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A Phase 1 Study to Assess Mass Balance and Absolute Bioavailability of Zimlovisertib in Healthy Male Participants Using a ^{14}C-Microtracer Approach

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Abstract

Zimlovisertib (PF-06650833) is a selective, reversible inhibitor of interleukin-1 receptor-associated kinase 4 (IRAK4) with anti-inflammatory effects. This phase 1, open-label, fixed-sequence, two-period, single-dose study aimed to evaluate the mass balance and excretion rate of zimlovisertib in healthy male participants using a ^{14}C-microtracer approach. All six participants received 300 mg ^{14}C-zimlovisertib with lower radioactivity per mass unit orally in Period A, then unlabeled zimlovisertib 300 mg orally and ^{14}C-zimlovisertib 135 μg intravenously (IV) in Period B. Study objectives included extent and rate of excretion of ^{14}C-zimlovisertib, pharmacokinetics, and safety and tolerability of oral and IV zimlovisertib. Total radioactivity recovered in urine and feces was 82.4% $\pm$ 6.8% (urine 23.1% $\pm$ 12.3%, feces 59.3% $\pm$ 9.7%) in Period A. Zimlovisertib was absorbed rapidly following oral administration, with the fraction absorbed estimated to be 44%. Absolute oral bioavailability of the 300-mg dose was 17.4% (90% confidence interval 14.1%, 21.5%) using the dose-normalized area under the concentration–time curve from time 0 to infinity. There were no deaths, serious adverse events (AEs), severe AEs, discontinuations or dose reductions due to AEs, and no clinically significant laboratory abnormalities. These results demonstrate that zimlovisertib had low absolute oral bioavailability and low absorption (<50%).

Keywords

bioavailability, IRAK4 inhibitor, mass balance, zimlovisertib, microtracer accelerator mass spectrometry

Zimlovisertib is a first-in-class, highly selective, reversible inhibitor of Interleukin-1 receptor-associated kinase 4 (IRAK4)[1] that is being evaluated as a potential treatment of different inflammatory conditions, including hidradenitis suppurativa and rheumatoid arthritis (RA).[2, 3] The structure of zimlovisertib[1] is shown in Figure 1a. Zimlovisertib was investigated in two phase 1 studies—a single ascending dose (SAD) and a multiple ascending dose (MAD) study[4]—and exhibited clinically meaningful reductions in signs and symptoms in patients with moderately to severely active RA in a phase 2 study.[3]

In healthy participants, zimlovisertib exposure (area under the concentration–time curve [AUC] and maximum concentration [C_{max}]) increased in a dose-related manner up to a 100-mg dose, with less than proportional increases observed at higher doses.[4] Accumulation ranged from 0.9-fold to 1.4-fold for AUC_{τ} and 0.9-fold to 1.3-fold for C_{max} in the MAD study. Less than 1% of the dose was recovered unchanged in urine, with renal clearance ranging from 14 to 23 mL/min ($\leq$750 mg BID for immediate-release and 300 mg QD for modified-release).[4]

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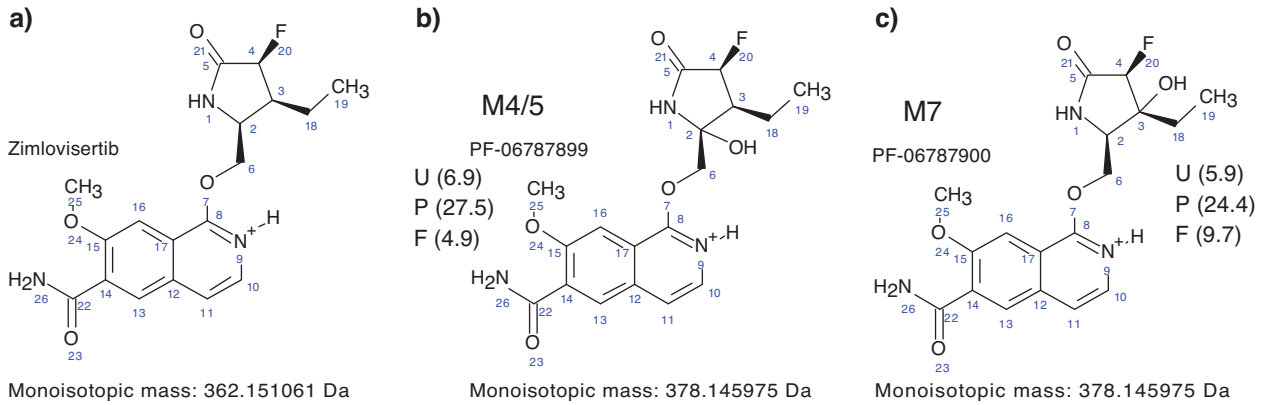

Figure 1. Structure of zimlovisertib (a) and its metabolites, PF-06787899 (b) and PF-06787900 (c). For urine and feces, numbers in parentheses indicate the percentage of oral dose. For plasma, numbers in parentheses indicate the percentage of chromatographic radioactivity. F, feces; P, plasma; U, urine.

Mass balance excretion studies are a standard part of new chemical entity development and are designed to investigate the total fate of the molecule, including mass balance, routes of excretion, and delineation of metabolic pathways.[5] Typical clinical use of a 100 μCi ^{14}C label requires dosimetry assessments (including rat distribution studies), which impact study timelines due to the requirement for preclinical studies.[6, 7] However, application of sensitive ^{14}C-labeled microtracer accelerator mass spectrometry (AMS), which can be used to detect ultra-low microdoses of radiolabel (0.1–1 μCi), circumvents the need for toxicology and dosimetry assessments prior to human dosing and allows for generation of disposition data faster than conventional high-dose ^{14}C approaches.[7, 8, 9, 10] These microdoses of ^{14}C radiolabel are considered insignificant in terms of radioactivity, therefore eliminating the need for animal studies.[6, 7, 11, 12] In addition, ^{14}C-microtracer approaches may present several advantages, such as measurement of multiple pharmacokinetic (PK) parameters with reduced interpatient variability (due to a cross-over type design) and lower cost compared with traditional phase 1 studies. Consequently, this approach is being increasingly used in the evaluation of mass balance of new drugs.[8, 9, 13]

Using a ^{14}C-microtracer approach, we report the absorption, excretion, absolute oral bioavailability, and mass balance of zimlovisertib in healthy male participants. A more detailed description of metabolites and clearance mechanisms will be reported separately.

