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# Safety, Tolerability, and Pharmacokinetics of Multiple Repeated Oral Doses of the $\alpha$2/3/5-Subtype Selective $GABA_A$-Positive Allosteric Modulator PF-06372865 in Healthy Volunteers

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**Abstract**

Multiple-dose pharmacokinetics (PK) and safety were investigated in this phase 1 study of PF-06372865, a positive allosteric modulator of $\alpha$2/3/5 subunit-containing $\gamma$-aminobutyric acid A receptors (NCT03351751). In 2 cohorts (7-8 PF-06372865 and 2 placebo in each cohort), healthy adult subjects received twice-daily oral doses of PF-06372865 for 21 days, which included titration in the first 7 days, followed by a maintenance dose of 25 mg twice daily (Cohort 1) and 42.5 mg twice daily (Cohort 2) for 14 days. Serial PK samples were collected on days 1 and 21. Nineteen subjects were assigned to study treatments; 18 completed the study. Approximate dose-proportional increases in maximum plasma concentratin and area under the plasma concentration–time curve over the dosing interval were observed. PF-06372865 was rapidly absorbed with a median time to maximum concentration of 1 to 2 hours following both single- and multiple-dose administration. Mean terminal elimination half-life on day 21 was approximately 11 hours in both cohorts. All adverse events were mild; the most frequently reported was dizziness. After titration, there were no reports of somnolence. There were no clinically significant safety findings, including a lack of withdrawal symptoms on discontinuation of treatment. These results demonstrate that PF-06372865 is safe and well tolerated at doses estimated to achieve high receptor occupancy (>80%), a profile differentiated from nonselective benzodiazepines.

**Keywords**

CVL-865, GABA, epilepsy, $GABA_A$, PF-06372865

Benzodiazepines (BZDs), nonselective positive allosteric modulators (PAMs) of $\gamma$-aminobutyric acid A ($GABA_A$) receptors, are highly efficacious in epilepsy and anxiety but have significant side effects that limit their clinical utility in these indications. For example, BZD use is often associated with sedation, somnolence, and cognitive impairment, along with the risk of development of physical and psychological dependence. In epilepsy, loss of efficacy (tolerance) is a particular issue, which means that many BZDs are used only acutely. The majority of $GABA_A$ receptors present in the central nervous system (CNS) contain 2 $\alpha$, 2 $\beta$, and a single $\gamma$ subunit, and those that contain an $\alpha$1, $\alpha$2, $\alpha$3, or $\alpha$5 subunit in conjunction with a $\gamma$2 subunit are sensitive to BZDs.[1] Molecular studies in which $GABA_A$ receptors containing specific $\alpha$ subunits have been rendered unresponsive to diazepam have elucidated the contribution of those subunits to different aspects of the in vivo pharmacology.[2] These studies, together with subtype-selective tool compounds, have attributed the sedative effects of BZD to $\alpha$1 activity,[3] anticonvulsant activity to $\alpha$1/2 subunits,[4–6] anxiolysis and analgesic activity to

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$\alpha$2/3 subunits,[5,7,8] the development of efficacy tolerance in epilepsy populations to high intrinsic activity at the $\alpha$1 subunit,[9] and physiological and psychological dependence in part to $\alpha$1 subunits.[10,11]

There has been a concerted effort to identify to subtype-selective PAMs, with negligible potentiation of GABA via the $\alpha$1 subunit–containing receptors, and while the desired in vitro profile may appear unambiguous based on the robust preclinical evidence, the translation of minimal $\alpha$1 potentiation in vitro to a lack of dose-limiting sedation or somnolence in the clinic has not always proved to be the case. For example, MK-0343 is reported to exhibit $\alpha3 > \alpha2 = \alpha5 > \alpha1$ subtype selectivity, with low intrinsic efficacy of the latter ($\sim$20% of diazepam) and was demonstrated to be anxiolytic in vivo at doses corresponding to $\sim$35% to 65% receptor occupancy (RO).[7] However, dose-limiting sedation was observed in the clinic at levels of RO below the limit of detection.[12] Subsequent compounds that exhibited lower functional efficacy at $\alpha$1 subunits than MK-0343, TPA023 and TPA023B, also possessed dose-limiting adverse events (AEs) including drowsiness, which precluded dosing beyond 60% RO.[7] Furthermore, ocinaplon (subsequently discontinued) was found to be a nonsedating anxiolytic in 2 trials in patients with generalized anxiety disorder (GAD), which was at odds with the in vitro profile which showed significant functional efficacy at $\alpha$1.[13,14] The reasons for the differences in the clinical tolerability are not clear, and there remains an incomplete understanding of the effects of PAMs on $GABA_A$ receptors at the biophysical level. The in vitro functional activity is assessed in recombinant cell lines in which the level of intrinsic activity is determined by peak potentiation of low concentrations of GABA. However, GABA concentrations are very high in the synapse following action potential–induced exocytosis of GABA in vivo. As such, the chloride current potentiation is more related to changes in area under the curve of the inhibitory postsynaptic potential than changes in the peak current, and the rate at which the inhibitory postsynaptic potential returns to baseline will depend on many factors that may be influenced for $GABA_A$ PAMs, including channel-gating kinetics like opening time.[15] Such information is not available but could help develop an understanding as to why similar PAMs possess different clinical profiles.

