Pharmacokinetic and Pharmacodynamic Effects of a γ-Secretase Modulator, PF-06648671, on CSF Amyloid-β Peptides in Randomized Phase I Studies

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γ-Secretase modulators (GSMs) represent a promising therapy for Alzheimer’s disease by reducing pathogenic amyloid-β (Aβ) peptide production. Three phase I studies (NCT02316756, NCT02407353, and NCT02440100) investigated the safety/tolerability, pharmacokinetics (PKs), and pharmacodynamics (PDs) of the oral GSM, PF-06648671. A PK/PD indirect-response model was developed (using biomarker data) to simultaneously characterize differential effects of PF-06648671 on multiple Aβ species in cerebrospinal fluid (CSF). Healthy subjects (n = 120) received single doses or multiple-ascending doses of PF-06648671/placebo for 14 days. No serious adverse events occurred; severe adverse events were deemed not drug related. PF-06648671 decreased Aβ42 and Aβ40 concentrations in CSF, with greater effects on Aβ42, and increased Aβ37 and Aβ38 levels, particularly Aβ37. No significant change in total Aβ was observed. The PK/PD model well described the tendency of observed CSF Aβ data and the steady-state effects of PF-06648671, supporting its use for predicting central Aβ effects and optimal dose selection for GSMs in future trials.

Study Highlights

**WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?**

✔ In Alzheimer’s disease, γ-secretase modulators (GSMs) have been identified as potential therapies; through modulating γ-secretase activity, the balance of amyloid-β (Aβ) species production can be altered to reduce Aβ-associated pathogenicity. However, quantitative models predictive of changes in various Aβ species in human cerebrospinal fluid (CSF) with respect to GSMs are not available to date.

**WHAT QUESTION DID THIS STUDY ADDRESS?**

✔ Using pharmacokinetics (PKs) and pharmacodynamics (PDs) data from three studies in healthy subjects, we aimed to quantitatively characterize differential effects of the GSM PF-06648671 on CSF Aβ species simultaneously using a population PK/PD model.

**WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?**

✔ PF-06648671 decreased CSF levels of the more pathogenic Aβ42 and Aβ40 species and increased levels of less pathogenic Aβ37 and Aβ38. The PK/PD model adequately described observed data on multiple CSF Aβ species and predicted the effects of PF-06648671 on CSF Aβ levels and ratios.

**HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?**

✔ The PK/PD model developed here may be used to inform dose selection in future PF-06648671 clinical trials.

Dementia is a progressive, neurodegenerative disorder affecting ~50 million people worldwide, of which Alzheimer’s disease (AD) is most common.1 Treatments including cholinesterase inhibitors and N-methyl-d-aspartate receptor antagonists help with AD symptoms.2 However, a medical need exists for agents capable of modifying and slowing AD progression.

AD pathology in the brain is characterized by senile, amyloid plaques comprising a core of amyloid-β (Aβ) peptide fibrils,34 and neurofibrillary tangles of primarily hyperphosphorylated tau protein.45 Amyloid precursor protein (APP) is a transmembrane protein that is cleaved during processing by γ-secretase to generate Aβ peptides of various lengths, including Aβ37, 38, 40, and 42.45 Aβ42 has a higher aggregation

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The aim was to develop a pharmacokinetic; q.d., once daily. PK, pharmacokinetic; PD, pharmacodynamic; PK, pharmacokinetic; q.d., once daily. CSF, cerebrospinal fluid; LP, lumbar puncture; PD, pharmacodynamic; PK, pharmacokinetic; q.d., once daily.

A tendency than the other peptides \(^5\) and is a major component of amyloid plaques. \(^9\) Aggregated A\(\beta\) oligomers interact with synaptic receptors, \(^10\) causing nerve-cell disruption and death. \(^11\) The shorter A\(\beta\)37 and A\(\beta\)38 species are believed to be less pathogenic than A\(\beta\)42. \(^12\) Increased A\(\beta\)42 production and an increased ratio of A\(\beta\)42:A\(\beta\)40 has been associated with an aggressive familial form of AD. \(^13\) and A\(\beta\)42:A\(\beta\)40 or A\(\beta\)42:A\(\beta\)38 ratios in cerebrospinal fluid (CSF) are useful biomarkers for AD pathology and predictors of AD. \(^14,15\)

Reducing A\(\beta\)42 and A\(\beta\)40 by targeting \(\gamma\)-secretase is a promising therapeutic strategy, leading to the development of \(\gamma\)-secretase inhibitors (GSIs) and, more recently, \(\gamma\)-secretase modulators (GSMs). GSIs inhibit APP cleavage and A\(\beta\)42 and A\(\beta\)42 production \(^16,17\) but are associated with detrimental effects on cognitive functioning and toxicities, including gastrointestinal bleeding, skin cancer, and immunosuppression, likely due to off-target effects on other processes, such as Notch proteolysis. \(^18–23\) Efforts have been made to design “Notch-sparing” GSMs that are APP-selective, \(^24\) but their safety has not been demonstrated. Moreover, GSIs can increase production of C-terminal fragments of APP, potentially causing toxic effects on synaptic function and cognition. \(^20,25\)