Methods

The study was conducted between December 2017 and March 2018 at a single center (PRA Health Sciences, Groningen, Netherlands). It was conducted in accordance with the protocol, legal and regulatory requirements, and the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002), International Conference on Harmonization Good Clinical Practice Guidelines, and the Declaration of Helsinki. The study protocol was approved by the Independent Ethics Committee at the study center in Groningen, Netherlands. All participants provided written, informed consent.

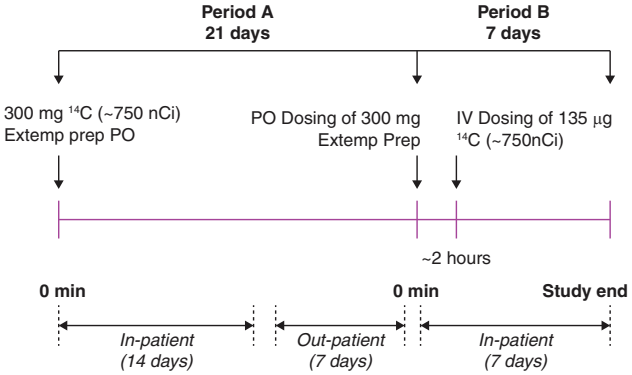

Figure 2. Two-period, fixed-sequence study design. Extemp prep, extemporaneous preparation; IV, intravenous; PO, oral administration.

Overall Study Design and Objectives

This mass balance study was a phase 1 open-label, fixed-sequence, two-period, single-dose study of zimlovisertib (Figure 2). The objective was to assess the absorption, metabolism, and excretion of ^{14}C-labeled zimlovisertib and the absolute oral bioavailability (F) and fraction absorbed (F_a) of zimlovisertib following intravenous (IV) ^{14}C-zimlovisertib 135 μg and oral ^{14}C-zimlovisertib 300 mg with lower radioactivity per mass unit (LR). The low radioactivity (750 nCi) per mass unit in Period A was designed to keep the total radioactivity the same as IV (135 μg) in both periods due to the low solubility of zimlovisertib. The primary objective

was to characterize the rates and extent of excretion of total radioactivity in urine and feces after single oral administration of ^{14}C-zimlovisertib 300 mg LR, including percentage radioactivity excreted at each timepoint, total percentage dose excreted, and percentage recovery of total radioactivity, based on total dose administered. Secondary objectives included determining the single-dose pharmacokinetics of zimlovisertib and its two major metabolites (PF-06787899 and PF-06787900; Figure 1b,c) and determining the safety and tolerability of zimlovisertib in both periods.

Participants

Participants were eligible for inclusion if they were healthy males who had provided informed consent, were aged 18–55 years, and had a body mass index of 17.5–30.5 kg/m^2 and total body weight >50 kg. Exclusion criteria included evidence or history of any clinically significant comorbid disease, including hematological, pulmonary, gastrointestinal, renal, endocrine, hepatic, psychiatric, neurologic, allergic, and cardiovascular diseases; positive hepatitis B surface antigen, hepatitis C antibody, or human immunodeficiency disease results; screening supine blood pressure ≥140 mm Hg (systolic) or ≥90 mm Hg (diastolic) after ≥5 minutes of rest; use of prescription or nonprescription drugs within 7 days or five half-lives (whichever was longer) of the first dose of zimlovisertib; total plasma ^{14}C radioactivity of >11 mBq/mL; or active or latent infection, including tuberculosis, human immunodeficiency virus, and hepatitis viruses.

Treatments

This study was conducted in two parts, Period A and Period B (Figure 2). In Period A, all participants received regimen A (^{14}C-zimlovisertib 300 mg LR orally containing approximately 750 nCi ^{14}C) on day 1. Participants were to remain in the clinic until 90% of dose, or <1% of dose per day for 2 consecutive days, had been recovered from urine and feces. No participant was to be confined beyond day 21, regardless of recovery. Participants returned to the clinic from day −1 until day 8 of Period B, which began within 21–24 days of zimlovisertib administration in Period A. In Period B, participants received regimen B (unlabeled zimlovisertib 300 mg orally followed by a 5-minute IV infusion of ^{14}C-zimlovisertib 135 μg containing approximately 750 nCi ^{14}C at approximately 2 hours after the oral dose) on day 1. Participants were to abstain from food and drink, with the exception of water, for 4 hours prior to laboratory evaluations and for 8 hours prior to the collection of pre-dose PK samples. Lunch and dinner were provided 4 and 10 hours post-oral dose in both periods, respectively. There was no placebo group in the study. The duration of Periods A and B could be up to 21 and 7 days, respectively, with a washout period of at least 21 days between regimen A and B.

Assessments

In Period A, blood, feces, and urine samples were collected over several time points: pre-dose and 0–24 hours post-dose (fecal samples); pre-dose, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours post-dose on day 1 (blood samples); pre-dose, 0–12 hours, and 12–24 hours post-dose on day 1 (urine samples); and at least once daily over the following 8 days for all samples (up to 168 hours for blood samples; blood samples were not collected on days 4 and 6). Up to three additional blood samples were also collected between day 8 and day 21 for ^{14}C AMS and metabolite profiling. In Period B, blood samples were collected over several time points (pre-dose and 0.25, 0.5, 1, and 1.5 hours post-oral dose; 2 hours post-oral dose/pre-IV dose; 3, 4, 6, 8, and 12 hours post-oral dose; and 2.08, 2.17, 2.33, 2.5, 2.75, 3, 3.5, 4, 5, 6, 8, 10, 12, and 18 hours post-IV dose on day 1. Urine and fecal samples were collected 2 hours post-oral dose immediately prior to IV dose, 12 hours post-IV dose and between 0–10 and 10–22 hours (urine) and 0–24 hours (feces) post-IV dose on day 1. All samples were collected once daily over the following 8 days (up to 168 hours; blood samples were not collected on days 4 and 6).