PF-06372865 (now known as CVL-865) is a novel small-molecule high-affinity ligand for the BZD site of the $GABA_A$ receptor and demonstrates functional selectivity in vitro for receptors containing $\alpha$2/3/5 subunits compared with those containing an $\alpha$1 subunit, a profile relatively similar to that of TPA023.[15] PF-06372865 is structurally unrelated to BZDs (structure is disclosed in Nickolls et al[15]) and has demonstrated efficacy in preclinical models of anxiety, pain, and epilepsy.[6,16] Based on in vitro studies using human hepatocytes, microsomes, and recombinant cytochrome P450s (CYPs), CVL-865 is primarily metabolized by CYP3A4 with minor contributions from CYP1A2 and CYP2B6. No active metabolites have been identified. The potential for drug interactions with coadministered CYP3A4 inducers and inhibitors will be evaluated in future clinical studies.

In a single-ascending-dose study up to 100 mg in healthy volunteers, it was demonstrated that PF-06372865 was well tolerated, and the majority of AEs were mild and did not increase in severity with increasing RO (as would be expected with a classic BZD).[15] In contrast to TPA023, dose-limiting CNS AEs were not observed, even at RO estimated to be >80%. In that first clinical study, robust pharmacodynamic changes in markers of $\alpha$2/3 pharmacology were observed (ie, occupancy-dependent decreases in saccadic peak velocity and increase in quantitative electroencephalogram $\beta$ frequency). Both preclinical efficacy data and the effect on saccadic peak velocity observed in healthy volunteers suggested that at approximately 50% RO was sufficient to drive efficacy. Thus, doses to achieve similar exposure levels were selected for the proof-of-concept studies with PF-06372865 in chronic low back pain[17] and GAD.[18] However, disappointingly, a lack of efficacy was reported in both trials potentially either due to low RO exposures clinically (vs a minimum 50% RO effective range in preclinical studies) or a lack of translation from the in vitro to clinical profile. However, the accumulating scientific evidence[19] together with a lack of efficacy in the pain and anxiety clinical trials has highlighted the importance of achieving RO >50% with PAMs of $GABA_A$ receptors that have relatively low intrinsic efficacy (compared to BZDs). In contrast to the pain and anxiety patient trials, robust anticonvulsant activity of PF-06372865 at single doses (of 17.5 and 52.5 mg) achieving $\sim$50% and 80% RO has been established in a clinical trial in patients with epilepsy in the photosensitive epilepsy model.[20] Pharmacologic effects in this model have been demonstrated to substantially increase the likelihood that efficacy will be seen in the clinical epilepsy population for several anticonvulsant mechanisms and has been used as a reliable and early indicator in antiepileptic drug discovery, including aiding dose selection for future phase 2 and 3 epilepsy trials.[21]

The promising efficacy in the photoepilepsy model, the ability to achieve maximal pharmacodynamic effects at RO >50%, and the emerging data that also suggest a RO >50% may be required in other indications means further exploration of the profile of PF-06372865 was warranted.[19] The current study was designed to assess the safety, tolerability, and pharmacokinetic (PK) profile of PF-06372865 at doses that would achieve RO levels previously not achieved with

**Table 1.** Dose Administration in Multiple Dose Cohorts and Titration Schedule

| Cohort | Number of Subjects | Maintenance Dose of PF-06372865 Twice Daily |
|---|---|---|
| 1 | 8 active; 2 placebo | 25 mg |
| 2 | 8 active; 2 placebo | 42.5 mg |
| Cohort | Study Day | Dose of PF-06372865 twice daily[a] |
| 1 | 1–3 | 5 mg |
| 2 | 4–7 | 12.5 mg |
| | 8–21 | 25 mg |
| | 1–2 | 5 mg |
| | 3–4 | 12.5 mg |
| | 5–7 | 25 mg |
| | 8–21 | 42.5 mg |

[a] Doses administered twice daily except for the last dose, when a single morning dose was received.

multiple doses in the clinical programs for GAD and pain.