GSMs modulate, rather than inhibit, \(\gamma\)-secretase and shift A\(\beta\) production from A\(\beta\)42 and A\(\beta\)40 to A\(\beta\)37 and A\(\beta\)38, without altering overall A\(\beta\) levels. \(^12,26,27\) GSMs interact with \(\gamma\)-secretase to increase “processivity” (successive cleavage) of A\(\beta\), leading to these less pathogenic forms of A\(\beta\). \(^28–30\) It has been demonstrated \(in vitro\) that the aggregation propensity of A\(\beta\)42 is greatly reduced by co-incubation with A\(\beta\)37 and A\(\beta\)38, raising the possibility that shorter A\(\beta\) peptides may be beneficial in preventing or slowing amyloid plaque formation. \(^30\) Importantly, by modulating rather than inhibiting \(\gamma\)-secretase, GSMs can avoid the mechanism-based toxicity associated with GSIs. \(^31\)

Several GSMs are in development; \(^26,30,32–35\) to date, published clinical studies of one GSM have provided proof of the GSM mechanism in humans and have confirmed translation of pharmacologic activity from animal models to humans. \(^32,33\) However, quantitative models predictive of A\(\beta\) changes in human CSF are yet to be published.

PF-06648671 is a novel, brain-penetrable, small-molecule GSM and a potent modulator of \(\gamma\)-secretase \(in vivo\) and \(in vitro\). In cell-based assays, PF-06648671 reduced A\(\beta\)42 and A\(\beta\)40, with concomitant increases in A\(\beta\)37 and A\(\beta\)38, without inhibiting the cleavage of Notch or other substrates. \(^36\) Research in animals demonstrated reduced A\(\beta\)42 within the brain and CSF following acute oral administration of PF-06648671. \(^36\) This change in the CSF A\(\beta\) profile represents mechanism-relevant pharmacodynamic (PD) endpoints suitable for inclusion in translational research studies in humans.

This analysis utilized data from three phase I studies of PF-06648671: two single-dose studies, B7991001 (clinicaltrials.gov NCT02316756) and B7991003 (NCT02407353), and a multiple-dose study, B7991002 (NCT02440100). The aim was to develop a pharmacokinetic (PK)/PD model to characterize the differential effects of PF-06648671 on the various CSF A\(\beta\) species in healthy humans.

**RESULTS**

**Study populations**

In B7991001, 18 subjects were randomized and received the study drug; all were white men, with a mean age of 35.3 years (range 22–55 years) and mean weight of 79.1 kg (range 52.8–104.0 kg).

Subjects received PF-06648671 doses of 2, 4, 12, and 40 mg in cohort 1, or 120, 240, 360 mg in cohort 2 (\(n = 6\), except 4 mg, where \(n = 5\)); eight received placebo (Table 1). One subject discontinued from each of the 2 mg and 4 mg groups and was replaced. All subjects were analyzed for PK (except those receiving placebo); all were included in PD and safety analyses.

In B7991003, 22 subjects were randomized and received single-dose PF-06648671 150 mg (\(n = 3\)), 300 mg (\(n = 11\)), and placebo (\(n = 8\); Table 1). All were men, except one woman randomized to placebo. Eleven subjects were white, eight were black, one was Asian, and two were classified as “other.” Mean age was 36.1 years (range 21–55 years), mean weight was 76.0 kg (range 56.0–96.6 kg). All subjects completed the study, except one assigned placebo who was no longer willing to participate and was replaced. All subjects were analyzed for PK (except those receiving placebo), PD, and safety.

B7991002 comprised two parts: one in healthy subjects aged 18–55 years, and one in healthy elderly subjects aged 65–85 years.

| Table 1 | Overview of the PF-06648671 phase I studies used in this analysis |
|---------|-----------------------------------------------------------------------------|
| **B7991001:** First-in-human (single-ascending dose) |
| **Design** | Crossover, placebo controlled, placebo substitution |
| **Subjects** | Healthy |
| **Data collection** | PK, safety, tolerability, plasma biomarkers |
| **PF-06648671 doses** (\(n = 5–8\) per dose) |
| Cohort 1 | 2, 4, 12, 40 mg, placebo |
| Cohort 2 | 120, 240, 360 mg, placebo |
| **B7991003:** CSF biomarker |
| **Design** | Single dose, parallel, placebo controlled, lumbar catheterization for serial CSF sampling |
| **Subjects** | Healthy |
| **Data collection** | PK, safety, tolerability, serial CSF sampling for time-course CSF biomarker effects (≤36 hours) |
| **PF-06648671 doses** (\(n = 3\), 300 mg (\(n = 11\)), placebo (\(n = 8\)) |
| **B7991002:** Multiple-ascending doses |
| **Design** | Parallel group, placebo controlled, single LP for CSF sample collection |
| **Subjects** | Healthy (part 1) and healthy elderly (part 2) |
| **Data collection** | Safety, tolerability, PK, PD (through CSF biomarkers) at: Day -3 (baseline) and predose on day 14 in one cohort of 40 mg q.d. 24 Hours postdose on day 14 (in cohorts of 100, 200, and 360 mg q.d.) (steady state) |
| **PF-06648671 doses** |
| Part 1 | 4, 12, 40, 40 (no LP), 100, 200, 360 mg (\(n = 8\) for each), or placebo q.d. (\(n = 2\)) for 14 days |
| Part 2 | 200 mg (\(n = 8\)) or placebo (\(n = 2\)) q.d. for 14 days |

CSF, cerebrospinal fluid; LP, lumbar puncture; PD, pharmacodynamic; PK, pharmacokinetic; q.d., once daily.