Mass Balance. Urine samples were collected and refrigerated, and the total volume was recorded after each collection period. Fecal samples were collected in polypropylene containers and frozen at −20°C, and the total mass was recorded for each 24-hour collection period. Total ^{14}C in urine and feces samples was measured by a sensitive microtracer approach using AMS at the Netherland Organization for Applied Scientific Research (TNO). In Period A, urine and feces were collected until the total radioactivity recovered was ≥90% or <1% on 2 consecutive days. Radioactivity in urine and feces was reported as the percentage of the administered radioactivity excreted at each time interval and the total percentage of dose excreted.

Plasma and Urine PK Sample Analysis

Total ^{14}C (both periods) and ^{14}C-zimlovisertib (Period B only) in plasma samples were measured by a sensitive microtracer approach using AMS at TNO (the Netherlands). Plasma and urine concentrations of zimlovisertib were measured by liquid chromatography tandem mass spectrometric method (LCMS). An AB SCIEX API 6500 QTRAP system was used for MS. Transitions monitored for zimlovisertib were mass-to-charge ratio (m/z) 362.2 → m/z 219.1 (±0.2 for each m/z) for urine and 362.1→ m/z 219.1 (±0.2 for each m/z) for plasma. For the plasma concentration

measurement, the chromatographic separation on high-performance liquid chromatography (HPLC) was achieved using Phenomenex, Synergy^{TM} 4 μm Hydro-RP 80 Å, 30 × 2 mm column and a mobile phase gradient consisting of 0.1% formic acid in 5.0 mM ammonium formate (component A) and 0.1% formic acid in acetonitrile (component B) delivered at 0.5 mL/min. The gradient started in isocratic mode with component A:component B in 80:20 ratio up to 0.60 minutes, followed by an increase in B to 40% at 2.50 minutes and 95% at 2.55 minutes, which was maintained up to 3.55 minutes, then the percentage of B was decreased to achieve a starting condition at 3.60 minutes, which was maintained up to the run end (5.00 minutes). For urine concentration measurement, the chromatographic separation was achieved using Phenomenex Gemini C18 50 × 3 mm 3 μm column with a mobile phase gradient consisting of 0.1% formic acid in 10 mM ammonium formate (component A) and 0.1% formic acid in acetonitrile (component B) delivered at 0.6 mL/min. The gradient started in isocratic mode with component A:component B in 75:25 ratio up to 0.50 minutes, followed by an increase in B to 90% at 3.00 minutes, which was maintained up to 3.50 minutes, then the percentage of B was decreased to achieve a starting condition at 3.60 minutes, which was maintained up to the run end (4.30 minutes).

The lower limit of quantification (LLOQ) for zimlovisertib by the LCMS method was 1.00 ng/mL in urine and 0.100 ng/mL in plasma whereas the LLOQ for ^{14}C-zimlovisertib in plasma using AMS was 0.485 mBq/mL. The internal standard (IS) for zimlovisertib was a stable-isotope-labeled IS, zimlovisertib-[^{13}C, ^{2}H]. The method of extraction for urine was supported liquid extraction, and for plasma was liquid–liquid extraction. In plasma, within-day and between-day variability were percentage coefficient of variation (%CV) ≤13.5% (relative error [RE] −13.4%–6.0%) and %CV 13.0% (RE −3.4%), respectively. In urine, within-day and between-day variability were %CV ≤6.3% (RE −6.3%–9.0%) and %CV ≤8.1% (RE 3.0%), respectively.

PK Analyses. Plasma and urine pharmacokinetics were assessed during Periods A and B using non-compartmental analysis of concentration–time data. Specifically, plasma PK parameters following oral administration were AUC to the time of the last quantifiable concentration (AUC_{last}), AUC from time 0 to infinity (AUC_{inf}), C_{max}, time to reach C_{max} (T_{max}), terminal elimination half-life ($t_{1/2}$), and apparent oral clearance (CL/F). The urine PK parameters of zimlovisertib calculated after oral administration were amount of zimlovisertib excreted unchanged in urine (Ae), percentage of total dose excreted unchanged in urine (Ae%), and renal clearance (CLr). Following IV infusion, the plasma PK parameters calculated were AUC_{last}, AUC_{inf}, C_{max}, T_{max}, $t_{1/2}$, volume of distribution (Vd [area]), steady-state volume of distribution (V_{ss}), and clearance (CL). The PK parameter analysis population was defined as participants who had at least one of the zimlovisertib PK parameters of interest. F was estimated as the ratio of adjusted geometric means of dose-normalized AUC_{inf} for oral unlabeled zimlovisertib and IV ^{14}C-zimlovisertib in Period B. The natural log transformed dose-normalized AUC_{inf} and AUC_{last} were analyzed using a mixed-effects model with treatment as a fixed effect and participant as a random effect. The estimates of the adjusted (least squares [LS]) mean differences and corresponding 90% confidence interval (CI) were obtained from the mixed-effect model. The adjusted mean differences and 90% CI for the differences were exponentiated to provide estimates of the ratio of adjusted geometric means and 90% CI for the ratio.

F_a was estimated as the ratio of the percentage of administered radioactive dose excreted into the urine following oral and IV administrations of ^{14}C-zimlovisertib in Periods A and B, respectively. The elucidation of metabolic pathways of zimlovisertib were also investigated and will be reported elsewhere.

Safety. Safety and tolerability assessments of zimlovisertib following oral/IV administration included monitoring for adverse events (AEs; any untoward medical occurrence in a participant receiving zimlovisertib, whether related to zimlovisertib or not) and serious AEs (SAEs) at day 1 and days 8–21 in Periods A and B, and a follow-up phone call 28–35 days after last dose in Period B. Any AEs occurring following the initiation of treatment or increasing in severity were counted as treatment emergent AEs (TEAEs). All participants were included in the safety analyses. TEAEs were summarized descriptively. Electrocardiograms (ECGs), vital signs, and laboratory evaluations (chemistry, hematology, and urinalysis) were also monitored throughout the study. Vital signs and laboratory evaluations had to meet predefined criteria to be considered clinically meaningful.