## Methods

The study was conducted at the Pfizer Clinical Research Unit in New Haven, Connecticut, in accordance with US Food and Drug Administration guidelines and Good Clinical Practice as established by the International Conference of Harmonisation and the principles of the World Medical Association of Helsinki (2013). The study protocol and informed consent forms were reviewed by the Integreview Institutional Review Board, Austin, Texas. All study subjects provided written informed consent before performance of any study procedures.

### *Study Design*

This study was a randomized, double-blind, sponsor-open, placebo-controlled, multiple, repeated-dose study using a spray-dried dispersion tablet formulation of PF-06372865. The primary objective was to investigate the safety, tolerability, and PK of ascending repeated doses of PF-06372865. The study consisted of 2 cohorts, which included dose titrations for 1 week followed by 2 weeks at a maintenance dose of 25 mg twice daily in cohort 1 and 42.5 mg twice daily in cohort 2 (Table 1). Subjects remained inpatient at the clinical research site from 1 day before the first dose of PF-06372865 until 3 days after the last administration. In cohort 1, the titration regiment consisted of administering 5 mg twice daily for 3 days, then 12.5 mg twice daily for 4 days, and then 25 mg twice daily for 14 days. In cohort 2, the titration regiment consisted of administering 5 mg twice daily for 2 days, then 12.5 mg twice daily for 2 days, then 25 mg twice daily for 3 days, and then 42.5 mg twice daily for 14 days. A sample size of approximately 10 subjects per cohort (~8 on active treatment and ~2 on placebo) was chosen empirically to provide adequate safety, toleration, and PK information at each repeated dose level.

All subjects were administered PF-06372865 or placebo with ambient water to a total volume of ~240 mL. To standardize conditions, all subjects were fasted overnight before the morning dose on days 1 and 21 and were required to refrain from eating and drinking beverages other than water during the first 4 hours after morning dosing. The evening dose was administered with water without regard to meals.

### *Study Subjects*

Women of non–childbearing potential and men aged 18 to 55 years with a body mass index of 17.5–30.5 kg/m$^2$ were eligible if they were determined to be healthy based on medical history and physical examination, electrocardiogram (ECG), vital signs, and clinical laboratory testing at screening. Subjects were excluded for any clinically significant medical condition, including clinically significant gastrointestinal pathology, which could interfere with absorption, distribution, metabolism, or excretion of study drug. Use of any prescription medication or nonprescription drugs within 7 days or 5 half-lives (whichever is longer) and use of dietary supplements within 28 days before the first dose of the study medication were prohibited. Consumption of alcohol within 24 hours before admission to the Clinical Research Unit was prohibited. Consumption of caffeine-containing products and use of tobacco- or nicotine-containing products within 24 hours before the start of dosing was prohibited. Fertile male subjects were required to use a highly effective method of contraception for the duration of the study and for at least 60 days after the last dose of the investigational product.

### Study Drugs

The doses of PF-06372865 selected for this study were selected based on margins to toxicology findings, clinical toleration, and safety data from previous phase 1 and phase 2 clinical studies with PF-06372865 (https://clinicaltrials.gov/ct2/results?term=PF-06372865), pharmacology data generated from NeuroCart, and RO data.[15] The doses examined in this study were approximately 3 to 6 times higher than those used in the previous phase 2 multiple-dose patient studies.

The dose for cohort 2 was planned to be ≤2-fold the 25-mg twice-daily dose used in cohort 1 and was reduced to 42.5 mg twice daily based on projected exposures relative to the predefined exposure limits.

### Study Assessments

Subjects had a physical examination and clinical laboratory testing (chemistry, hematology, and urinalysis) at the screening visit, at baseline, 72 hours after the last dose of the investigational product, and at the follow-up visit. At screening, tests were conducted for HIV and hepatitis B and C viruses. At screening, at 24 and 72 hours after the final dose of the investigational product, and at the follow-up visit, a 12-lead ECG and vital signs (blood pressure, heart rate) were obtained. AEs were recorded when reported. In women, serum follicle-stimulating hormone was obtained at screening. Drug and alcohol screenings, the latter at the discretion of the investigator, were run at screening and baseline. Suicidal ideation and behavior assessments including the Columbia Suicide Severity Rating Scale were performed at screening, at baseline, 72 hours after the last dose of the study drug (before discharge from the Clinical Research Unit), and at the follow-up visit.

### Pharmacokinetic Analysis

Blood samples (~3 mL collected by venipuncture) to provide ~1 mL of plasma for analysis of PF-06372865 plasma concentrations were collected into tubes containing $K_2$ ethylenediaminetetraacetic acid. Serial blood samples were collected before dosing and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours after administration of the morning dose on days 1 and 21 and at 24, 48, and 72 hours after the final dose on day 21. Blood samples were also collected before the morning dose on days 2, 7, 10, and 13 for assessment of time to steady state. A cumulative urine sample was also collected over 0 to 12 hours after dosing on day 21 for assessment of PF-06372865 urine concentrations. All samples were stored at −80°C until analysis.