*Dosed in fed and fasted states.*
In part 1, 70 subjects were randomized; 8 were assigned to each PF-06648671 dose, and 14 to placebo. The 40 mg q.d. dose was repeated in a second cohort without lumbar puncture (LP), to investigate the underlying cause for severe adverse events (AEs) experienced in three subjects in the first 40 mg q.d. cohort (details below). Six subjects discontinued, four due to AEs (details below). All subjects were men, 63 were white, 4 were black, and 3 were Asian; mean age was 32.2 years (range 18–55 years), and mean weight was 76.1 kg (range 52.4–107.2 kg). In part 2, 10 elderly subjects were randomized to PF-06648671 200 mg q.d. (n = 8) or placebo (n = 2); 4 were women (all received PF-06648671). Mean age was 68.0 years (range 65–75 years) and mean weight was 72.3 kg (range 54.9–91.3 kg); nine were white and one was Asian. All subjects completed part 2. All subjects in B7991002 were analyzed for PK (except those receiving placebo), PD, and safety.

**Safety**

In B7991001, 14 treatment-emergent AEs were reported in 12 subjects in cohort 1 (9 treatment-related), and 13 AEs in 11 subjects in cohort 2 (8 treatment-related; Table S1). No deaths, serious AEs (SAEs), severe AEs, dose reductions, or temporary discontinuations due to AEs were reported; one subject discontinued due to a non-treatment-related AE (pneumonia).

In B7991003, 14 subjects reported 32 AEs, of which 1 was considered treatment related (in the placebo group; Table S1). No deaths, SAEs, discontinuations, dose reductions, or temporary discontinuations due to AEs occurred. Two subjects receiving placebo had severe AEs of post-LP syndrome and procedural pain; neither was deemed treatment related.

In B7991002, 177 AEs were reported in 65 subjects, of which 103 were considered treatment related (Table S1). No deaths, SAEs, or dose reductions due to AEs occurred. Severe AEs were reported in three subjects receiving PF-06648671 40 mg q.d.: nausea and headache in two subjects (one discontinued the study, one temporarily discontinued the study drug), and postural dizziness and headache in one subject (the subject discontinued the study); all were considered related to LP rather than the study drug. Two additional subjects discontinued the study due to AEs: one due to mild hunger in the 40 mg q.d. group and one due to pregnancy of the subject’s partner, which was reported during treatment in the 100 mg q.d. group; both were unrelated to the study drug.

The most common laboratory abnormality across all studies was low-density lipoprotein cholesterol elevated >1.2-times upper limit of normal; no abnormalities were considered clinically significant by investigators. Vital signs or electrocardiogram data abnormalities were infrequent and not considered clinically significant by investigators.

**PK analysis**

PF-06648671 was rapidly absorbed following single and multiple doses; T\(_{\text{max}}\), median time to reach maximal plasma concentration (C\(_{\text{max}}\)), was ≤1.5 hours across studies in the fasting state (Table S2). In the single-dose studies, plasma C\(_{\text{max}}\) and area under the concentration-time curve from time 0 to infinity (AUC\(_{0-\text{inf}}\)) increased with dose (Table S2). Mean terminal half-life (t\(_{1/2}\)) values were 12.8–23.1 hours. With food, median T\(_{\text{max}}\) was delayed by 3 hours, C\(_{\text{max}}\) decreased, and AUC\(_{0-\text{inf}}\) increased slightly (Table S2). PF-06648671 plasma concentration over time in B7991003 is shown in Figure 1a. In B7991003, the ratio of PF-06648671 CSF exposure (measured as area under the concentration-time curve from time of administration up to the time of the last quantifiable concentration (AUC\(_{0-\text{last}}\)) to that in plasma (~0.003) was substantially lower than the free fraction in plasma (~0.013) and the ratio observed in B7991002 (~0.013; data not shown). This lower ratio was primarily attributed to nonspecific binding of PF-06648671 to the long tubing used in serial CSF sample collection, with likely substantial drug loss, whereas no tubing was used in the B7991002 single-sampling procedure. Therefore, PF-06648671 CSF concentration was not used in PK/PD modeling.

In B7991002, steady-state plasma C\(_{\text{max}}\) and AUC from time 0 to time \(\tau\) (AUC\(_{0-\tau}\); \(\tau = 24\) hours for q.d. dosing) increased with dose (Figure 2a), with mean t\(_{1/2}\) values of 12.0–20.8 hours across doses (Table S2). Observed accumulation ratios for AUC\(_{0-\tau}\) and C\(_{\text{max}}\) were 1.2–1.8 on day 14. Following multiple ascending doses, plasma PK parameters in elderly subjects were generally similar to those in younger subjects (Table S2). PF-06648671 concentrations in CSF of nonelderly subjects at day 14 ranged from mean coefficient of variation) 3.49 ng/mL (81%) with 40 mg q.d., to 27.85 ng/mL (31%) with 360 mg q.d. The mean ratio of CSF:total plasma concentration ranged from 0.0124–0.0143, approximately equal to the unbound fraction in plasma (0.013).

**PD analysis**

In B7991003, data from serial CSF sampling were highly variable (Figure 1b–f), and a baseline drift of A\(\beta\) levels with placebo was observed. Nevertheless, dose-dependent decreases in A\(\beta\)42, and to a lesser extent in A\(\beta\)40, were observed up to 36 hours postdose; dose-dependent increases in A\(\beta\)37, and to a lesser extent A\(\beta\)38, were observed (Figure 1b–e and Table S2). Total CSF A\(\beta\) levels in some subjects fluctuated substantially over the collection period (Figure 1f). However, no difference was observed between PF-06648671 and placebo groups. Total A\(\beta\) levels did not change significantly with either dose (Figure 1f and Table S2). A delay between PK T\(_{\text{max}}\) and the time to maximum PD effect was noted (Figure 1a vs. 1b–e). Following single doses, no rebound effect of plasma A\(\beta\) levels over baseline was observed.

