the ratio of geometric LS means for AUC_{24} and AUC_{τ} were contained within 80% to 125%. However, the 90% CI of the ratio of geometric LS means for AUC_{∞} was not contained within 80% to 125%. Geometric mean AUC_{∞} following a single dose administration of 5 mg CVN424 increased by 19% in presence of food.

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 $C_{\max}$ over the dose range 1 to 25 mg and for AUC_{24} over the dose range 1 to 75 mg. Statistical analyses of $C_{\max}$ and AUCs (AUC_{24} , AUC_t , 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.

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 h 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), $C_{\max,ss}$, $C_{\min,ss}$ and AUC_{τ} increased with increase in dose in 25 mg to 150 mg dose range. Mean plasma $C_{\max,ss}$ values were 315 ng/mL, 776 ng/mL, and 1097 ng/mL and $C_{\min,ss}$ 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 AUC_{tau} values on Day 7 were 2954 h*ng/mL, 9425 h*ng/mL, and 16200 h*ng/mL for 25 mg, 75 mg, and 150 mg MAD cohorts, respectively. Median T_{max} ranged from 1.75 to 2.5 hours and mean t_{1/2} ranged from 30.6 to 34.1 hours on Day 7 across MAD cohorts.

Point estimates of C_{max} and AUCs (AUC₂₄, AUC_t) for both Day 1 and Day 7 using a power model suggest a less than dose-proportional increase in the dose range of 25 mg to 150 mg for MAD cohorts.

Mean plasma C_{max} and AUC accumulation ratios ranged from 1.75 to 2.47 following once-daily dose of CVN424 oral suspension for 7 days across MAD cohorts, suggesting moderate accumulation of CVN424 as expected given the drug's half-life. Concentrations of CVN424 with once-daily dosing reached steady-state levels by Day 4 or 5.

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 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 (**Error! Reference source not found.**). 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_{max}) and total (AUC_{24} , AUC_t , and AUC_{∞}) 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_{max} and a delay in t_{max} with food. A small increase in the overall exposure was noted when given with food (comparison of AUC_{∞} , 19%), although AUC_{24} and AUC_t 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 (75mg 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 will be observed in Parkinson's disease patients, who may be elderly 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 physiological rise in heart rate linked to each degree Celsius rise in febrile patients in previous studies (Karjalainen J, 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 μ M for adrenergic α 1 receptors (maximum inhibition seen was 70% for α 1D) and the sigma receptor (Brice et al., 2021), although it was not determined if this inhibition in binding translates to a functional effect. The maximum total plasma concentration achieved in this trial was 1097 ng/ml (2.3 μ M) at the top dose of the SAD cohort. Although adrenergic receptors are known to effect heart rate it is unlikely that the free drug concentration was sufficient to modulate these receptors. Therefore, 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 suspension formulation, while use of solid form in future clinical studies may require bridging PK analysis to assess bioequivalence.

While GPR6 expression is most prominent in striatal indirect 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 published pre-clinical data has shown that CVN424 is efficacious in models relevant to Parkinson's disease such as the rat 6-OHDA lesion model, achieves good levels of brain exposure with a total plasma concentration that achieves 50% receptor occupancy (RO_{50}) 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 Parkinson's disease patients with motor fluctuations, currently ongoing (NCT04191577).

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budgets and contracting; 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 and Carlton

Conducted experiments: Davidson

Performed data analysis: Davidson and Margolin

Contributed to the writing of the manuscript: Margolin , Brice, Matthews, Carlton and Davidson

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Figure Legends

Figure 1: A, Loss of dopaminergic input causes hyperactivity of indirect pathway and hypoactivity of direct pathway, with excessive inhibition of thalamocortical motor signals. GPR6 drives constitutive cAMP increase, exacerbates hyperactivity of indirect pathway, and is unopposed because of dopamine loss.

B, CVN424 (GPR6 inverse agonist) lowers cAMP level even in absence of dopamine D2 receptor signaling, reducing imbalance between direct and indirect pathways and relieving excessive inhibition of thalamocortical motor signals.

Figure 2: A, Single dose cohorts, beginning Day 1. B, Multiple dose cohorts, beginning Day 7. C, Multiple dose cohorts, trough (pre-dose) values.

Figure 3: A, Mean (+/-SD) change from Baseline in Temperature. B, Mean (+/-SD) change from Baseline in pulse rate.