Plasma and urine concentrations of PF-06372865 were determined using validated liquid chromatography coupled to tandem mass spectrometry methods in compliance with Pfizer standard operating procedures at York Bioanalytical Solutions (York, UK). For plasma, the isotopically labeled internal standard of PF-06372865 (PF-06739550) was added to 50 $\mu$L of plasma and extracted by protein precipitation with 250 $\mu$L of acetonitrile containing 0.2% formic acid. After centrifugation, a 100-$\mu$L aliquot of the supernatant was mixed with 400 $\mu$L of 10 mM ammonium formate with 0.2% formic acid and centrifuged. For urine, 10% orthophosphoric acid and Triton X-100:acetonitrile (1:1) was added to urine samples before use to minimize nonspecific binding. Internal standard was then added to 25 $\mu$L of treated urine and mixed with 200 $\mu$L of methanol with 0.2 % formic acid and 10 mM ammonium formate with 0.2% formic acid and centrifuged. Aliquots of plasma (5 $\mu$L) and urine (10 $\mu$L) extracts were injected onto a Poroshell high-performance liquid chromatography column (120 EC-C18, 3.0 × 50 mm, 2.7 $\mu$m; Agilent, Santa Clara, California) maintained at 50°C. Analytes were eluted using a step gradient with mobile phases comprising 10 mM of ammonium formate with 0.2% formic acid (solvent A) and methanol with 0.2% formic acid (solvent B) at a flow rate of 0.6 mL/min. Detection was performed by positive ion tandem mass spectrometry on a Sciex API 4000 or API 5000 mass spectrometer (Applied Biosystems, Foster City, California) with multiple reaction monitoring (m/z 441.2–348.0 for PF-06372865 and m/z 446.2–353.1 for internal standard). The calibration curve ranges of the plasma and urine assays were 1.00 to 1000 ng/mL and 0.050–50.0 ng/mL, respectively, using a weighted (1/concentration2) linear regression. The lower limit of quantification for PF-06372865 in plasma and urine were 1.00 and 0.050 ng/mL, respectively. Samples with concentrations above the upper limit of quantification were diluted into calibration range. For plasma samples, assay accuracy expressed as percent bias for the quality control (QC) samples ranged from −1.9% to 1.7% and assay precision expressed as percentage coefficient of variation for QC samples was ≤15.1%. For urine QC samples, the between-day assay accuracy ranged from −0.4% to 6.0%, and assay precision was ≤10.6%.

Actual PK sample times were used in the derivation of PK parameters and were calculated using standard noncompartmental methods. The maximum plasma concentration ($C_{max}$) and time to $C_{max}$ ($t_{max}$) were observed directly from the data. The area under the plasma concentration-time curve over the dosing interval ($AUC_{tau}$) was calculated using the linear/log trapezoidal method, and linear regression of the terminal phase of the PK profile was used to determine the terminal elimination half-life ($t_{\frac{1}{2}}$). The peak-to-trough ratio was calculated by dividing $C_{max}$ by predose trough concentrations on day 21. The accumulation ratios for $AUC_{tau}$ ($R_{ac}$) and $C_{max}$ ($R_{ac,Cmax}$) were calculated using dose-normalized values obtained on day 21 divided by

dose-normalized day 1 values for $AUC_{tau}$ and $C_{max}$, respectively. The amount of PF-06372865 recovered unchanged in the urine during the dosing interval ($Ae_{tau}$) on day 21 was determined by multiplying urine concentration by urine volume and then dividing it by dose to calculate $Ae_{tau}$%. Renal clearance was defined as $Ae_{tau}$ divided by $AUC_{tau}$. PF-06372865 concentrations below the limit of quantification were set as zero for the PK analysis. Descriptive statistics of all PK parameters were calculated for single doses (day 1) and multiple doses (day 21). The percentage coefficient of variation was calculated by dividing the standard deviation by arithmetic mean for each parameter.

Scatterplots of the relationships between dose-normalized $C_{max}$ and $AUC_{tau}$ were used to assess dose proportionality. Predose trough plasma concentrations obtained over the 21 days of dosing were used to determine the time to reach steady state.