Results

Participants

In total, six healthy, male participants (100% white, mean [standard deviation (SD)] age 33.67 [11.43], BMI 24.2 [3.81] kg/m^2) were assigned to open-label treatment and received regimens A and B (Table S1). All participants completed the study and were included in the PK and safety analyses for both periods. All participants had met the discharge criteria (>90% of recovered radioactivity or <1% of radioactivity recovered on 2 consecutive days) by day 8, but their stay was longer due to delay in the availability of the data. All participants

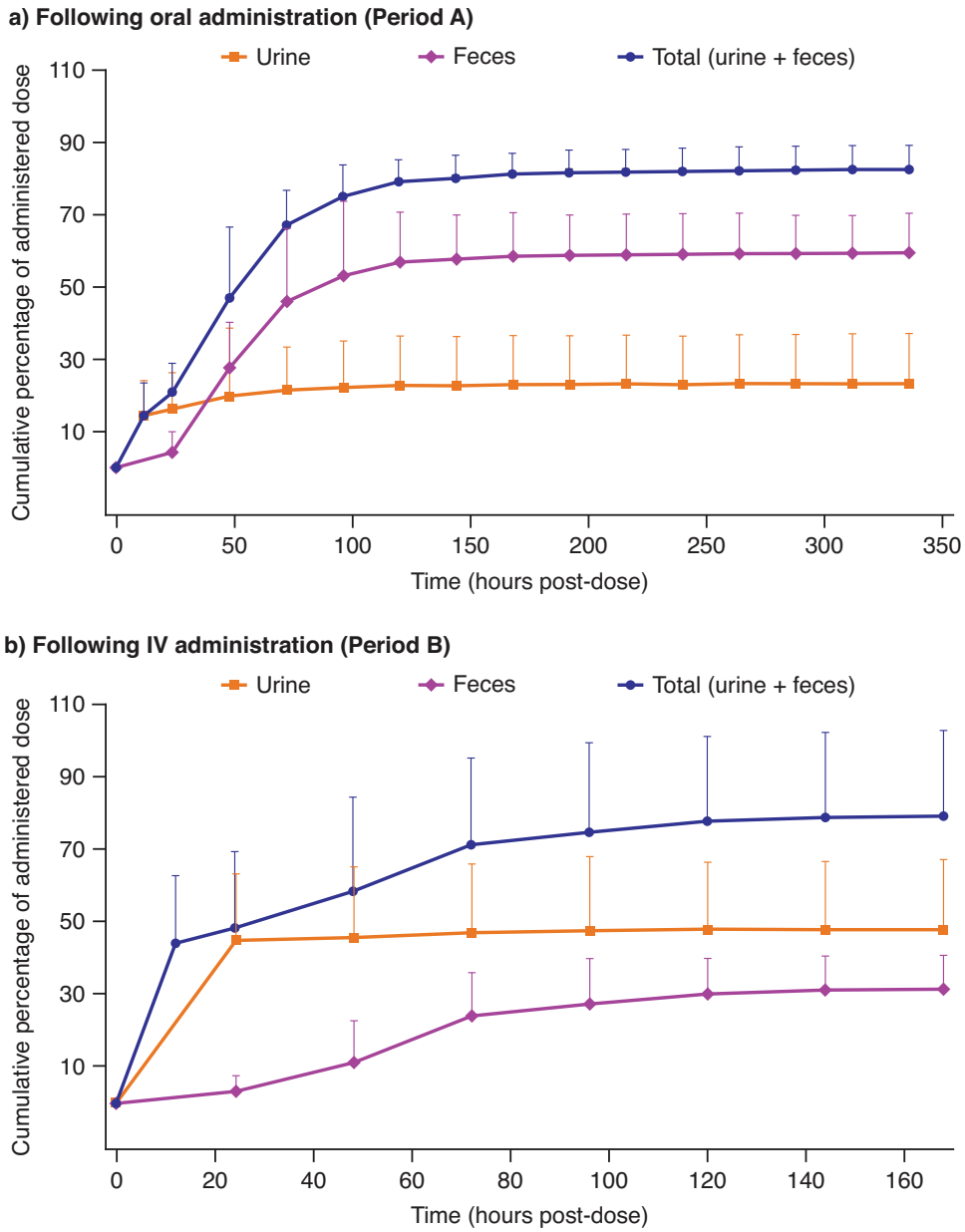

Figure 3. Mean (SD) cumulative elimination of radioactivity in urine, feces, and total (urine and feces combined) plot. Error bars represent +SD. IV, intravenous; SD, standard deviation.

were discharged by day 14 in Period A and by day 8 in Period B.

Mass Balance. In Period A, total recovery over a period of 336 hours post-dose was 82.4% ± 6.8% (urine 23.1% ± 12.3%, feces 59.3% ± 9.7%). The corresponding total recovery of administered radioactivity in Period B over 168 hours following IV administration was 82.6% ± 22.8% (urine 51.2% ± 18.6%, feces 31.4% ± 8.8%). In Period A, 35.5% of radioactivity was from zimlovisertib recovered following analysis of pooled human feces, indicating low absorption (Figure 3).

Plasma PK. Mean plasma concentration profiles of zimlovisertib in Period A and Period B are shown in Figure 4. The mean plasma concentration–time profiles in Period B of total ^{14}C (by AMS), ^{14}C-zimlovisertib (by AMS), and zimlovisertib (by LCMS) are shown in Figure 5. Total ^{14}C concentration–time profiles (Periods A and B) are shown in Figure 6. Concentration–time profiles for unlabeled zimlovisertib and total ^{14}C were comparable in Periods A and B.