In B7991002 (in healthy young subjects), single CSF samples were obtained and analyzed at baseline and steady state in cohorts receiving placebo, 40, 100, 200, and 360 mg q.d. Robust dose-dependent changes in A\(\beta\) were observed with a negligible placebo effect (Figure 2b–e, 0 mg dose). Across all PF-06648671 doses, placebo-adjusted A\(\beta\)42 and A\(\beta\)40 levels decreased, with a commensurate dose-dependent increase in CSF A\(\beta\)37 and A\(\beta\)38 levels (Table S2). No consistent changes in total A\(\beta\) levels were observed across doses (Figure 2f and Table S2).

**Supplementary Text** provides further details.

**PK/PD model**

A PK/PD indirect-response model was used to simultaneously characterize the differential effects of PF-06648671 on multiple CSF A\(\beta\) species, including A\(\beta\)42, A\(\beta\)40, A\(\beta\)38, and A\(\beta\)37 (Figure 3). The placebo response was empirically characterized to...
account for the baseline drift observed over time; parameter estimates for placebo response are listed in Table S3.

Inhibitory effects of PF-06648671 on CSF Aβ42 were greater than on Aβ40, demonstrated by higher maximum inhibition achievable (I_{max}) and smaller plasma concentration at half I_{max} (IC_{50}) values with Aβ42 vs. Aβ40 (Table 2). Also, PF-06648671-induced increases in Aβ37 levels were greater than those for Aβ38, demonstrated by higher maximum response achievable (E_{max}) and lower plasma concentration at half E_{max} (EC_{50}) values for Aβ37 (Table 2).

Goodness-of-fit plots of observed Aβ concentrations vs. population-predicted and individual-predicted Aβ concentrations showed that the PK/PD model adequately described the observed Aβ species concentrations from single-dose and multiple-dose studies (Figure S2); no apparent bias or systematic trends were evident (Figure S3). Visual predictive check (VPC) revealed that the observed and simulated medians were in good agreement overall for multiple-dose data (Figure 4), although variability tended to be higher in the simulation than in the observed data. VPC of single-dose data also showed a congruence between the simulated and observed data for the central tendency; however, overestimation of variability was greater (data not shown).

Using the PK/PD model, the differential effects of PF-06648671 at steady state on Aβ species in CSF were investigated. Reduction in Aβ42 was expected to plateau at ~70% at high doses studied. Although the estimated maximum reductions were similar for CSF Aβ42 and Aβ40 (74% and 73%), the doses required to achieve the same level of reduction were considerably higher for Aβ40 than Aβ42 due to higher estimated IC_{50} for Aβ40 vs. Aβ42. For instance, it was predicted that an average 50% reduction in CSF Aβ42 would be achieved at PF-06648671 75 mg q.d. (Figure 5a), whereas PF-06648671 250 mg q.d. would be required to reduce CSF Aβ40 to the same extent (Figure 5b). In addition, an increase in CSF Aβ37 of up to nearly 500% was predicted within the simulated dose ranges of PF-06648671 (Figure 5c). Predicted steady-state effects of PF-06648671 on Aβ38 are not shown, due to large uncertainty in its parameter estimates, especially in EC_{50} and γ (sigmoidicity; Table 2).

The ratio of Aβ42:Aβ40 decreased maximally by 30% with PF-06648671 between 150 and 200 mg q.d. (Figure 5d). Similarly, reductions in Aβ42:Aβ37 and Aβ42:Aβ38 ratios were predicted to reach maximum between 150 and 200 mg q.d. (Figure 5e, f). The median ratios expected at 200 mg q.d. were ~0.09, 0.10, and 0.11 vs. 0.13, 0.42, and 1.42 at 0 mg doses, for Aβ42:Aβ40, Aβ42:Aβ38, and Aβ42:Aβ37, respectively (data not shown).

A statement regarding data sharing is in the Supplementary Text.

Figure 1. PK and PD plots from study B7991003 (serial CSF sampling biomarker study). (a) Plasma concentration of PF-06648671 over time. (b–f) CSF concentration over time for (b) Aβ42, (c) Aβ40, (d) Aβ38, (e) Aβ37, and (f) total Aβ. Mean profiles are shown as bold lines, profiles from individual subjects are shown as thinner lines. Aβ, amyloid-β; CSF, cerebrospinal fluid; PD, pharmacodynamic; PK, pharmacokinetic.
Figure 2  PK and PD plots from study B7991002 at days 0 and 14 (multiple-ascending dose). (a) Plasma concentration of PF-06648671 by dose. (b–f) CSF concentration by dose for (b) Aβ42, (c) Aβ40, (d) Aβ38, (e) Aβ37, and (f) total Aβ. Box plot shows median (bold horizontal line) as well as quartiles (25% and 75%), with whiskers to the last data point within 1.5 times the IQR. Black dots represent the outlier values (values outside 1.5 times the IQR above the 75% quartile and below the 25% quartile). Aβ, amyloid-β; CSF, cerebrospinal fluid; IQR, interquartile range; PD, pharmacodynamic; PK, pharmacokinetic.