RO (total and at $\alpha 2$ subunit-containing receptors) and PF-06372865 concentrations were previously determined in a clinical PET study using the radioligand [$^{11}$C]-flumazenil and by using an $E_{max}$ model incorporating receptor binding at each $\alpha$ subunit. The RO achieved at both dose levels in this study were calculated based on the parameter estimates from the previously developed model.[15] In the previous study, the maximum $RO_{max}$ was estimated to be 95.6% by assuming the ratios of Occ50,$\alpha 1$/Occ50,$\alpha 2$/Occ50 and $\alpha 3$/Occ50,$\alpha 2$ were same as the $K_i$ ratios determined in vitro for $\alpha 1/\alpha 2$ (0.18/2.92) and $\alpha 3/\alpha 2$ (1.06/2.92), respectively. Maximum RO was assumed to be the same for the different $\alpha$ subunits. The abundance of $\alpha 1$, $\alpha 2$, and $\alpha 3$ receptors was approximated to be 50%, 25%, and 25%, respectively, based on total $GABA_A$ receptors in the whole brain.[1] The Occ50,$\alpha 2$ was estimated to be 53.2 ng/mL, and Occ50,$\alpha 1$ and Occ50,$\alpha 3$ were 3.28 and 19.3 ng/mL, respectively. RO in this study was calculated using these exposure-RO parameters and observed geometric mean PF-06372865 concentrations from this study.

**Table 2.** Baseline Demographics

| Characteristic | Subjects (n = 19) |
|---|---|
| Age, y, mean (SD) | 37.8 (8.8) |
| Male, n (%) | 17 (89.5) |
| Hispanic/Latino, n (%) | 5 (26.3) |
| Race, n (%) | |
| White | 6 (31.6) |
| Black | 13 (68.4) |
| Body mass index, kg/m$^2$, mean (SD) | 26.6 (3.0) |
| Weight, kg, mean (SD) | 84.1 (13.9) |

SD, standard deviation.
Continuous variables reported where appropriate as mean (SD; range).

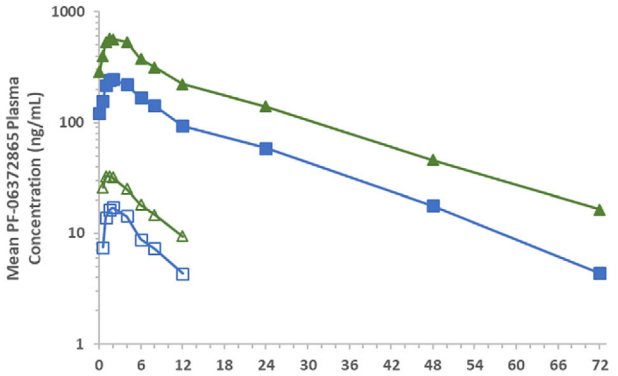


**Figure 1.** Arithmetic mean plasma concentration-time profiles following single and multiple oral doses of PF-06372865 on days 1 and 21 (open squares: day 1 cohort 1; open triangles: day 1 cohort 2; closed squares: day 21 cohort 1; and closed triangles: day 21 cohort 2). Cohort 1 (25 mg twice daily PF-06372865) titration: 5 mg twice daily for 3 days, 12.5 mg twice daily for 4 days, and 25 mg twice daily for 14 days. Cohort 2 (42.5 mg twice daily PF-06372865) titration: 5 mg twice daily for 2 days, 12.5 mg twice daily for 2 days, 25 mg twice daily for 3 days, and 42.5 mg twice daily for 14 days.

## Results

### *Study Subjects*

Nineteen healthy adult subjects were enrolled between November 8, 2017, and February 28, 2018, and 18 completed the study. One subject (cohort 1) was withdrawn for noncompliance with study requirements and was thus excluded from the PK analysis. Baseline demographic characteristics were consistent with a population of healthy subjects (Table 2).

### *Pharmacokinetics*

Arithmetic mean PF-06372865 plasma concentration–time profiles following single (day 1) and multiple (day 21) oral twice-daily doses are presented in Figure 1. PK parameters for each treatment are summarized descriptively in Table 3.

PF-06372865 was rapidly absorbed with a median $t_{max}$ of 1–2 hours following both single- and multiple-dose administration. The mean $t_{\frac{1}{2}}$ on day 21 ranged from 11.2 to 11.5 hours for both treatments. Urinary recovery of unchanged PF-06372865 was low, with <0.6% of the dose recovered in the 12-hour dosing interval on day 21. In general, plasma PF-06372865 exposures appeared to increase in an approximate dose-proportional manner from 25 to 42.5 mg twice-daily dosing.