Tables

Table 1. Demographic characteristics of the study subjects

SAD Subjects						
Parameter (unit)	Placebo * (N=10)	CVN424				
		1 mg (N=6)	5 mg (N=6)	25 mg (N=6)	75 mg (N=6)	225 mg (N=6)
Age (years)	38.0 (10.1)	31.3 (7.8)	36.5 (9.8)	35.3 (8.2)	34.2 (7.1)	30.3 (8.0)
Female, No. (%)	3 (30.0)	2 (33.3)	2 (33.3)	2 (33.3)	0	1 (16.7)
Male, No. (%)	7 (70.0)	4 (66.7)	4 (66.7)	4 (66.7)	6 (100.0)	5 (83.3)
Race, No. (%)						
White	9 (90.0)	4 (66.7)	5 (83.3)	3 (50.0)	5 (83.3)	4 (66.7)
Black or African American	1 (10.0)	2 (33.3)	0	3 (50.0)	1 (16.7)	2 (33.3)
American Indian or Alaska Native	0	0	1 (16.7)	0	0	0
Ethnicity, No. (%)						
Hispanic or Latino	6 (60.0)	1 (16.7)	4 (66.7)	2 (33.3)	2 (33.3)	3 (50.0)
Not Hispanic or Latino	4 (40.0)	5 (83.3)	2 (33.3)	4 (66.7)	4 (66.7)	3 (50.0)
Height (cm)	170.0 (10.4)	176.0 (12.3)	167.3 (9.0)	169.5 (9.9)	175.4 (7.1)	169.3 (7.5)
Weight (kg)	75.7 (11.9)	78.1 (12.2)	72.6 (8.1)	72.0 (8.2)	75.6 (3.9)	73.6 (7.5)
Body mass index (kg/m ²)	26.1 (2.6)	25.2 (2.5)	25.9 (1.9)	25.1 (2.8)	24.7 (2.1)	25.7 (2.4)

MAD Subjects				
Parameter (unit)	Placebo * (N=6)	CVN424		
		25 mg (N=6)	75 mg (N=6)	150 mg (N=6)
Age (years)	34.5 (7.71)	36.8 (8.93)	31.7 (9.81)	38.0 (7.87)
Female, No. (%)	1 (16.7)	2 (33.3)	0	0
Male, No. (%)	5 (83.3)	4 (66.7)	6 (100.0)	6 (100.0)
Race, No. (%)				
White	2 (33.3)	4 (66.7)	5 (83.3)	3 (50.0)
Black or African American	4 (66.7)	2 (33.3)	1 (16.7)	3 (50.0)
American Indian or Alaska Native	0	0	0	0
Ethnicity, No. (%)				
Hispanic or Latino	2 (33.3)	1 (16.7)	2 (33.3)	1 (16.7)
Not Hispanic or Latino	4 (66.7)	5 (83.3)	4 (66.7)	5 (83.3)
Height (cm)	171.6 (9.7)	173.0 (8.7)	174.7 (5.5)	175.8 (5.0)
Weight (kg)	79.1 (9.4)	84.0 (9.7)	83.0 (7.3)	83.9 (9.2)
Body mass index (kg/m ²)	26.9 (2.1)	28.0 (1.1)	27.2 (1.9)	27.1 (1.7)

Values are mean (s.d.) unless otherwise noted.

Abbreviations: SAD, single ascending dose. MAD, multiple ascending dose. No., number.

* Subjects who received placebo were pooled across single-dose cohorts, and similarly for multiple-dose cohorts.

Table 2. Mean (CV) Plasma Pharmacokinetic Parameters of CVN424: Single Dose Cohorts

Parameter (unit)	Treatment					
	1 mg (N=6)	5 mg Fasted (N=6)	5 mg Fed (N=6)	25 mg (N=6)	75 mg (N=6)	225 mg (N=6)
C_{max} (ng/mL)	11.11 (31.1)	44.03 (9.3)	29.43 (14.3)	206.8 (25.6)	386.0 (13.3)	682.3 (39.7)
t_{max} (h) ^(a)	1.500 (1.00, 1.52)	1.517 (1.50, 3.00)	3.000 (1.50, 5.00)	1.500 (1.50, 2.00)	2.000 (1.50, 2.00)	1.500 (1.00, 3.00)
AUC_{24} (h*ng/mL)	75.89 (23.9)	325.3 (9.7)	344.6 (14.1)	1608 (15.3)	4076 (21.8)	7511 (21.2)
AUC_t (h*ng/mL)	124.8 (25.9)	554.4 (9.5)	635.0 (16.3)	2613 (18.4)	7134 (32.2)	17950 (19.9)
AUC_{∞} (h*ng/mL)	169.5 (33.2)	706.0 (19.9) ^(b)	859.7 (29.2) ^(b)	3323 (35.3)	8797 (41.6)	24720 (27.1) ^(c)
$t_{1/2z}$ (h)	39.4 (32.6)	35.9 (34.8) ^(b)	36.7 (40.4) ^(b)	32.0 (50.4)	29.6 (21.2)	41.4 (12.8) ^(c)
λ_z (1/h)	0.0198 (41.1)	0.0214 (36.5) ^(b)	0.0214 (37.0) ^(b)	0.0262 (42.7)	0.0243 (20.7)	0.0169 (12.3) ^(c)
CL/F (L/h)	6.607 (40.9)	7.320 (20.5) ^(b)	6.247 (30.1) ^(b)	8.177 (28.3)	9.622 (34.7)	9.605 (29.4)
V_z/F (L)	339.7 (18.1)	360.3 (20.6) ^(b)	306.2 (22.5) ^(b)	314.1 (28.0)	391.6 (27.7)	560.4 (18.1)

Abbreviations: CV, coefficient of variation; SAD, single ascending dose;

Notes: Arithmetic means were displayed in the table.