Figure 3  PK/PD model schematic. γ, sigmoidicity constant; Aβ, amyloid-β; Conc, plasma concentration of drug; CSF, cerebrospinal fluid; EC_{50}, plasma concentration at half E_{max}; E_{max}, maximum response achievable; IC_{50}, plasma concentration at half I_{max}; I_{max}, maximum inhibition achievable; k_{in}, zero-order rate of Aβ production; k_{out}, first-order turnover constant of Aβ; PD, pharmacodynamic; PK, pharmacokinetic.
In three phase I studies of PF-06648671, no significant drug-related safety concerns were noted with single-dose and multiple-ascending daily doses for up to 14 days in healthy subjects, including elderly subjects. Severe AEs (headache, nausea, and dizziness) were considered to be secondary to the LP procedure rather than drug-related. The most common AEs of headache, back pain, dizziness, and musculoskeletal discomfort in B7991002 were deemed in most cases to result from post-LP syndrome. Symptoms resolved with supportive treatment in all subjects. No clinically significant laboratory and electrocardiogram abnormalities were observed.

PF-06648671 was rapidly absorbed and plasma $T_{\text{max}}$ was prolonged under fed conditions, consistent with other GSMs. The $t_{1/2}$ of ~20 hours favored once-daily dosing. The mean CSF:plasma concentration ratio at day 14 of multiple dosing was roughly equivalent to the unbound fraction in plasma, indicating that PF-06648671 has excellent CSF penetration.

PF-06648671 resulted in robust inhibition of CSF $A\beta_{42}$ and $A\beta_{40}$ production, and a commensurate increase in $A\beta_{37}$ and $A\beta_{38}$, with no net change in total CSF $A\beta$. These observations are consistent with the GSM mode of action. The greater reduction in CSF $A\beta_{42}$ than $A\beta_{40}$ following single and multiple doses of PF-06648671 over the clinically relevant dose range led to the decrease in $A\beta_{42}:A\beta_{40}$ ratio, indicating that PF-06648671 had a stronger inhibitory effect on $A\beta_{42}$ than $A\beta_{40}$. Similar to other GSMs, PF-06648671 did not lead to the plasma $A\beta$-rebound effect observed with GSIs.

Table 2 PD parameter estimates in the PK/PD indirect-response model

| Species | Parameters | Estimates | RSE, % |
|---------|------------|-----------|--------|
| Aβ42   | Baseline (pg/mL) | 1,001     | 5.19   |
|        | $k_{\text{out}}$ (/hour) | 0.121     | 14.8   |
|        | $I_{\text{max}}$ | 0.744     | 5.92   |
|        | $IC_{50}$ (ng/mL) | 421       | 28.1   |
| Aβ40   | Baseline (pg/mL) | 7,584     | 4.52   |
|        | $k_{\text{out}}$ (/hour) | 0.0835    | 15.3   |
|        | $I_{\text{max}}$ | 0.725     | 15.2   |
|        | $IC_{50}$ (ng/mL) | 1,228     | 38     |
| Aβ38   | Baseline (pg/mL) | 2,404     | 4.22   |
|        | $k_{\text{out}}$ (/hour) | 0.144     | 24.3   |
|        | $E_{\text{max}}$ | 0.869     | 87.2   |
|        | $EC_{50}$ (ng/mL) | 1,765     | 142    |
|        | Gamma ($\gamma$) | 1.4       | 97.3   |
| Aβ37   | Baseline (pg/mL) | 702       | 4.3    |
|        | $k_{\text{out}}$ (/hour) | 0.0964    | 9.93   |
|        | $E_{\text{max}}$ | 5.63      | 21     |
|        | $EC_{50}$ (ng/mL) | 1,285     | 37     |
|        | Gamma ($\gamma$) | 1.42      | 25.8   |

$\gamma$, sigmoidicity constant; Aβ, amyloid-β; EC$_{50}$, plasma concentration at half $E_{\text{max}}$; $E_{\text{max}}$, maximum response achievable; IC$_{50}$, plasma concentration at half $I_{\text{max}}$; $I_{\text{max}}$, maximum inhibition achievable; $k_{\text{out}}$, first-order turnover constant of Aβ; PD, pharmacodynamic; PK, pharmacokinetic; RSE, relative standard error.

DISCUSSION

In three phase I studies of PF-06648671, no significant drug-related safety concerns were noted with single-dose and multiple-ascending daily doses for up to 14 days in healthy subjects, including elderly subjects. Severe AEs (headache, nausea, and dizziness) were considered to be secondary to the LP procedure rather than drug-related. The most common AEs of headache, back pain, dizziness, and musculoskeletal discomfort in B7991002 were deemed in most cases to result from post-LP syndrome. Symptoms resolved with supportive treatment in all subjects. No clinically significant laboratory and electrocardiogram abnormalities were observed.

PF-06648671 was rapidly absorbed and plasma $T_{\text{max}}$ was prolonged under fed conditions, consistent with other GSMs. The $t_{1/2}$ of ~20 hours favored once-daily dosing. The mean CSF:plasma concentration ratio at day 14 of multiple dosing was roughly equivalent to the unbound fraction in plasma, indicating that PF-06648671 has excellent CSF penetration.