**Table 3.** Summary of Plasma and Urine PF-06372865 Pharmacokinetic Parameter Values Following Single and Multiple Doses

| | Parameter Summary Statistics[a] by Treatment | |
|---|---|---|
| Parameter, Units | PF-06372865 25 mg Twice Daily Titrated[b] | PF-06372865 42.5 mg Twice Daily Titrated[c] |
| Day 1 (single dose) | | |
| N[d] | 8 | 7 |
| $C_{max}$, ng/mL | 18.9 ± 5.1 | 36.2 ± 19.4 |
| $t_{max}$, h | 2.0 (0.5–4.0) | 1.0 (0.5–2.0) |
| $AUC_{tau}$, ng • h/mL | 116 ± 35 | 233 ± 140 |
| Day 21 (multiple dose) | | |
| N[d], n[e] | 7, 7 | 7, 7 |
| $C_{max}$, ng/mL | 253 ± 108 | 612 ± 351 |
| $t_{max}$, h | 1.5 (1.0–4.0) | 2.0 (1.0–4.0) |
| $AUC_{tau}$, ng • h/mL | 2026 ± 977 | 4713 ± 2995 |
| PTR | 3.1 ± 0.9 | 3.3 ± 0.9 |
| $t_{\frac{1}{2}}$, h | 11.2 ± 3.5 | 11.5 ± 5.1 |
| $R_{ac}$[f] | 3.2 ± 0.8 | 2.5 ± 0.9 |
| $R_{ac,Cmax}$[f] | 2.7 ± 0.9 | 2.0 ± 0.7 |
| $Ae_{tau}$ % | 0.40 ± 0.51 | 0.52 ± 0.34 |

$Ae_{tau}$, amount of PF-06372865 recovered unchanged in the urine during the dosing interval; $AUC_{tau}$, area under the plasma concentration–time curve over the dosing interval; $C_{max}$, maximum plasma concentration; PTR, peak-to-trough ratio; $R_{ac}$, accumulation ratio for $AUC_{tau}$; $R_{ac,Cmax}$, accumulation ratio for $C_{max}$; $t_{\frac{1}{2}}$, terminal elimination half-life; $t_{max}$, time to maximum concentration.

Accumulation ratios ($R_{ac}$ and $R_{ac,Cmax}$) were calculated based on dose-normalized parameters due to the titration scheme.

[a]Arithmetic mean ± standard deviation for all parameters, except median (range) for $t_{max}$.

[b]The titration scheme for treatment PF-06372865 25 mg twice daily was 5 mg twice daily for 3 days, 12.5 mg twice daily for 4 days, and 25 mg twice daily for 14 days.

[c]The titration scheme for treatment PF-06372865 42.5 mg twice daily was 5 mg twice daily for 2 days, 12.5 mg twice daily for 2 days, 25 mg twice daily for 3 days, and 42.5 mg twice daily for 14 days.

[d]N = number of subjects in the treatment group and contributing to the mean.

[e]n = number of subjects with reportable $t_{\frac{1}{2}}$.

[f]Accumulation ratios were calculated based on dose-normalized parameters due to the titration scheme.

Intersubject variability for PF-06372865 exposures based on percentage coefficient of variation was moderate to high, ranging between 31% and 64% for $AUC_{tau}$ and 27% and 57% for $C_{max}$. Variability was greater with the 42.5-mg twice-daily dose and was generally higher on day 21 as compared to day 1 in both treatment groups. Single-dose PK observed in this study was consistent with previously reported PK,[15] and multiple-dose PK was also consistent with a previous multiple-dose study (NCT02070289; unpublished data).

Accumulation ratios ($R_{ac}$ and $R_{ac}$, $C_{max}$) were calculated based on dose-normalized parameters due to the titration dosing scheme. Plasma PF-06372865 accumulation was <3.5-fold following twice-daily dosing (Table 3). Geometric mean accumulation ratios ranged from 2.5 to 3.2 for $AUC_{tau}$ ($R_{ac}$) and 2.0–2.7 for $C_{max}$ ($R_{ac}$, $C_{max}$), respectively on day 21. The time to reach steady state was confounded by the titration dosing regimen and could not be determined based on observed trough concentrations but was estimated to be reached within approximately 3 days based on the observed terminal $t_{1/2}$.

RO was calculated to be approximately 78% and 87% at $C_{max}$ on day 21 for 25 mg twice daily and 42.5 mg daily, respectively. In addition, trough RO was calculated to be ~67% and 81%, reflective of the ~11-hour terminal $t_{1/2}$ and small peak-to-trough ratio of approximately 3.

### *Safety/Tolerability*

None of the subjects experienced a serious AE. There were no permanent discontinuations due to AEs, and no deaths occurred during this study. The maximum tolerated dose was not defined; however, a further increase in dose was precluded based on predefined exposure limits in preclinical toxicity studies available at that time. There were no clinically significant findings in clinical laboratory assessments, vital signs, ECGs, physical examinations, or any suicidal behaviors as measured by the Columbia Suicide Severity Rating Scale.