PK parameters of zimlovisertib and its two major metabolites, PF-06787899 and PF-06787900, are summarized in Table 1. Zimlovisertib was absorbed

Table 1. Plasma pharmacokinetic parameter values of zimlovisertib and its two major metabolites, PF-06787899 and PF-06787900, following administration of ^{14}C-zimlovisertib LR 300 mg orally (Period A) and unlabeled zimlovisertib 300 mg orally and ^{14}C-zimlovisertib 135 μg IV (Period B)

Parameter Summary Statistics by Treatment									
	^{14}C-zimlovisertib LR 300 mg PO Period A				**Unlabeled zimlovisertib 300 mg PO + ^{14}C-zimlovisertib 135 μg IV[a] Period B**				
	Zimlovisertib		**Metabolites**		**Zimlovisertib**			**Metabolites**	
Parameter (Units)	**Zimlovisertib**	**Total ^{14}C PO**	**PF-06787899**	**PF-06787900**	**^{14}C-zimlovisertib**	**Zimlovisertib**	**Total ^{14}C IV**	**PF-06787899**	**PF-06787900**
N, n	6, 4	6, 1	6, 3	6, 3	6, 6	6, 5	6, 1	6, 5	6, 3
AUC_{inf} (ng·h/mL[b]), geometric mean (geometric %CV)	1510 (41)	48.2[c]	2109 (32)	867.6 (63)	3.660 (22)	1481 (15)	164[c]	2097 (20)	883.4 (24)
AUC_{inf} (ng·h/mL[b]), arithmetic mean (SD)	1592 (533.88)	NC	2183 (735.69)	975.3 (599.00)	3.737 (0.87)	1494 (223.23)	NC	2130 (418.57)	898.7 (194.38)
AUC_{last} (ng·h/mL[b]), geometric mean (geometric %CV)	1453 (33)	47.63 (37)	1878 (28)	792.7 (46)	3.640 (22)	1303 (33)	131.2 (11)	1909 (22)	786.8 (42)
AUC_{last} (ng·h/mL[b]), arithmetic mean (SD)	1513 (430.51)	50.27 (18.37)	1940 (568.61)	864.0 (418.70)	3.72 (0.88)	1355 (371.36)	131.8 (13.96)	1948 (443.01)	842.8 (355.89)
C_{max} (ng/mL[b]), geometric mean (geometric %CV)	283.8 (29)	3.572 (30)	237.2 (36)	127.6 (38)	6.052 (77)	209.9 (11)	67.97 (77)	256.6 (35)	120.3 (27)
C_{max} (ng/mL[b]), arithmetic mean (SD)	293.7 (86.21)	3.70 (1.01)	249.2 (82.25)	134.6 (45.48)	7.13 (3.77)	211.0 (23.43)	79.93 (42.08)	269.0 (88.38)	123.7 (29.42)
T_{max} (h), median (range)	0.500 (0.500–2.00)	1.00 (0.500–2.00)	2.00 (1.00–2.05)	1.00 (0.500–2.00)	0.0830 (0.0830–0.0830)	1.00 (0.500–1.50)	0.083 (0.083–0.500)	1.50 (0.500–1.97)	1.50 (0.500–1.50)

(*Continued*)

Table 1. Continued

Parameter Summary Statistics by Treatment									
	^{14}C-zimlovisertib LR 300 mg PO Period A				Unlabeled zimlovisertib 300 mg PO + ^{14}C-zimlovisertib 135 μg IV[a] Period B				
	Zimlovisertib		Metabolites		Zimlovisertib			Metabolites	
Parameter (Units)	Zimlovisertib	Total ^{14}C PO	PF-06787899	PF-06787900	^{14}C-zimlovisertib	Zimlovisertib	Total ^{14}C IV	PF-06787899	PF-06787900
$t_{1/2}$ (h), arithmetic mean ± SD	19.58 ± 7.6	52.5[c]	21.50 ± 7.1	22.10 ± 4.9	1.987 ± 0.4	18.22 ± 5.7	96.9[c]	22.14 ± 5.6	23.47 ± 2.5
CL/F (mL/min) oral, geometric mean (geometric %CV)	3372 (41)	NC	NC	NC	NC	3379 (15)	NC	NC	NC
CL/F (mL/min) oral, arithmetic mean (SD)	3593 (1593.3)	NC	NC	NC	NC	3408 (494.6)	NC	NC	NC
CL (mL/min) IV, geometric mean (geometric %CV)	NC	NC	NC	NC	587.5 (22)	NC	NC	NC	NC
CL (mL/min) IV, arithmetic mean (SD)	NC	NC	NC	NC	598.7 (121.23)	NC	NC	NC	NC
Vd(area) (L) IV, geometric mean (%CV)	NC	NC	NC	NC	99.27 (11)	NC	NC	NC	NC
Vd(area) (L) IV, arithmetic mean (SD)	NC	NC	NC	NC	99.83 (12.12)	NC	NC	NC	NC
V_{ss} (L) IV, geometric mean (geometric %CV)	NC	NC	NC	NC	66.56 (26)	NC	NC	NC	NC
V_{ss} (L) IV, arithmetic mean (SD)	NC	NC	NC	NC	68.43 (17.31)	NC	NC	NC	NC

Period B dosing: the ^{14}C-zimlovisertib IV dose was administered as an infusion over approximately 5 minutes starting at approximately 2 hours after the unlabeled oral dose.

%CV, percent coefficient of variation; AUC_{inf}, area under the concentration–time curve from time 0 to infinity; AUC_{last}, area under the concentration–time curve from time 0 to the time of the last quantifiable concentration; CL, systemic clearance; CL/F, apparent clearance; C_{max}, maximum plasma concentration; dpm, disintegrations per minute; IV, intravenous; LR, lower radioactivity per mass unit; N, number of participants in the treatment group; n, number of participants where $t_{\frac{1}{2}}$, AUC_{inf}, CL, CL/F, Vd(area), and V_{ss} were determined where applicable; NC, not calculated; PO, oral administration; SD, standard deviation; $t_{\frac{1}{2}}$, terminal half-life; T_{max}, time to reach maximum plasma concentration; Vd, volume of distribution; V_{ss}, steady-state volume of distribution.

[a]Note actual ^{14}C-zimlovisertib IV doses were less than 135 μg. Actual dose for all participants was 129 μg.

[b]Units for radioactivity parameters are dpm/mL (C_{max}) or dpm • h/mL (AUC). Period A and Period B total ^{14}C-zimlovisertib only.

[c]Only one observation, no summary statistics calculated.