PF-06648671 resulted in robust inhibition of CSF $A\beta_{42}$ and $A\beta_{40}$ production, and a commensurate increase in $A\beta_{37}$ and $A\beta_{38}$, with no net change in total CSF $A\beta$. These observations are consistent with the GSM mode of action. The greater reduction in CSF $A\beta_{42}$ than $A\beta_{40}$ following single and multiple doses of PF-06648671 over the clinically relevant dose range led to the decrease in $A\beta_{42}:A\beta_{40}$ ratio, indicating that PF-06648671 had a stronger inhibitory effect on $A\beta_{42}$ than $A\beta_{40}$. Similar to other GSMs, PF-06648671 did not lead to the plasma $A\beta$-rebound effect observed with GSIs.

| Species | Parameters | Estimates | RSE, % |
|---------|------------|-----------|--------|
| Aβ42   | Baseline (pg/mL) | 1,001     | 5.19   |
|        | $k_{\text{out}}$ (/hour) | 0.121     | 14.8   |
|        | $I_{\text{max}}$ | 0.744     | 5.92   |
|        | $IC_{50}$ (ng/mL) | 421       | 28.1   |
| Aβ40   | Baseline (pg/mL) | 7,584     | 4.52   |
|        | $k_{\text{out}}$ (/hour) | 0.0835    | 15.3   |
|        | $I_{\text{max}}$ | 0.725     | 15.2   |
|        | $IC_{50}$ (ng/mL) | 1,228     | 38     |
| Aβ38   | Baseline (pg/mL) | 2,404     | 4.22   |
|        | $k_{\text{out}}$ (/hour) | 0.144     | 24.3   |
|        | $E_{\text{max}}$ | 0.869     | 87.2   |
|        | $EC_{50}$ (ng/mL) | 1,765     | 142    |
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| Aβ37   | Baseline (pg/mL) | 702       | 4.3    |
|        | $k_{\text{out}}$ (/hour) | 0.0964    | 9.93   |
|        | $I_{\text{max}}$ | 5.63      | 21     |
|        | $EC_{50}$ (ng/mL) | 1,285     | 37     |
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$\gamma$, sigmoidicity constant; Aβ, amyloid-β; EC$_{50}$, plasma concentration at half $E_{\text{max}}$; $E_{\text{max}}$, maximum response achievable; IC$_{50}$, plasma concentration at half $I_{\text{max}}$; $I_{\text{max}}$, maximum inhibition achievable; $k_{\text{out}}$, first-order turnover constant of Aβ; PD, pharmacodynamic; PK, pharmacokinetic; RSE, relative standard error.

Figure 4 Visual predictive check for multiple-dose data on day 14 of B7991002. (a) Aβ42. (b) Aβ40. (c) Aβ38. (d) Aβ37. Circles represent observed data; gray solid and dashed lines represent observed median and 90% percentiles; black lines represent the corresponding simulation percentiles. Baseline was not presented for better visualization of dose response. Aβ, amyloid-β; CSF, cerebrospinal fluid.
The placebo response in CSF Aβ observed in B7991003 was likely an artifact of a small CSF volume and frequent sampling, as observed with serial Aβ sampling from CSF elsewhere. Alternatively, the drift could relate to the number of samples obtained over the study, with potential impact on intracranial pressure and/or CSF redistribution to lumbar subarachnoid space. Reducing the sampling frequency may minimize such effects. The placebo response was negligible in B7991002 using single LP collection.

The PK/PD indirect-response model was developed to quantitatively and simultaneously characterize differential effects of PF-06648671 on four forms of CSF Aβ, and total Aβ, and potentially provide mechanistic understanding of these effects. PF-06648671 inhibited CSF Aβ42 and Aβ40 while increasing Aβ37 and Aβ38, with no effect on total Aβ. To our knowledge, this is the first PK/PD model using clinical data to describe differential effects of a GSM on multiple Aβ species. Although simultaneous characterization of all Aβ species is more challenging than using separate models for each species, it is advantageous as the model can account for correlations in baseline levels or drug-induced responses between Aβ species. For example, a patient with high baseline CSF Aβ42 levels most likely has high levels of other Aβ species. Furthermore, a patient whose response is greater in one species is likely to have greater responses in other species. Therefore, our model allowed accurate prediction of drug effects not only on the individual Aβ species but also Aβ ratios of interest, and accounted for the observed delay between PK and PD responses in a semimechanistic manner. However, variability tended to be overpredicted because the number of subjects may have been insufficient for robust characterization of inter-individual variability.

Figure 5 Predicted average steady-state effects of PF-06648671 on CSF samples. (a) Aβ42 percent change from baseline. (b) Aβ40 percent change from baseline. (c) Aβ37 percent change from baseline. (d) Ratio of Aβ42:Aβ40. (e) Ratio of Aβ42:Aβ38. (f) Ratio of Aβ42:Aβ37. Dotted line represents the median, with the shaded area representing the 95% CI. *95% CI for ratio of Aβ42:Aβ38 prediction was truncated due to missing values. Aβ, amyloid-β; CI, confidence interval; CSF, cerebrospinal fluid.
The PK/PD model predicted a 50% reduction in CSF Aβ42 levels with PF-06648671 75 mg, but quadrupling the dose to 300 mg would only give a predicted reduction of 65%, in keeping with the observed data. The plateau observed in Figure 5a suggests that dose levels approached the limit of the GSM mechanism in inhibiting Aβ42 production and that higher doses would not lead to greater inhibition. If Aβ42 inhibition or the ratio of Aβ42 to Aβ40 or other shorter species is important to elicit a clinical benefit with GSM (not yet proven), this model would inform optimal dosing regimens in future efficacy trials.