A total of 53 treatment-emergent adverse events (TEAEs) were reported by 16 subjects following treatment of PF-06372865 or placebo (Table 4). Of these, 30 were considered to be treatment related. All

**Table 4.** Incidence of Treatment-Emergent Adverse Events

| | Placebo (N = 4) | PF-06372865 25 mg Twice Daily Titrated (N = 8) | PF-06372865 42.5 mg Twice Daily Titrated (N = 7) |
|---|---|---|---|
| Total TEAEs reported (treatment related) | 5 (3) | 26 (12) | 22 (15) |
| Number of subjects with any AE (treatment related) | 3 (1) | 7 (7) | 6 (4) |
| TEAEs reported by ≥2 subjects (treatment related) | | | |
| Constipation | 1 (0) | 3 (0) | 1 (0) |
| Back pain | 0 | 2 (0) | 0 |
| Dizziness | 1 (1) | 2 (2) | 3 (3) |
| Headache | 0 | 1 (0) | 2 (1) |
| Somnolence | 0 | 3 (3) | 0 |

AE, adverse event; TEAEs, treatment-emergent adverse events.
Subjects were counted only once per treatment per event. The titration scheme for treatment PF-06372865 25 mg twice daily was 5 mg twice daily for 3 days, 12.5 mg twice daily for 4 days, and 25 mg twice daily for 14 days. The titration scheme for treatment PF-06372865 42.5 mg twice daily was 5 mg twice daily for 2 days, 12.5 mg twice daily for 2 days, 25 mg twice daily for 3 days, and 42.5 mg twice daily for 14 days. Medical Dictionary for Regulatory Activities (version 20.1) coding dictionary applied.

**Table 5.** Somnolence and Dizziness Adverse Events by Cohort and Dosing Period

| | Reaction | Week 1 (Titration) | Week 2 (Maintenance) | Week 3 (Maintenance) | Follow-Up |
|---|---|---|---|---|---|
| Placebo | Dizziness | 0/4 | 0/4 | 1/4 | 0/4 |
| | Somnolence | 0/4 | 0/4 | 0/4 | 0/4 |
| 25 mg twice daily | Dizziness | 2/8 | 1/8 | 0/8 | 0/8 |
| | Somnolence | 3/8 | 0/8 | 0/8 | 0/8 |
| 42.5 mg twice daily | Dizziness | 3/7 | 1/7 | 1/7 | 1/7 |
| | Somnolence | 0/7 | 0/7 | 0/7 | 0/7 |

Cohort 1 (25 mg twice daily PF-06372865) titration: 5 mg twice daily for 3 days, 12.5 mg twice daily for 4 days, and 25 mg twice daily for 14 days. Cohort 2 (42.5 mg twice daily PF-06372865) titration: 5 mg twice daily for 2 days, 12.5 mg twice daily for 2 days, 25 mg twice daily for 3 days, and 42.5 mg twice daily for 14 days.

AEs were mild in severity, and the relative number of AEs did not increase with increasing dose. The most frequent TEAEs reported were transient dizziness and somnolence. Dizziness was reported by 6 subjects (1 subject after dosing placebo, 2 subjects after dosing with PF-06372865 twice-daily titrated treatment, and 3 subjects after dosing with 42.5 mg twice-daily titrated treatment), and the majority of reports occurred during the 1-week titration period (Table 5). Somnolence was reported by 3 subjects in the 25-mg twice-daily cohort and occurred only during the titration period. Of note, there were no reports of sedation by any subjects participating in the study, and somnolence was not reported in the subjects of cohort 2 who were administered 42.5 mg twice daily. Following abrupt discontinuation, there were no reports of AEs associated with withdrawal symptoms.

## Discussion

To our knowledge, this is the first reported study describing the PK profile of PF-06372865 following multiple doses exceeding 7.5 mg twice daily, which achieved only ~50% RO at $\alpha$2-containing $GABA_A$ receptors at $C_{max}$. In the current study, RO up to ~87% $\alpha$2-containing $GABA_A$ receptors were evaluated, allowing further characterization of safety, tolerability, and PK of PF-06372865. Observed PK in this study was generally consistent with previously reported single-dose PK up to 100 mg.[15] Single doses of PF-06372865 were associated with approximate dose-proportional increases in AUC and $C_{max}$ in this study. Following single- and multiple-dose administration, median maximal plasma concentrations were achieved 1 to 2 hours after dosing on days 1 and 21.