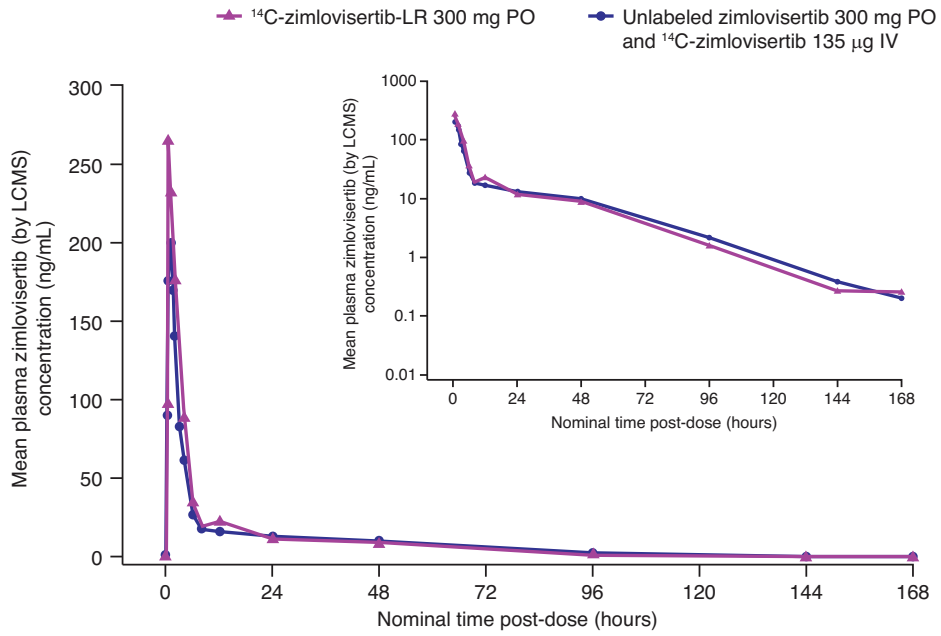

Figure 4. Mean plasma zimlovisertib concentration (by LCMS) over time. Outer and inner panels are linear and semilogarithmic, respectively. IV, intravenous; HR, hour; LCMS, liquid chromatography tandem mass spectrometric method; LR, lower radioactivity per mass unit; PO, oral administration.

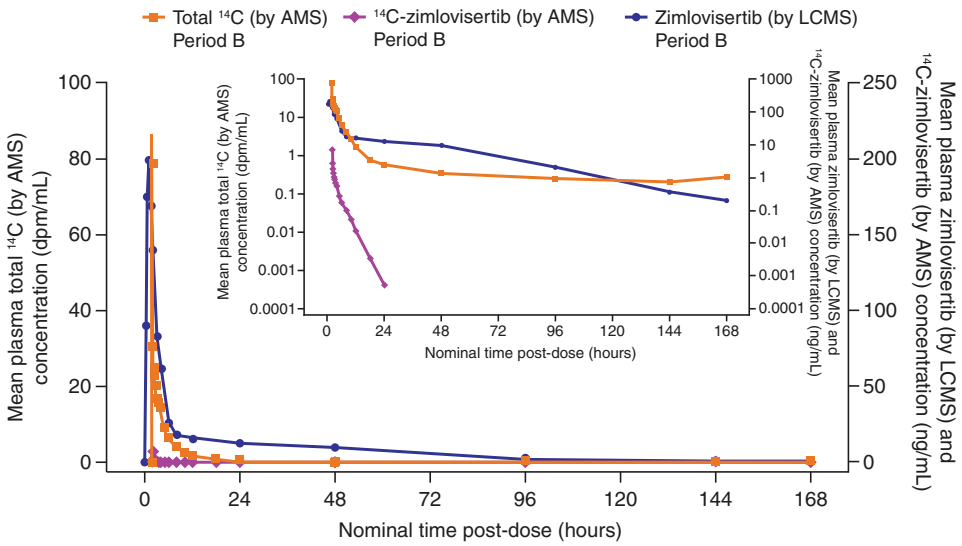

Figure 5. Mean plasma zimlovisertib concentration (by LCMS) over time, total ^{14}C (by AMS) and ^{14}C-zimlovisertib (by AMS) following administration of unlabeled zimlovisertib 300 mg PO + ^{14}C-zimlovisertib 135 μg IV (Period B). Outer and inner panels are linear and semilogarithmic, respectively. Planned times for ^{14}C-zimlovisertib IV were relative to the PO dose (start of the IV infusion was approximately 2 hours post-zimlovisertib PO and infusion took around 5 minutes). AMS, accelerator mass spectrometry; HR, hour; IV, intravenous; LCMS, liquid chromatography tandem mass spectrometric method; PO, oral administration.

rapidly following oral administration (median T_{max} of 0.5 hours [range 0.5–2.0 hours] in Period A and 1.0 hour [range 0.5–1.5 hours] in Period B). Median T_{max} was 1.0 hour [range 0.5–2.0 hours] for total ^{14}C in Period A. Unlabeled plasma zimlovisertib arithmetic and geometric mean AUC and C_{max} exposures were overlapping in Periods A and B. Mean apparent terminal $t_{\frac{1}{2}}$ for ^{14}C-zimlovisertib 135 μg IV in Period B was only 1.987 hours, possibly due to the concentration falling below the LLOQ of 0.485 mBq/mL. The corresponding terminal $t_{\frac{1}{2}}$ for zimlovisertib was 19.58 and 18.22 hours, respectively, in Periods A and B. Median plasma concentrations of ^{14}C were similar after oral and IV administration. Apparent arithmetic mean CL/F for