The reductions in Aβ42 and Aβ40, and increases in Aβ37 and Aβ38, correspond with other GSMS. Decreases in CSF Aβ42 and Aβ40 of up to 77% and 74%, and increases in CSF Aβ37 and Aβ38 of up to 545% and 69%, respectively, were reported with multiple-ascending doses of BMS-932481 over 28 days in healthy subjects. Single dosing of BMS-932481 in healthy subjects led to a decrease of up to 50% in Aβ42 and up to 12-fold and 2-fold increases in Aβ37 and Aβ38, respectively. Another study using single-ascending doses of E2212 reported a decrease in Aβ42 AUC0–24 hours values of up to 44.1%.

Our analysis has limitations and challenges. Data were obtained from healthy subjects, whose PD responses may differ from those of patients with AD. The number of subjects in each cohort was small and data variability was large. Study designs differed between single-dose and multiple-dose studies: rich (serial) PD samples from B7991003 provided information on CSF Aβ dynamics but did not provide a clear exposure–response relationship due to placebo baseline drift and large variability; steady-state information on the PD response with more robust dose-response relationships was obtained from B7991002 but without the Aβ time course. Another challenge was characterizing exposure-response for Aβ38 due to its step-like response; PD parameter estimates for Aβ38 were estimated with large uncertainty. Nevertheless, we believe a single PK/PD model developed from these pooled data provides valuable information and utility in designing future studies and selecting doses.

In conclusion, the GSM PF-06648671 had a good safety profile at doses up to 360 mg q.d. for up to 14 days in healthy young and elderly subjects, was well absorbed, and could be administered once daily irrespective of food. PF-06648671 resulted in robust, dose-dependent reductions in CSF Aβ42 and Aβ40 and elevations in Aβ37 and Aβ38, with no effect on total Aβ. The PK/PD model adequately described the relationship between PF-06648671 exposure and responses across multiple Aβ species and provided quantitative mechanistic understanding of the differential effects. This model could support dose selection and optimization in future PF-06648671 clinical trials testing the therapeutic benefit of modulating levels of Aβ species in AD.

METHODS

Compound
PF-06648671 (2-[[1S]-1-[[25,58]-5-4-chloro-5-fluoro-2-[trifluoromethyl]phenyl]tetrahydrofuran-2-yl]ethyl]-7-(4-methyl-1H-imidazol-1-yl)-3,4-dihydro-2H-pyrido[1,2-a]pyrazine-1,6-dione; Figure S1) was prepared using methods described in a Pfizer patent (WO2014045156, 2014; https://patentscope.wipo.int/search/en/detail.jsf?docId=W02014045156&recNum=262&docAn=1B201 3058347&queryString=EN_ALL:nmr%20AND%20PA:pfi zer&maxRec=8201).

Study design

Studies were phase 1, randomized, investigator blind and subject blind, sponsor open, and placebo controlled (Table 1). Primary and secondary objectives included safety/tolerability, PK characterization, and PF-06448671 effects on Aβ species (PD).

B7991001 was conducted between December 2014 and March 2015 in healthy subjects aged 18–55 years, with a single-ascending oral dose with placebo substitution, in a crossover design. Subjects received a single dose of PF-06648671 or placebo after fasting (all doses) or after a high-fat meal (120 mg fed arm).

B7991003 was conducted between October 2015 and March 2016 in healthy subjects aged 18–55 years. A single oral dose of PF-06648671 or placebo was administered after fasting.

B7991002 was conducted between May 2015 and October 2016 in healthy subjects aged 18–55 years (part 1) and healthy elderly subjects aged 65–85 years (part 2). PF-06648671 was administered orally once daily for 14 days, after fasting (days 1, 7, and 14) or >1 hour before/after a standard breakfast (all other times).

A sample size of 8 subjects per cohort (B7991001), 30 subjects (B7991003), and 10 subjects per cohort (B7991002) aimed to minimize exposure of a new chemical entity yet provide adequate safety, tolerability, and PK data at each dose (B7991001, B7991002), or based on variability estimates from sponsor-conducted studies (B7991003).

Before dosing, randomization numbers were allocated corresponding to a treatment schedule determined by a sponsor-generated randomization code.

Studies were performed at Pfizer Clinical Research Units in Belgium (B7991001 and B7991002) and the United States (B7991003), in compliance with the ethical principles of the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. The protocol was approved by the Independent Ethics Committee at each unit. All subjects provided informed consent.

Safety

AEs, and their severity and potential causality, were recorded by investigators. SAEs were defined as any that resulted in death or were life-threatening, required inpatient hospitalization or prolongation of existing hospitalization, or resulted in persistent or significant disability/incapacity or in congenital anomaly/birth defects. Laboratory evaluations, physical examinations, electrocardiogram monitoring, and vital sign assessments were performed.

PK/PD characterization

For plasma PK analysis, blood samples were collected predose and at regular intervals postdose on day 1 and once daily on days 2–4 (B7991001); predose and at regular intervals on day 1 and at 12–24-hour intervals to day 4 (B7991003); and predose and at regular intervals postdose on days 1, 7, and 14, and once daily on days 2–6, 8–13, and 15–17 (B7991002).

In B7991003, serial CSF samples for PK and PD were collected 2 hours predose and at 0, 2, 4, 8, 12, 16, 20, 24, 28, and 36 hours postdose via PharMed® (Saint-Gobain Biopharm, Aurora, OH) tubing connected to a catheter in each subject’s lumbar. In B7991002, a single LP was performed 72 hours pre-first dose and 24 hours post-dose 1+4 dose, or day 14 predose for those receiving 40 mg q.d.