The incidence of TEAEs was not dose related, and all AEs across the cohorts were mild in severity. The most frequently occurring TEAEs were somnolence and dizziness, which is in concordance with previously reported trials in healthy volunteers.[15] A 1-week dose titration step was used in this trial to mitigate against the potential occurrence of central nervous system AEs. Indeed, a titration was used in both chronic low back

pain (NCT02262754) and GAD (NCT02310568) trials, with 2.5 mg twice daily being administered for 1 week before initiating 7.5 mg twice daily.[17,18] In the current trial, due to the inpatient design, a relatively fast titration (7 days) was used to enable a quick ascent to the target doses of 25 mg twice daily in cohort 1 and 42.5 mg twice daily in cohort 2. Once the target dose was reached, twice-daily dosing continued at the top dose for an additional 14 days. The titration scheme was well tolerated and no issues were encountered, suggesting that a similar regimen may be used in subsequent clinical trials with PF-06372865. While the safety and tolerability of the doses examined in this trial have not been examined without a titration and may be well tolerated, the cumulative experience of single-dose exposure discussed in Nickolls et al,[15] and in the proof of concept trials would suggest that titration is of utility to mitigate AEs. Indeed, titration regimens are routinely used when initiating chronic treatment with BZDs to treat anxiety, for example, and it is not clear whether this reduces CNS-related AEs with BZDs to the extent reported here with PF-06372865.

The exposures achieved in this trial resulted in estimated ROs at $GABA_A$ receptors $\alpha 2$ subunits of $>80\%$, which is in contrast to ROs achieved with BZDs, which are limited to $<20\%$ to avoid AEs associated with sedation and somnolence in some patient populations. Indeed, no somnolence was observed in the cohort administered 42.5 mg twice daily, and only transient, mild somnolence was reported in the 25-mg twice-daily cohort during the titration, providing further evidence of differentiation of PF-06372865 from BZDs. For example, in a sample of $\sim$3500 patients treated for anxiety with lorazepam, one of the most commonly prescribed BZDs, the most frequent adverse reaction was sedation (15.9%),[22] and at doses that achieve RO $<20\%$. The $\alpha 1$-preferring sedative-hypnotic zolpidem does somewhat validate the hypothesis that $\alpha 1$-conatining receptors are associated with sedation. While the data in this trial supports the hypothesis that activity at $\alpha 1$ subunits is involved in the sedative properties of BZDs, there does remain some disconnect between the $\alpha 1$-sparing functional efficacy reported from in vitro functional assays and the clinical profile of some subtype-selective PAMs that exhibit dose-limited CNS AEs in the clinic at RO $<65\%$.[7] Data demonstrating that TPA023, TPA023B, and PF-06372865 do not generalize to an interoceptive cue in zolpidem-trained rats in drug discrimination studies, in contrast to ocinaplon, suggests alignment between the lack of $\alpha 1$ subunit activation in vitro and in vivo, but the reason for the misalignment in the clinic remains unknown.[15,23,24]

The data with PF-06372865 demonstrate that it is nonsedating both preclinically and clinically, and there are data to support anticonvulsant potential clinically[20]; however, little is known whether the minimized $\alpha 1$ activity and selective functional efficacy at $\alpha 2/3/5$ as measured from in vitro assays will result in efficacy in patients with anxiety and/or pain. A clinical trial in patients with drug-resistant focal-onset seizures (NCT04244175) has been initiated with PF-06372865 (CVL-865), and a study is planned in an experimental model of panic in healthy volunteers (NCT0459253), both studies will include doses expected to achieve $\sim$60% and 80% RO.

An attractive attribute associated with subtype-selective PAMs with relatively low intrinsic efficacy vs BZDs, is that there is no convincing evidence that chronic administration leads to symptoms of withdrawal following abrupt discontinuation or the development of tolerance of anticonvulsant efficacy preclinically, and thus are potentially unlikely to lead to withdrawal or tolerance clinically. This trial adds weight to that evidence as no subject reported any AEs associated with the symptoms of withdrawal following abrupt discontinuation of PF-06372865. This is in contrast with clobazam, for example, where withdrawal-related AEs were observed in phase 1 studies following abrupt discontinuation at therapeutic and subtherapeutic dosages.[25]

The current study supports ongoing clinical evaluation of PF-06372865 at doses that achieve receptor occupancies $\geq 50\%$, enabling the possibility to fully test the mechanism of action and realize the therapeutic potential of subtype selective $GABA_A$ receptor PAMs.

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## Conflicts of Interest

The trial was sponsored by Pfizer Inc. RG, MW, HW, ZS, and AO are or were employees of Pfizer at the time of this research and may own stock in the company.

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## Author Contributions

All authors contributed to the design, analysis, and interpretation of data associated with this clinical trial and contributed to the development of this manuscript.

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