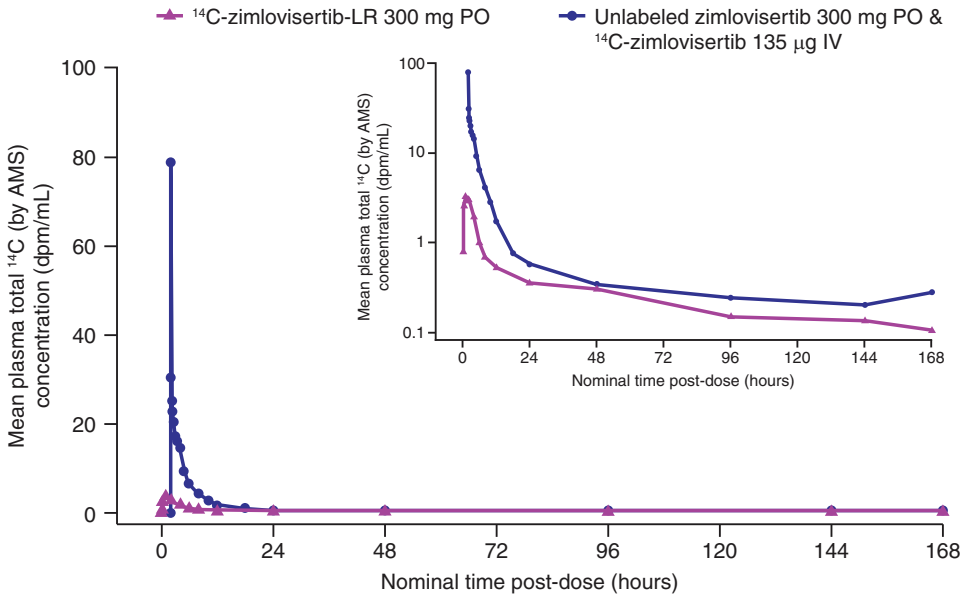

Figure 6. Mean plasma zimlovisertib total ^{14}C concentration over time (by AMS). Outer and inner panels are linear and semilogarithmic, respectively. Planned times for ^{14}C-zimlovisertib IV were relative to the PO dose (start of the IV infusion was approximately 2 hours post-zimlovisertib PO and infusion took around 5 minutes). AMS, accelerator mass spectrometry; HR, hour; IV, intravenous; LCMS, liquid chromatography tandem mass spectrometric method; LR, lower radioactivity per mass unit; PO, oral administration.

zimlovisertib was 3593 and 3408 mL/min in Periods A and B, respectively. Apparent geometric mean CL/F for zimlovisertib was 3372 and 3379 mL/min in Periods A and B, respectively. Arithmetic mean CL for ^{14}C IV dosing during Period B was 598.7 mL/min. Geometric mean CL for ^{14}C IV dosing during Period B was 587.5 mL/min. Arithmetic mean (SD) Vd (area) following IV administration was 99.83 (12.12) L. Mean V_{ss} for ^{14}C IV dosing during Period B was 66.56 L. Variability in total zimlovisertib exposure was moderate for AUC_{inf} (oral unlabeled drug geometric %CV 41% vs IV ^{14}C-labeled drug 22%). Geometric %CV was also moderate to high for C_{max} (oral unlabeled drug 29% vs IV ^{14}C-labeled drug 77%). F of the 300-mg dose was 17.4% (90% CI 14.1%, 21.5%) using dose-normalized AUC_{inf}. F_a based on dose-normalized total ^{14}C values was 44.4%. The F and F_a values were <50%, indicating low absorption.

Median T_{max} for the metabolites PF-06787899 and PF-06787900 was 2.00 and 1.00 hours, respectively, for Period A and 1.50 hours for both metabolites in Period B. Mean apparent terminal $t_{\frac{1}{2}}$ for PF-06787899 and PF-06787900 was 21.50 and 22.10 hours, respectively, for Period A, and 22.14 and 23.47 hours, respectively, for Period B. Variability in exposure of the metabolites was moderate, based on %CV of AUC_{inf} and C_{max} for oral labeled and unlabeled administration of zimlovisertib (Table 1).

Urinary excretion of zimlovisertib was a minor route of elimination (0.29% of parent drug excreted unchanged in the urine) over a 336-hour collection period. Urine PK parameters are summarized in Table S2. Geometric mean renal clearance was 577 mL/h (19% CV). Secretion of zimlovisertib in the gastrointestinal tract was low after IV administration, with only 1.7% of the dose detected unchanged in feces.

Safety

Zimlovisertib was well tolerated with an acceptable safety profile. There were no deaths, other serious AEs, severe AEs, discontinuations due to AEs, temporary discontinuations or dose reductions due to AEs, and no medication errors reported during the study. There were 13 TEAEs in the study, nine following oral administration of ^{14}C-zimlovisertib 300 mg LR and four following oral unlabeled zimlovisertib 300 mg plus ^{14}C-zimlovisertib 135 μg IV, all of which were mild in severity and resolved by the end of the study. Of these, four were considered related to treatment (three events of headache, one event of nausea). TEAEs were experienced across both periods in two participants (two participants experienced two events of headache each [one in Period A and one in Period B] and one of these participants also experienced one event of neck pain continuously for around 31 days across Periods A and B). No conclusions could be drawn from the incidence of TEAEs in the two treatment periods because of the small numbers of both AEs and participants. There were no clinically significant laboratory abnormalities, and none of the absolute values or changes from

baseline in vital signs or ECGs met predefined categorical criteria.

Discussion

In this study, a sensitive ^{14}C-labeled microtracer approach was successfully used to investigate the mass balance, absolute bioavailability, and pharmacokinetics of zimlovisertib in healthy male participants. Due to the low solubility of zimlovisertib,[1] a ^{14}C-labeled zimlovisertib IV dose of 135 μg with low radioactivity was administered in Period B to have the same radioactivity as that in 300 μg of IV dose in Period A. PK profiles of zimlovisertib (measured by LCMS) administered as ^{14}C-zimlovisertib 300 mg LR in Period A and as zimlovisertib in Period B were comparable, indicating that both materials had similar absorption. The total radioactivity recovered was 82.4% and 82.6% after oral and IV administration of ^{14}C-labeled zimlovisertib, respectively, suggesting acceptable recovery of zimlovisertib.