Supplementary Text summarizes sample and statistical analyses.

PK/PD modeling

Analyses were performed using NONMEM version 7.3 (ICON Development Solutions, Gaithersburg, MD), with a first-order conditional estimation with interaction method. PK was characterized by using a two-compartment model with linear elimination and first-order absorption (Table S4). In estimating PD effects, PK data were included but population PK parameters were fixed (Table S4), using the population PK parameters and data approach.
In B7991003, an empirical placebo model was developed for each Aβ species using placebo data, to account for baseline drift with placebo (Eq. 1):

$$\text{Placebo}_{ij} = (\theta_{P_{\text{max}}} + \eta_i) \cdot t^{\gamma_{ij}}$$

(Eq. 1)

(\(i\), \(j\)th individual; \(j\), \(j\)th time; \(\theta\), fixed-effects parameter; \(P_{\text{max}}\), coefficient describing magnitude of placebo response; \(\eta\), random-effects parameter; \(K'\), exponent of time to describe placebo response rate.) Placebo parameters (\(P_{\text{max}}, \theta\), and \(\eta\)) and interindividual variance in \(P_{\text{max}}\) were fixed when treatment effects were estimated.

A PK/PD indirect-response model was used to characterize effects of PF-06648671 (Figure 3). CSF Aβ responses were described according to whether the production rate of Aβ species decreased (\(A_{1-42}\) and \(A_{1-40}\); Eq. 2) or increased (\(A_{1-42}\) and \(A_{1-40}\); Eq. 3) with respect to PF-06648671 plasma concentration:

$$\frac{dA_{1-40,42}}{dt} = k_{\text{in}} \cdot \left(1 - \frac{\text{Conc}}{I_{50} + \text{Conc}}\right) - k_{\text{out}} \cdot A_{1-40,42}$$

(Eq. 2)

$$\frac{dA_{1-38,37}}{dt} = k_{\text{in}} \cdot \left(1 + \frac{\text{Conc}^2}{E_{50}^2 + \text{Conc}^2}\right) - k_{\text{out}} \cdot A_{1-38,37}$$

(Eq. 3)

\(k_{\text{in}}\), zero-order rate of Aβ production; \(I_{50}\), zero-order turnover rate constant of Aβ (thus, baseline = \(k_{\text{in}}/k_{\text{out}}\)); \(\gamma\), sigmoidicity constant.

Overall PD response was the sum of baseline, placebo (single-dose PD data), and treatment effects. Unique PD parameters were estimated for each Aβ species.

**Model evaluations**

Concordance plots between observed and model-predicted values were created to assess the model’s goodness-of-fit. The model was evaluated by VPC, where 1,000 simulations were performed to check agreement between observed data and final model predictions (simulated data).

**Model predictions**

From the final PK/PD model parameter estimates and variance-covariance matrix, 1,000 sets of population parameters were sampled assuming a multivariate normal distribution. Aβ responses from the sampled parameters were simulated; 2.5th and 97.5th percentiles were taken to construct 95% confidence intervals.

**SUPPORTING INFORMATION**

Supplementary information accompanies this paper on the Clinical Pharmacology & Therapeutics website (www.cpt-journal.com).

**Figure S1.** Chemical structure of PF-06648671: (2-[[((1S)-1-[(25S,5R)-5-[(4-chloro-5-fluoro-2-(trifluoromethyl)phenyl][tetrahydrofuran-2-yl)]ethy]l-7-(4-methyl-1H-imidazol-1-yl)]-3,4-dihydro-2H-pyrind[1,2-al]pyrazine-1,6-dione.

**Figure S2.** Goodness-of-fit plot for observed Aβ species concentration vs. (a–d) population-predicted and (e–h) individual-predicted Aβ species concentration following PF-06648671 single (150, 300 mg; B7991003) or multiple (40, 100, 200, 360 mg q.d.; B7991002) dosing, using the PK/PD indirect-response model. (a, e) Aβ42. (b, f) Aβ40. (c, g) Aβ38. (d, h) Aβ37. Solid line represents a line of identity, and dashed line represents loess smooth. Aβ, amyloid-β; PK/PD, pharmacokinetic-pharmacodynamic.

**Figure S3.** Conditional weighted residual plots. (a) Aβ42. (b) Aβ40. (c) Aβ38. (d) Aβ37. The dashed line represents loess smooth. Aβ, amyloid-β; LOESS, locally weighted scatterplot smoothing.

**Table S1.** Overview of all causality (treatment-related) treatment-emergent adverse events.

| Aβ Species | Treatment-Related EAs |
|------------|-----------------------|
| Aβ1-42     | 18%                   |
| Aβ1-40     | 3%                    |

**CONFLICTS OF INTEREST**

J.E.A., C.C., F.D.C., T.F., C.K., J.M., L.MdC., and R.Q. are employees of, and hold stock options for, Pfizer Inc. P.H., E.H.-K., C.L., and R.L. were employees of, and held stock options for, Pfizer Inc at the time of the research.

**AUTHOR CONTRIBUTIONS**

J.E.A., T.F., E.H.-K., P.H., C.L., J.M., L.MdC., and R.Q. wrote the manuscript. T.F., E.H.-K., C.L., J.M., L.MdC., and R.Q. designed the research. C.C., F.D.C., P.H., C.K., C.L., L.MoC., and R.Q. performed the research. J.E.A., T.F., E.H.-K., P.H., C.L., J.M., and R.Q. analyzed the data.

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