^(a) For t_{max} , the median (minimum, maximum) values are presented.

^(b) n = 5

^(c) n = 3

Table 3. Mean (CV) Plasma Pharmacokinetic Parameters of CVN424: Multiple Dose Cohorts

Parameter (unit)	Treatment					
	25 mg (N=6)		75 mg (N=6)		150 mg (N=6)	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
C _{max} (ng/mL)	188.5 (29.2)	–	407.2 (30.7)	–	561.5 (23.2)	–
C _{max,ss} (ng/mL)	–	314.7 (47.8)	–	776.0 (37.3) ^(c)	–	1097 (29.6)
C _{min,ss} (ng/mL)	–	73.83 (26.7)	–	241.0 (41.6) ^(c)	–	496.8 (35.9)
t _{max} (h) ^(a)	1.750 (1.50, 3.00)	1.750 (1.00, 3.00)	2.000 (1.50, 2.08)	2.000 (1.50, 5.00) ^(c)	2.008 (1.50, 3.00)	2.500 (1.50, 3.00)
AUC ₂₄ (h*ng/mL)	1377 (6.7)	–	4567 (27.4)	–	6594 (23.9)	–
AUC _{tau} (h*ng/mL)	–	2954 (25.5)	–	9425 (42.5) ^(c)	–	16200 (31.4)
AUC _∞ (h*ng/mL)	2157 (15.5) ^(b)	–	NE	–	NE	–
t _{1/2z} (h)	15.4 (17.4) ^(b)	33.9 (19.4) ^(c)	NE	30.6 (22.8) ^(d)	NE	34.1 (56.2)
λ _z (1/h)	0.0456 (17.4) ^(b)	0.0211 (21.1) ^(c)	NE	0.0234 (20.2) ^(d)	NE	0.0316 (79.8)
R _{ac} (C _{max})	–	1.75 (61.6)	–	2.17 (35.0) ^(c)	–	1.99 (28.4)
R _{ac} (AUC)	–	2.16 (29.0)	–	2.13 (23.1) ^(c)	–	2.47 (26.4)

Abbreviations: CV, coefficient of variation; MAD, multiple-ascending dose; NE, not estimable; –, not calculated.

Notes: R_{ac}(AUC) calculated as AUC_{tau} Day 7/AUC₂₄ Day 1. Arithmetic means were displayed in the table.

^(a) For t_{max}, the median (minimum, maximum) values are presented.

^(b) n = 2

^(c) n = 5

^(d) n = 3

Table 4. Summary of Treatment-Emergent Adverse Events

SAD Subjects							
	Placebo (N=10) n (%)	CVN424					
		1 mg (N=6) n (%)	5 mg Fasted (N=6) n (%)	5 mg Fed (N=6) n (%)	25 mg (N=6) n (%)	75 mg (N=6) n (%)	225 mg (N=6) n (%)
Any TEAE	2 (20.0)	1 (16.7)	0	1 (16.7)	0	1 (16.7)	1 (16.7)
Feeling hot	0	0	0	0	0	1 (16.7)	0
Tooth abscess	0	0	0	0	0	1 (16.7)	0
Dizziness	0	1 (16.7)	0	0	0	0	0
Headache	0	0	0	0	0	0	1 (16.7)
Dyspepsia	0	0	0	1 (16.7)	0	0	0
Medical device site dermatitis	1 (10.0)	0	0	0	0	0	0
Tinea cruris	1 (10.0)	0	0	0	0	0	0
MAD Subjects							
	Placebo (N=6) n (%)	CVN424					
		25 mg (N=6) n (%)	75 mg (N=6) n (%)	150 mg (N=6) n (%)			
Any TEAE	0	3 (50.0)	1 (16.7)	2 (33.3)			
Any TEAE Leading to Early Discontinuation	0	0	1 (16.7) *	0			
Medical device site dermatitis	0	1 (16.7)	0	1 (16.7)			
Chills	0	0	0	1 (16.7)			
Back pain	0	1 (16.7)	0	1 (16.7)			
Neck pain	0	0	0	1 (16.7)			
Dysphagia	0	0	1 (16.7)	0			

Upper respiratory tract infection	0	1 (16.7)	0	0
Headache	0	1 (16.7)	0	0

Abbreviations: AE, adverse event; SAD, single ascending dose; TEAE, treatment-emergent AE.

Note: 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.

Figure 2.

Figure 3.

A

B

Abbreviations: MAD, multiple ascending dose.