The majority of orally administered zimlovisertib was not absorbed (F and F_a were 17% and 44%, respectively) and was eliminated unchanged via feces (59%). The fact that 31.4% of the radioactivity was recovered in feces after IV administration of ^{14}C-zimlovisertib 135 μg, but unchanged zimlovisertib contributed <2% of the radioactivity, suggests that the metabolites of zimlovisertib undergo biliary excretion or intestinal secretion. Urinary excretion is a minor route of elimination of orally administered zimlovisertib, with <1% of zimlovisertib being recovered unchanged in the urine. Similarly, <1% of zimlovisertib was recovered unchanged in urine in the first-in-human study.[4] Following IV administration, secretion of zimlovisertib in the gastrointestinal tract was low, with <2% recovered unchanged in feces. Of the total recovered radioactive dose, 51.2% was recovered in urine after IV administration, indicating that the majority of metabolites are excreted in the urine for this route of administration.

The terminal phase of median total ^{14}C time profile, as measured by AMS, was similar after oral and IV administration. Only one participant in each period met the criteria for calculation of terminal half-life due to variability in the data. Although the slope of the terminal phase of total radioactivity appears shallow, all participants met the discharge criteria (>90% radioactivity recovery or <1% radioactivity recovered on 2 consecutive days) by day 8, five out of six participants had >80% recovery of radioactivity by day 8, and one participant had 72.1% recovery in the same time period. The shorter apparent terminal phase following the IV dose may have been due to concentrations falling below LLOQ. No metabolites with long half-lives were identified in the plasma. The formation of two major metabolites (PF-06787899 and PF-06787900) was mediated by CYP3A4. A complete in vitro and in vivo metabolic characterization will be communicated separately.

In this study, zimlovisertib was generally well tolerated following oral or simultaneous oral/IV administration, with a safety profile consistent with previously conducted clinical studies.

Conclusions

Intestinal absorption was <50% and bioavailability <20% following oral administration of zimlovisertib. Recovery in urine and feces was 82.4% ± 6.8% (urine 23.1% ± 12.3%, feces 59.3% ± 9.7%). Fecal excretion is the primary route of excretion of unabsorbed, unchanged zimlovisertib. Major metabolites of zimlovisertib undergo biliary excretion and/or intestinal secretion. Renal excretion of unchanged zimlovisertib is low.

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Conflicts of Interest

All authors are employees and may hold stock in Pfizer Inc. Medical writing support, under the guidance of the authors, was provided by Neil Cockburn, BSc, and Molly MacFadyen, MSc, at CMC Connect, McCann Health Medical Communications, and was funded by Pfizer Inc, New York, NY in accordance with Good Publication Practice (GPP3) guidelines (*Ann Intern Med*. 2015;163[6]:461–464).

Data Availability Statement

Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions, and exceptions, Pfizer may also provide access to the related individual de-identified participant data. See https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information.

References

1. Lee KL, Ambler CM, Anderson DR, et al. Discovery of clinical candidate 1-{[(2S,3S,4S)-3-ethyl-4-fluoro-5-oxopyrrolidin-2-yl]methoxy}-7-methoxyisoquinoline-6-carboxamide (PF-06650833), a potent, selective inhibitor of interleukin-1 receptor associated kinase 4 (IRAK4), by fragment-based drug design. *J Med Chem*. 2017;60(13):5521–5542.

2. ClinicalTrials.gov A study to evaluate the safety and efficacy of PF-06650833, PF-06700841, and PF-06826647 in adults with hidradenitis suppurativa. Available at: https://clinicaltrials.gov/ct2/show/study/NCT04092452
3. Danto SI, Shojaee N, Singh RSP, et al. Efficacy and safety of the selective interleukin-1 receptor associated kinase 4 inhibitor, PF-06650833, in patients with active rheumatoid arthritis and inadequate response to methotrexate [abstract 2909]. *Arthritis Rheumatol*. 2019;71(suppl 10):5177.
4. Danto SI, Shojaee N, Singh RSP, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of PF-06650833, a selective interleukin-1 receptor-associated kinase 4 (IRAK4) inhibitor, in single and multiple ascending dose randomized phase 1 studies in healthy subjects. *Arthritis Res Ther*. 2019;21(1):269.
5. Roffey SJ, Obach RS, Gedge JI, Smith DA. What is the objective of the mass balance study? a retrospective analysis of data in animal and human excretion studies employing radiolabeled drugs. *Drug Metab Rev*. 2007;39(1):17–43.
6. Lozac'h F, Fahrni S, De Maria D, et al. Evaluation of cAMS for 14C microtracer ADME studies: opportunities to change the current drug development paradigm. *Bioanalysis*. 2018;10(5):321–339.
7. Spracklin DK, Chen D, Bergman AJ, Callegari E, Obach RS. Mini-review: comprehensive drug disposition knowledge generated in the modern human radiolabeled ADME study. *CPT Pharmacometrics Syst Pharmacol*. 2020;9(8):428–434.
8. Lappin G, Noveck R, Burt T. Microdosing and drug development: past, present and future. *Expert Opin Drug Metab Toxicol*. 2013;9(7):817–834.
9. Wang X, Chen J, Reyes J, Zhou S, Palmisano M, Li Y. A phase 1, open-label study in healthy subjects to evaluate the absolute bioavailability of AG-221 by a microtracer approach. *Oncol Ther*. 2020;8(1):91–102.
10. van Duijn E, Sandman H, Grossouw D, Mocking JAJ, Coulier L, Vaes WH. Automated combustion accelerator mass spectrometry for the analysis of biomedical samples in the low attomole range. *Anal Chem*. 2014;86(15):7635–7641.
11. Madeen E, Corley RA, Crowell S, et al. Human in vivo pharmacokinetics of [(14)C]dibenzo[def,p]chrysene by accelerator mass spectrometry following oral microdosing. *Chem Res Toxicol*. 2015;28(1):126–134.
12. Brown K, Tompkins EM, White INH. Applications of accelerator mass spectrometry for pharmacological and toxicological research. *Mass Spectrom Rev*. 2006;25(1):127–145.
13. Boulton DW, Kasichayanula S, Keung CFA, et al. Simultaneous oral therapeutic and intravenous ^{14}C-microdoses to determine the absolute oral bioavailability of saxagliptin and dapagliflozin. *Br J Clin Pharmacol*. 2013;75(3):763–768.

Supplemental Information

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